Animal Legal Defense Fund

525 East Cotati Avenue, Cotati, California 94931 T 707.795.2533 F 707.795.7280

ANIMAL LEGAL DEFENSE FUND'S COMMENTS TO NIH NOT-OD-16-128

Request for Public Comment on the Proposed Changes to the NIH Guidelines for Human Stem Cell Research and the Proposed Scope of an NIH Steering Committee's Consideration of Certain Human-Animal Chimera Research

I. BACKGROUND

A. ANIMAL LEGAL DEFENSE FUND

Animal Legal Defense Fund (ALDF) is a national nonprofit organization founded in 1979 to protect the lives and advance the interests of animals through the legal system. ALDF's membership is comprised of thousands of dedicated attorneys and more than 100,000 supporters throughout the United States. Every day, ALDF works to protect animals through litigation, legal assistance, administrative action, the promotion of the study of animal law, and public education.

Pursuant to this mission, ALDF works on a broad range of animal law issues including animal research generally, and research on humanized chimeric and transgenic animals specifically. As explained in the following subsection, this work includes submitting a rulemaking petition to NIH's parent agency, HHS.

In the present near-void of regulation and oversight, ALDF also expends substantial organizational resources monitoring progress in this important area of research to ensure compliance with applicable legal standards. ALDF therefore possesses a strong organizational interest in the NIH and HHS's appropriate regulation of this field of research.

B. ALDF'S 2013 RULEMAKING PETITION TO HHS

In December 2013, ALDF submitted a petition for rulemaking to HHS asking it to adopt regulations that expressly recognize the unique status of humanized chimeras, and implement an oversight framework to balance concerns related to the use of such beings.[1] Specifically, ALDF asked HHS to promulgate regulations pursuant to the PHSA recognizing that humanized chimeras

[1] A copy of *ALDF's 2013 Petition for Rulemaking to HHS* is appended to these comments as Enclosure 1.

with high level cognitive capacity qualify as human research subjects. ALDF further asked HHS to require ongoing IRB review of all experiments involving chimeras where there was a substantial possibility of a chimera obtaining high level cognitive capacity. The purpose of IRB review in that rulemaking petition is to safeguard against the possibility of creating a chimera with high level cognitive capacity, and thus violating that individual's rights.

HHS did not acknowledge or respond to ALDF's rulemaking petition. ALDF continued monitoring this field of research to ensure adequate protection of humanized chimeras. In conjunction with this continued monitoring, ALDF submitted a supplement to its HHS rulemaking petition in April 2015. That supplement highlighted recently published research where certain human cells almost completely took over the brains of research mice in an experiment.

ALDF's supplemental petition also asked HHS to expand the scope of the proposed rule to include protection and oversight related to the use of humanized transgenic animals.[2] ALDF cited recently published research where a humanized language gene caused changes that did or could affect cognition. In one study, mice with a humanized FOXP2 gene demonstrated accelerated learning by enhancing transitions from declarative to procedural performance. In another study, fetal mice with a humanized HARE5 gene had significantly larger brains than mice with a chimpanzee version of the HARE5 gene.

HHS responded to ALDF's rulemaking petition supplement in May of 2015, acknowledging receipt of the supplement while not promising to do more.[3]

Presently, ALDF's rulemaking petition is still pending before HHS. The arguments and evidence made in that enclosed rulemaking petition and related documents should be reviewed and considered as part of these comments as the two processes are interrelated. ALDF is concurrently providing a copy of these enclosed comments to HHS as part of the administrative record for that rulemaking petition.

II. ALDF'S COMMENTS TO NIH'S PROPOSED CHANGES TO THE *GUIDELINES* AND PROPOSED STEERING COMMITTEE

NIH suspended all funding for human-animal chimera research in September 2015 reportedly due to legal and ethical concerns about such research. In August 2016, NIH proposed funding such research again in tandem with proposed changes to its *NIH Guidelines for Human Stem Cell Research* (*Guidelines*) and the creation of a steering committee.[4]

---

[2] A copy of *ALDF's 2015 Petition for Rulemaking Supplement* is included as Enclosure 2.

[3] A copy of HHS's 2015 correspondence with ALDF is included as Enclosure 3.

[4] 81 Fed. Reg. 51921.

As part of these changes, NIH proposes expanding its prohibition of certain types of research involving human-animal chimeras. Under the proposal, NIH will not fund studies involving introduction of human stem cells into nonhuman primates through the blastocyst stage.[5] NIH will also not fund research involving breeding any of human-animal chimera where human cells may affect the germ line.[6]

In addition to expanding those prohibitions, NIH also proposes establishing a steering committee to provide general programmatic advice to NIH on human-animal chimera policy and research funding.

NIH's proposed changes and steering committee omit four important elements that are necessary to address significant legal and ethical issues surrounding this field of research.[7] First, NIH notes that the purpose of the *Guidelines* is to "help ensure that NIH-funded research in this area is ethically responsible, scientifically worthy, and conducted in accordance with applicable law", yet both the notice for comment and the proposed guideline changes fail to precisely articulate any ethical or legal concerns with the creation of human-animal chimeras. To address this concern, NIH should recognize that – at a minimum – humanized animals with *high level cognitive capacity* qualify as human research subjects protected under the Public Health Services Act (PHSA), and thus cannot be created or used in research.

Second, NIH narrowly focuses its policy on human-animal chimeras, but does not have any framework to address the use of humanized *transgenic* animals with humanized cognition. To address this concern, NIH should include humanized transgenic animals in its recognition of the rights of humanized animals with high level cognitive ability (see previous paragraph), and should also require oversight of research on humanized transgenic animals under the purview of the proposed steering committee.

Third, NIH's proposed steering committee will be inadequate to ensure regular and ongoing oversight of specific research projects. To address this concern, NIH should prohibit funding of research where there is no regular IRB review of research involving human-animal chimeras or humanized transgenic animals.

---

[5] The *Guidelines* only presently prohibit introduction of human pluripotent stem cells into blastocyst stage nonhuman primate embryos but does not prohibit introduction into pre-blastocyst stage nonhuman primate embryos.

[6] The *Guidelines* only presently prohibit breeding human-animal chimeras where human embryonic stem cells (hESCs) or human induced pluripotent stem cells may contribute to the germ line.

[7] Generally, ALDF' asks NIH to modify its proposed guideline changes to conform more closely to the regulations that ALDF proposed in its 2013 Petition for Rulemaking to HHS. As stated previously, that rulemaking petition and related correspondence is included as Enclosures 1 through 3. NIH should review those documents and consider them as a part of these comments.

1. NIH should explicitly recognize that humanized animals with high level cognitive capacity qualify as human research subjects protected by the PHSA, and prohibit funding research on such beings

The stated purpose of the *Guidelines* is to "help ensure that NIH-funded research in this area is ethically responsible, scientifically worthy, and conducted in accordance with applicable law." However, NIH's proposed changes fail to identify any specific ethical or legal problems that NIH seeks to avoid. NIH should look to the PHSA as a controlling authority on this legal issue, and recognize – at a minimum – that humanized chimeras (and other humanized animals) with high level cognitive capacity qualify as human research subjects. Moreover, NIH should ensure that this principle is reflected in any changes to the *Guidelines* by prohibiting the creation or research on such beings.

a) The PHSA provides strong legal rights to human research subjects

NIH should look to the PHSA to establish a controlling legal standard for the boundaries of legally permissible research on humanized research animals.

The PHSA – which is cooperatively implemented by HHS and NIH – generally requires administration of certain protections for "human research subjects."[8] The statutory duty to protect human research subjects under the PHSA led to the promulgation of a codified Basic HHS Policy for Protection of Human Research Subjects (sometimes referred to as the "Common Rule"), which requires continuing review by an Institutional Review Board (IRB) to ensure recognition of basic protections such as informed consent, confidentiality, minimal risk to subjects, and equitable selection of subjects.[9]

The legal question posed by research on humanized animals is: at what point do the animals become "human research subjects" as a matter of statutory law under the PHSA and associated regulations? This legal question underlies the tension that prompted NIH to suspend research on human-animal chimeras, and propose changes to its regulations at issue today.

ALDF proposes that the NIH adopt "high level cognitive capacity" as a standard to distinguish between humanized animals (i.e. animals with some human cells or genes)[10] that

---

[8] *See* 42 U.S.C. § 289(a); 42 U.S.C. § 289(a).

[9] 45 C.F.R. §§ 46.101, *et seq*.

[10] Although NIH's comments are tailored to research on human-animal chimeras, ALDF's comments propose expanding the scope of this policy to include human-animal transgenics as well as explained in the following section to these comments.

qualify as human research subjects under the PHSA, and those that do not. Specifically, ALDF proposes that NIH adopt the following definition of high level cognitive capacity:

> mental ability that is substantially similar to a normal human considering the individual's (i) linguistic ability; (ii) degree of self-awareness; (iii) sense of past and future self; (iv) moral agency; and / or (v) rational agency.

The articulated factors are ones commonly connected with adult human cognitive ability in relevant ethics literature. In particular, this standard is based on the work of ethicist Robert Streiffer who addressed this very question and suggested that "the introduction of human stem cells into a nonhuman animal that would substantially enhance its moral status is wrong [because] subsequent treatment of the subject likely will fall far below what its new moral status demands."[11] Streiffer notes that chimeric research has the potential to imbue the chimera recipient with "high level cognitive capacities" similar to what normal adult humans possess,[12] and that a chimera who obtained that high level cognitive capacity may have the moral status of a human.[13] Streiffer expresses concern that the research would be unethical unless the chimera was guaranteed enhanced protection in line with her new moral status. In that article, Streiffer cites related work supporting the use of the specific factors articulated above in assessing high level cognitive capacity.[14]

Additionally, this use of use of several factors rather than one bright-line test reduces definitional clarity but is nonetheless prudent in light of the difficulty of isolating a single trait as warranting additional protections. The standard that a humanized animal's cognitive capacity be "substantially similar" to a normal adult human's acknowledges the difficulty in defining a normal adult human's cognitive capacity and allows for flexibility in making a sound judgment.

ALDF's high level cognitive capacity standard for humanized animals is *a minimum* required under the PHSA, but NIH has authority to adopt even broader standards to define human research subjects in this context, and should consider doing so. For example, NIH could specify that any humanized animal with the cognition of a three-year old human child qualifies as a human research subject. Or NIH could more broadly specify that any humanized animal whose cognitive ability is simply substantially enhanced by human material qualifies as a human research subject.

---

[11] Streiffer, *At the Edge of Humanity: Human Stem Cells, Chimeras, and Moral Status*, KENNEDY INSTITUTE OF ETHICS JOURNAL, vol. 15, no. 4, 347 at 348 (Dec. 2005) (Enclosure 1: Appendix n.38).

[12] *Id*. at 352-354.

[13] *Id*. at 354.

[14] *Id*. at 354-355, *citing* Karpowicz et al., *Developing Human-Nonhuman Chimeras in Human Stem Cell Research: Ethical Issues and Boundaries*, KENNEDY INSTITUTE OF ETHICS JOURNAL vol. 15, 107-34 at 120 (2005) (Enclosure 1: Appendix n.59).

b) The proposed changes to the* Guidelines *are not adequately tailored to the concern that humanized animals with high level cognitive capacity would qualify as human research subjects, and should be amended accordingly

NIH's proposed changes to the *Guidelines* fail to articulate or reflect this important legal rule that, at the very least, humanized animals with high level cognitive capacity are protected under the PHSA. NIH's proposed changes allude to ethical and legal concerns but do not specify those concerns. For instance, NIH's proposed changes express concerns about human cells contributing to animal cognition or central nervous systems, but not explain why that is problematic. NIH's proposed changes also prohibit introducing human pluripotent stem cells into nonhuman primate embryos through the blastocyst stage – ostensibly as a prophylactic measure. However, the *Guidelines* do not explain what, exactly, this prohibition serves as a prophylaxis against.

There are several problems with this failure to articulate a precise standard (or standards) for when research on humanized animals becomes legally or ethically impermissible. One problem is that NIH risks publishing guidelines with under-inclusive prohibitions. For example, NIH's proposed prohibition on introducing human pluripotent stem cells into young nonhuman primate embryos is not the only way that a human-animal chimera could obtain high level cognitive capacity. Under the proposed changes to the *Guidelines*, researchers could remain free to introduce pluripotent human stem cells into the embryos of non-primate species – even animal species viewed as being relatively intelligent such as parrots and dolphins. Researchers may do so with the *intent* to displace the animal's brain cells with human brain cells, and qualify for NIH funding. Such research could plausibly give rise to human-animal chimeras with high level cognitive capacity.

ALDF's 2013 Petition for Rulemaking to HHS (Enclosure 1) and *ALDF's 2015 Petition for Rulemaking Supplement* (Enclosure 2) describe experiments where rodents have already been observed exhibiting signs of enhanced cognitive ability due to the introduction of human stem cells. In one remarkable study, human glial progenitor cells (GPCs) transplanted into day-old mouse pups almost entirely took over the mouse brains within a year.[15] Although cognitive effects were not measured in that study, another study involving healthy mice found that the addition of human GPCs caused a significant increase in mouse learning ability and change in behavior.[16] According to one publication, mere *self-restraint* prevented researchers in the former study from performing the same experiment with monkeys. Remarkably, such research involving human GPCs completely overtaking a baby monkey's brain would both be permitted under the proposed changes to the *Guidelines* (because the monkeys are beyond the blastocyst stage), *and* fall outside

---

[15] Windrem et al., *A Competitive Advantage by Neonatally Engrafted Human Glial Progenitors Yields Mice Whose Brains Are Chimeric for Human Glia*, THE JOURNAL OF NEUROSCIENCE, vol. 34 no. 48 pp.16153-16161 (Nov. 26, 2014) (Enclosure 2: Attachment A).

[16] Han et al., *Forebrain Engraftment by Human Glial Progenitor Cells Enhances Synaptic Plasticity and Learning in Adult Mice*, CELL STEM CELL Vol. 12, Iss. 3, 342-353 (Mar. 7, 2013) (Enclosure 1: Appendix n.34).

the scope of monitoring from the proposed steering committee (because the steering committee only reviews research with stem cells introduced up through the end of the gastrulation stage).

Another problem with failing to articulate a precise reason for when research on humanized animals is impermissible is that NIH's proposed steering committee will not have adequate direction on what it should be monitoring for, or why.

Therefore, NIH should clearly articulate that its overarching concern with using humanized animals in research is that those animals qualify as human research subjects under the PHSA when, at the very least, they obtain high level cognitive capacity.

Within the framework of the *Guidelines*, NIH could include a prohibition on research involving humanized animals with high level cognitive capacity as follows:

> IV. Research Not Eligible for NIH Funding:
>
> A. Research in which human pluripotent stem cells are introduced into non-human primate embryos up through the end of the blastocyst stage, is not eligible for funding.
>
> B. Research involving the breeding of animals where the introduction of human cells may contribute to the germ line, is not eligible for funding.
>
> C. NIH funding of the derivation of stem cells from human embryos is prohibited by the annual appropriations limitations on the funding of human embryo research (see e.g. Section 508, Omnibus Appropriations Act, 2016, Pub. L.114-113, 12/18/15), otherwise known as the Dickey Amendment.
>
> D. Research using hESCs derived from other sources, including somatic cell nuclear transfer, parthenogenesis, and/or IVF embryos created for research purposes, is not eligible for NIH funding.

> E. Research in any way involving animals where human stem cells (or other human genetic material*) has contributed or might contribute to high level cognitive capacity, where "high level cognitive capacity" is defined as mental ability that is substantially similar to a normal human considering the individual's (i) linguistic ability; (ii) degree of self-awareness; (iii) sense of past and future self; (iv) moral agency; and / or (v) rational agency.
>
> *(* The reference in this proposed language to animals with "other human genetic material" is intended to include human-animal transgenics, as justified in the following section.)*

2. NIH should expand the scope of the proposed steering committee and proposed changes to the *Guidelines* to include protection of humanized transgenic animals with high level cognitive capacity

The previous section established the standard that humanized animals with high level cognitive capacity qualify as human research subjects under the PHSA. With that standard in mind, NIH's proposed policy changes are unjustifiably narrow because NIH categorically excludes humanized *transgenic* animals from any protection or oversight even though research on such beings raises similar legal problems as research on chimeras. NIH should remedy this problem by requiring the proposed steering committee to monitor research on humanized transgenic animals, and by including humanized transgenic animals in ALDF's proposal[17] to prohibit funding on research involving animals where human stem cells or other human genetic material has contributed or might contribute to high level cognitive capacity.

a) Transgenic research has already caused significant effects to the cognition of humanized animals

Transgenic research involves taking genes or genetic sequences from one individual and inserting them into another individual's cells.[18] In human-animal transgenic research, an animal is given human genetic material, e.g. through direct insertion of human genetic material, or alteration of animal's genetic sequence to resemble human genes.

Many of the same ethical concerns that apply to human-animal chimeras applies to transgenics as well. Notably, NIH's request for public comment on its chimera policy expressed concern that "human cells might go in the developing animal and how they might function, such

[17] That proposal was articulated previously in these comments. *See* discussion, *supra* II.1.b.

[18] *Animals containing human material*, THE ACADEMY OF MEDICAL SCIENCES, p.18 (§ 2.2.1) (July 2011) (Enclosure 2: Attachment E).

as whether the human cells might contribute to the central nervous system and affect the cognition of the animal." These concerns apply to humanized transgenic animals as well because human genetic material can affect the development of an animal's function, including contribution to the central nervous system and cognition of the animal.

In 2014, one widely publicized study declared that "Humanized Foxp2 accelerated learning [in mice] by enhancing transitions from declarative to procedural performance."[19] In that study, researchers substituted the endogenous version of the FOXP2 gene in mice with the *humanized* FOXP2 gene which is understood to be an important gene "firmly linked to [human] speech and language development."[20] Astonishingly, researchers reported "marked effects of this humanization of Foxp2 on learning and striatal neuroplasticity." Specifically, the researchers found that the humanized mice learned stimulus-response associations significantly faster than their standard littermates in certain situations.[21] This cognitive enhancement was apparently entirely attributable to the humanized genetic material.

In another study, researchers created transgenic mice were given human or chimpanzee HARE5 enhancer gene to determine the effect on the neocortex.[22] The authors found that even compared to "chimpanzeed" mice with the HARE5 gene, the humanized mice developed brains that were *twelve percent larger*, and had noticeably larger neocortex size that was detectable by the naked eye.[23] Although the mice in this study were killed before maturing, one news report noted that some of the researchers planned to let the mice mature in future studies to test if the bigger brains made them smarter.[24]

---

[19] Schreiweis et al., *Humanized Foxp2 accelerates learning by enhancing transitions from declarative to procedural performance*, PROCEEDINGS OF THE NATIONAL ACADEMIES OF SCIENCE, vol. 111 no. 39 pp.14253-14258 (Sept. 30, 2014) (Enclosure 2: Attachment B).

[20] *Id*. at p.14253.

[21] *Id*. at pp.14253-14258.

[22] Boyd et al., *Human-Chimpanzee Differences in a FZD8 Enhancer Alter Cell-Cycle Dynamics in the Developing Neocortex*, CURRENT BIOLOGY 25, pp.772-770 (March 16, 2014) (Enclosure 2: Attachment C).

[23] *Id*.

[24] Pennisi, *Human DNA enlarges mouse brains*, SCIENCE (Feb. 19, 2015) (Enclosure 2: Attachment F).

b) NIH is not proposing to regulate the use of humanized transgenic animals, but should do so by including them within the scope of the proposed steering committee and ALDF's proposed prohibition on funding research involving humanized animals with high level cognitive capacity

NIH presently regulates the use of transgenic animals only as a biosafety issue,[25] but not as an ethical one as it now proposes to do with research on chimeras. Yet, as explained above, significant effects to animal cognition have already been observed in humanized transgenic research. Against this background, NIH's exclusion of transgenic animals from the same regulatory framework it proposes for chimeras is arbitrary.

NIH can easily remedy this unjustified omission by expanding the scope of the proposed steering committee to include research on humanized transgenic animals in addition to chimeras. The factors that the steering committee would need to consider could be amended as follows:

> (1) the characteristics of the human genetic material and cells to be introduced (including potency and any modifications of those cells); (2) characteristics of the recipient animal (e.g., species, stage of development, and any modifications that affect location or function of human cells); (3) other data relevant to the likely effects on the animal (e.g., changes in cognition, behavior, or physical appearance); (4) planned monitoring (including animal welfare assessments); and (5) any staging of proposed research (e.g., assessing the outcome of a particular experiment before conducting a further experiment).

Additionally, NIH should include transgenics in the funding prohibition that ALDF suggested in the previous section for research involving humanized animals with high level cognitive capacity. This may require that NIH amend the scope and title of its *Guidelines for Human Stem Cell Research* to reach beyond stem cell research to include transgenic research. Alternatively, NIH might instead issue independent guidelines regulating NIH funding of research on humanized animals. The PHSA provides the authority to protect humanized transgenic animals regardless of the approach that NIH takes to achieve that protective effect.

[25] *See NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules*.

3. In addition to general programmatic oversight by the proposed steering committee, NIH should require regular and ongoing IRB review of research involving humanized chimeras and transgenics

The PHSA generally requires IRB review of federally-funded research involving human subjects.[26] Because research on humanized animals implicates the PHSA's rights for human research subjects, NIH should ensure that its funding for research involving humanized chimeras and transgenics is likewise subject to IRB review.[27] It may accomplish this by working with HHS to promulgate regulations, or by including such a requirement in its *Guidelines*. For example, the *Guidelines* could be amended to read:

> IV. Research Not Eligible for NIH Funding:
>
> A. Research in which human pluripotent stem cells are introduced into non-human primate embryos up through the end of the blastocyst stage, is not eligible for funding.
>
> B. Research involving the breeding of animals where the introduction of human cells may contribute to the germ line, is not eligible for funding.
>
> C. NIH funding of the derivation of stem cells from human embryos is prohibited by the annual appropriations limitations on the funding of human embryo research (see e.g. Section 508, Omnibus Appropriations Act, 2016, Pub. L.114-113, 12/18/15), otherwise known as the Dickey Amendment.
>
> D. Research using hESCs derived from other sources, including somatic cell nuclear transfer, parthenogenesis, and/or IVF embryos created for research purposes, is not eligible for NIH funding.

[26] 42 U.S.C. § 289(a).

[27] ALDF expanded on these reasons for requiring IRB review in *ALDF's 2013 Petition for Rulemaking* (Enclosure 1 at pp.24-25.)

> E. Research in any way involving animals where human stem cells or other human genetic material has contributed or might contribute to high level cognitive capacity, where "high level cognitive capacity" is defined as mental ability that is substantially similar to a normal human considering the individual's (i) linguistic ability; (ii) degree of self-awareness; (iii) sense of past and future self; (iv) moral agency; and / or (v) rational agency.
>
> F. Research involving the introduction of human stem cells or other human genetic material that does not provide for regular oversight from an IRB to ensure that human stem cells or other human genetic material are not contributing to high level cognitive capacity in an animal.

NIH's proposal for a steering committee does not appear adequate to ensure the level of monitoring necessary to ensure compliance with the PHSA's protection for human research subjects. The stated purpose of the steering committee is generally to "provide programmatic input" to officials at the NIH and monitor trends in the general field of research. There is no assurance that specific research projects involving humanized chimeras and transgenics will receive consistent and regular scrutiny to safeguard against potential violations of the PHSA.

* * * * *

Thank you for your time and attention in considering these comments. Please feel free to contact me should you have any questions about this important issue.

Christopher A. Berry, Esq.
ANIMAL LEGAL DEFENSE FUND
cberry@aldf.org
(707) 795-2533 ext. 1041

Enclosures
(1) ALDF's 2013 Rulemaking Petition to HHS
(2) ALDF's 2015 Rulemaking Petition Supplement
(3) HHS's 2015 Correspondence with ALDF

ENCLOSURE 1

ALDF'S 2013 PETITION FOR RULEMAKING TO HHS

BEFORE THE UNITED STATES DEPARTMENT OF HEALTH AND HUMAN SERVICES

CITIZEN PETITION FOR RULEMAKING TO PROTECT HUMANIZED CHIMERAS UNDER THE PUBLIC HEALTH SERVICES ACT

ANIMAL LEGAL DEFENSE FUND,

Citizen petitioner,

170 E. Cotati Ave.

Cotati, CA 94931

Filing with:

KATHLEEN SEBELIUS,

In her official capacity as Secretary of the

United States Department of Health and Human Services,

200 Independence Avenue S.W.

Washington, D.C. 20201

TABLE OF CONTENTS

TABLE OF CONTENTS i

PROPOSED RULE TO PROTECT HUMANIZED CHIMERAS 1

I. ACTION REQUESTED 2

II. INTERESTS OF THE PETITIONER 2

III. FACTUAL BACKGROUND ON RESEARCH INVOLVING HUMAN-ANIMALS CHIMERAS 2

A. Non-Chimera Animal Research 3

B. Human-Animal Chimeras 5

1. Definition 5

2. Research Involving Human-Animal Chimeras 7

C. Ethical Implications of Using Human-Animal Chimeras 9

D. National Academy of Science Guidelines 12

IV. LEGAL BACKGROUND ON RESEARCH INVOLVING HUMAN SUBJECTS 15

A. The PHSA and the Belmont Report 15

1. PHSA Duty for HHS to Protect Human Research Subjects 15

2. The Belmont Report 16

B. Basic HHS Policy for Protection of Human Research Subjects 19

C. NIH Guidelines for Human Stem Cell Research 19

V. PROPOSED RULE FOR THE COMPREHENSIVE PROTECTION OF CHIMERAS WHO QUALIFY AS HUMAN SUBJECTS UNDER THE PHSA 21

§ 1 Scope. 21

§ 2 Definitions. 21

§ 3 IRB review. 22

§ 4 Humanized chimeras protected as research subjects. 22

VI. Legal Justification to Promulgate Proposed Rule 23

A. Scope 23

B. Definitions 23

C. IRB Review 24

D. Humanized Chimeras Protected As Research Subjects 25

VII. PROMULGATION OF THE PROPOSED RULE IS TIMELY WITH RESPECT TO THE PRESENT STATE OF SCIENCE 26

VIII. CONCLUSION 28

PROPOSED RULE TO PROTECT HUMANIZED CHIMERAS

The Animal Legal Defense Fund (ALDF) petitions the Secretary of the Department of Health and Human Services (HHS) to promulgate a rule under the Public Health Services Act (PHSA) that protects humanized chimeras. Specifically, ALDF asks the Secretary to clarify that the definition of "human subjects" under the PHSA[1] and its accompanying regulations[2] applies to all animals with human cells who have a substantially similar cognitive capacity to a normal adult human. Such human-animal chimeras with a high level cognitive capacity will be referred to throughout this petition as "humanized chimeras." ALDF further asks that the Secretary require an Institutional Review Board (IRB) procedure for all research involving human-animal chimeras to determine whether it carries a substantial risk of producing a humanized chimera.

The proposed rulemaking action under the PHSA is necessary to protect humanized chimeras as human research subjects in light of the scientific progress of chimera research. Chimera research involves splicing the cells of one animal species into another, and began in earnest during the 1980s. In the last decade, this research has expanded to include the introduction of human stem cells and cells derived therefrom into animal recipients, referred throughout this petition as "human-animal chimeras." This process of splicing human stem cells and their derivatives into nonhuman animals has already resulted in some human-animal chimeras with significantly improved cognition, and carries a non-negligible risk of producing humanized chimeras with high level cognitive capacity.

While research involving chimeras rapidly proliferates and expands in scope, HHS has failed to take action to ensure adequate protection of humanized chimeras under the PHSA. Current regulations do not require that the recipients of federal funds involved in human-animal chimera research assess the risk of producing a humanized chimera with high cognitive capacity,

[1] 42 U.S.C. § 289(a).

[2] 45 C.F.R. §§ 46.101, *et seq.*

much less minimize that risk, monitor the experiments for evidence that chimeras have acquired high cognitive capacity, or protect chimeras that have acquired such cognitive capacity. The rapid progress of this research within a regulatory vacuum has resulted in a serious risk of creating a humanized chimera with no recognized protections. Accordingly, HHS should invoke its rulemaking authority under the PHSA to resolve this problematic situation without delay.

I. ACTION REQUESTED

ALDF submits this petition pursuant to its First Amendment's right to petition the government,[3] the Administrative Procedure Act,[4] and the HHS implementing regulations.[5] ALDF implores HHS to promulgate rulemaking that: specifies that protection for human subjects under 42 U.S.C. § 289 of the PHSA applies to humanized chimeras (i.e chimeras with human cells who have substantially similar cognitive capacity to a normal adult human) and implements IRB procedures to accordingly ensure their protection. The proposed rule is set forth in detail *infra* at Section V of this petition.

II. INTERESTS OF THE PETITIONER

ALDF is a national nonprofit organization founded in 1979 to protect the lives and advance the interests of animals through the legal system. ALDF's membership is comprised of thousands of dedicated attorneys and more than 100,000 supporters throughout the United States. Every day, ALDF works to protect animals through litigation, legal assistance, administrative action, the promotion of the study of animal law, and public education.

III. FACTUAL BACKGROUND ON RESEARCH INVOLVING HUMAN-ANIMALS CHIMERAS

Recent advances in biotechnology involving the improvement of animal cognition with human stem cells or cells derived therefrom carry a significant risk of resulting in humanized

[3] U.S. CONST. AMEND. I.

[4] 5 U.S.C. § 553(e).

[5] 45 C.F.R. §§ 160.101, *et seq.*

chimeras with high level cognitive capacity. Scientists now recognize that many nonhuman animals used in invasive experiments already possess consciousness. Some of those animals are implanted with human stem cells or cells derived therefrom and are known as human-animal chimeras. These chimeras have acquired not only human cells but human qualities as well, including in some cases improved cognition attributable to the introduction human brain cells. This has prompted serious concern from leading scientists and ethicists that humanized chimeras created during the course of such research will not receive appropriate protection.

A. Non-Chimera Animal Research

It is widely recognized that animals receiving human cells in chimera research already possess remarkable cognitive capacity. A group of leading scientists that included Stephen Hawking recently conferred about animal cognition and issued The Cambridge Declaration on Consciousness (Cambridge Declaration), concluding that:

> [T]he weight of evidence indicates that humans are not unique in possessing the neurological substrates that generate consciousness. Non-human animals, including all mammals and birds, and many other creatures, including octopuses, also possess these neurological substrates.[6]

These scientists cited decades of research to support their finding concluded that many animals have the capacity for conscious experience, emotion, sensitivity to reward and punishment, and self-recognition.[7] They listed many impressive feats of animal intelligence including talking parrots, self-recognition in apes and magpies, tool-making in crows, and the complex social intelligence of

[6] THE CAMBRIDGE DECLARATION ON CONSCIOUSNESS (2012) (emphasis added), available online at http://fcmconference.org/img/CambridgeDeclarationOnConsciousness.pdf. The Cambridge Declaration was signed at the Francis Crick Memorial Conference. The conference website features videos on these topics, as well as information about the scientists and their conference presentations. *See* Francis Crick Memorial Conference, *Featured Videos*, available online at http://fcmconference.org/#videos (last accessed Nov. 25, 2013); Francis Crick Memorial Conference, *Speakers and Abstracts*, available online at http://fcmconference.org/#program (last accessed Nov. 25, 2013).

[7] CAMBRIDGE DECLARATION, *supra* n.6.

pigs.[8] Other recent studies have shown that dolphins call each other unique names with signature whistles[9] and that chimpanzees plan for the future.[10]

Consciousness is not limited to animals that are commonly recognized for their exceptional intelligence, such as cetaceans and great apes, but exists in species such as rodents and monkeys.[11] For example, recent studies have shown that rodents are capable of empathy and laughter.[12] Other studies reveal that rhesus macaque monkeys can pass variants of the mirror test thereby demonstrating their capacity for self-recognition.[13]

Despite these impressive cognitive capacities, millions of animals are used in invasive experimentation every year.[14] Research animals are put to manifold uses including studies aimed at uncovering information about diseases, psychology, drug effects, treatments, cosmetic safety, and almost any other conceivable scientific pursuit. The research is oftentimes invasive, causing great suffering and death.

---

[8] Francis Crick Memorial Conference, *supra* n.6.

[9] King et al., *Vocal copying of individually distinctive signature whistles in bottlenose dolphins*, PROCEEDINGS OF THE ROYAL SOCIETY B, vol. 280 no. 1757 (Apr. 22, 2013), available online at http://rspb.royalsocietypublishing.org/content/280/1757/20130053.full.pdf+html.

[10] Osvath and Karvonen, *Spontaneous Innovation for Future Deception in Male Chimpanzees*, PLOS ONE vol. 7 no. 5 (May 9, 2012), available online at http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0036782.

[11] CAMBRIDGE DECLARATION, *supra* n.6.

[12] Francis Crick Memorial Conference, *supra* n.6; Bartal et al., *Empathy and Pro-Social Behavior in Rats*, SCIENCE, vol. 335, pp. 1427-1430 (Dec. 9, 2011), abstract available online at http://www.sciencemag.org/content/334/6061/1427.

[13] Populin et al., *Rhesus Monkeys (Macaca mulatta) Do Recognize Themselves in the Mirror: Implication for the Evolution of Self-Recognition*, PLOS ONE, vol. 5 (September 29, 2010), available online at http://www.plosone.org/article/info:doi/10.1371/journal.pone.0012865.

[14] Although comprehensive statistics are not kept, most observers estimate that at least 25 million vertebrate animals are used each year for research in the United States. The vast majority are rodents but other animals such as nonhuman primates are commonly used as well. *See* Nat'l Assoc. for Biomedical Research, *The Humane Care and Use of Laboratory Animals*, at 1-2 (2008), available online from http://www.nabr.org.

These activities are morally questionable, at best, and deserve greater scrutiny in their own right. However, the harm takes on special significance in light of recent research involving the transplant of human cells into the brains of these already intelligent animals. This type of research has *already* improved the cognitive abilities of some human-animal chimeras and threatens to continue pushing them closer to human-like cognitive capacity.

B. Human-Animal Chimeras

1. Definition

The term chimera comes from Greek mythology and refers to a beast with the body of a lion, the head of a goat, and the tail of a serpent.[15] In the modern lexicon, it refers to an organism with at least two populations of genetically distinct cells originating from different individual zygotes.[16] Chimeras may be classified into the following categories:

- *animal-animal* (nonhuman animals containing cells from another genetically distinct nonhuman animal);
- *human-human* (human individuals containing cells from another genetically distinct human);
- *animal-human* (human individuals containing cells from a nonhuman animal); or
- *human-animal* (nonhuman animals containing cells from a human, the type of chimeras who are the focus of this Proposed Rule).

Naturally born chimeras are extremely rare and may occur, for example, when the embryos of fraternal twins merge into one body during the early stages of pregnancy resulting in an individual with two populations of genetically unique cells.[17] However, most chimeras are the

[15] *Chimera*, THEFREEDICTIONARY, available online at http://www.thefreedictionary.com/ Chimera (last accessed Nov. 25, 2013); Greely, *Defining Chimeras . . . and Chimeric Concerns*, AM. J. BIOETHICS, vol. 3, no. 3, 17-19 (Summer 2003), abstract available online at http://muse.jhu.edu/login?auth=0&type=summary&url=/journals/american_journal_of_bioethics/v003/3.3greely.html.

[16] Boklage, *Embryogenesis of chimeras, twins and anterior midline asymmetries*, HUMAN REPRODUCTION, vol. 21, no. 3, 579 (2006), available online at http://humrep.oxfordjournals.org/content/21/3/579.full.

[17] *Id.*

result of biotechnological manipulation for research purposes and accordingly begin with animal bases.

Since the 1980s, researchers in the United States have been creating animal-animal chimeras by introducing cells from other species.[18] One of the earliest examples of this type of research involved the combination of sheep and goat cells into a "geep" in 1984.[19] Physical variations in the geeps include the presence or absence of wool, sheep or goat-like head shape, and sheep or goat-like body shape.[20]

The precise effect of chimerization cannot be predicted before the changes occur.[21] However, the degree of influence that the implanted cells have on the recipient appears to be affected by several factors including: the versatility of the stem cell type used,[22] the life stage of the recipient when the cells are transplanted,[23] and the genetic similarity between the two species.[24] In other words, the early introduction of embryonic stem cells in the blastocyst of a closely related species will likely create a stronger chimeric effect than the introduction of a less versatile adult stem cell into the adult body of a distantly related recipient.

---

[18] Polzin et al., *Production of Sheep-Goat Chimeras by Inner Cell Mass Transplantation*, J. OF ANIMAL. SCI. vol. 65, pp. 325-330 (1987), available online at http://www.animal-science.org/content/65/1/325.full.pdf; *Science: It's a Geep*, TIME MAGAZINE (Feb. 27, 1984), available online at http://www.time.com/time/magazine/article/0,9171,921546,00.html.

[19] *Ibid.*

[20] *Ibid.*

[21] *See* Greely et al., *Thinking About the Human Neuron Mouse*, AM. J. BIOTECH. vol. 7 no. 5, 27-40 (May 2007), manuscript available online at http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2220020/pdf/nihms37941.pdf (stating that group was unable to rule out the possibility of a mouse with human brain cells obtaining "some aspects of human consciousness" even though that result seemed implausible.)

[22] Embryonic and pluripotent adult stem cells are more versatile than other types of stem cells. *See* Greely et al., *supra* n.21.

[23] Greene et al., *ETHICS: Moral Issues of Human-Non-Human Primate Neural Grafting*, SCIENCE, vol. 309, no. 5733, 385-386 (July 15, 2005), abstract available online at http://www.sciencemag.org/content/suppl/2005/08/03/309.5733.385.DC1.

[24] *See id.*

The site of the transplant also affects the degree of influence implanted cells will have on chimeric animals, since that is where the greatest change is likely to occur.[25] This consideration is especially significant when neural or pluripotent stem cells are introduced in the recipient's brain, since this can affect behavior and cognitive abilities.[26] This is the most controversial use of chimeras.

2. Research Involving Human-Animal Chimeras

The creation of human-animal chimeras by transplanting human cells into animal recipients is highly controversial. Human-animal chimeras are believed to be a more valuable research tool due to the possible application of the technology for human benefit.[27] Researchers find research on this type of chimera appealing for several purposes: (1) evaluating the potential for chimera to grow cells, tissues, and organs suitable for transplanting into humans; (2) studying biological development in vivo; and (3) testing therapies or cures for human diseases.[28]

Researchers are already studying ways to grow human organs in animal bodies for transplantation in human patients.[29] Researchers at the University of Nevada at Reno, for example, created sheep chimeras with half-human organs and almost fully human livers.[30] Researchers at the

---

[25] *See id.*

[26] Knowles, *Ethics of Research Using Hybrids, Chimeras and Cytoplasmic Hybrids*, STEM CELL NETWORK, 3 (2003), available online at http://www.stemcellschool.org/pdf/Ethics-of%20Research-Using-Hybrids.pdf (noting the ethical relevancy of grafting human brain tissue in a nonhuman primate.)

[27] *See* Hunter, *Chimeras: The Ethics of Creating Human-Animal Interspecifics*, Dissertation, 6 & 16-17, (Jan. 20, 2009), available online at http://edoc.ub.uni-muenchen.de/10022/1/Huther_Constanze.pdf.

[28] *Id.*

[29] Vince, *Pig-human chimeras contain cell surprise*, NEW SCIENTIST (Jan. 13, 2004), available online at http://www.newscientist.com/article/dn4558-pighuman-chimeras-contain-cell-surprise.html.

[30] *Id.*; Boyle, *Endowed by Their Creator? The Future of Constitutional Personhood*, BROOKINGS INSTITUTE, at 5-6 (Mar. 9, 2011), available online at http://www.brookings.edu/~/media/research/files/papers/2011/3/09%20personhood%20boyle/0309_personhood_boyle.pdf.

Mayo Clinic developed a line of pigs with human blood cells.[31] And stem cell researcher Irving Weissman developed a line of mice with almost fully human immune systems to study cancer and other diseases.[32]

In the last decade, researchers have made headlines by implanting cells derived from human stem cells into animal brains.[33] Chimeric animals with implanted human brain cells show evidence of improved cognitive ability. In a remarkable study involving healthy mice, researchers found that the addition of human glial progenitor cells (GPCs), a type of brain cell derived from stem cells, caused a significant increase in mouse learning ability and change in behavior:[34]

> [H]uman glial chimeric mice exhibit enhanced performance in four different learning tasks: [auditory fear conditioning (AFC)], [contextual fear conditioning (CFC)], Barnes maze [spatial memory task], and novel object location. Moreover, the analysis of AFC indicates that alloengraftment by mouse GPCs did not affect the learning of the recipient mice, supporting the notion that the improved learning in the humanized chimeras resulted from the presence of human glia, rather than from cell engraftment per se.[35]

Another experiment, involving the grafting of human brain cells into neurologically diseased mice, showed that their mental ability returned to normal.[36] Experiments like this are not limited to mice but are also being performed on monkeys.[37] These remarkable studies suggest a potential for ever

---

[31] Boyle, *supra* n.30 at 5-6.

[32] Vince, *supra* n.29.

[33] *See, e.g.,* Weiss, *Of Mice, Men and In-Between*, WASH. POST, (Nov. 20, 2004), available online at http://www.washingtonpost.com/wp-dyn/articles/A63731-2004Nov19.html; Mott, *Animal-Human Hybrids Spark Controversy*, NAT'L GEO. NEWS, (Jan. 25, 2005), available online at http://news.nationalgeographic.com/news/2005/01/0125_050125_chimeras.html.

[34] Han et al., *Forebrain Engraftment by Human Glial Progenitor Cells Enhances Synaptic Plasticity and Learning in Adult Mice*, CELL STEM CELL Vol. 12, Iss. 3, 342-353 (Mar. 7, 2013), available online at http://download.cell.com/cell-stem-cell/pdf/PIIS1934590913000076.pdf?intermediate=true.

[35] *Id.* at 351.

[36] Blurton-Jones et al., Presentation, *Restoration of memory in mouse models of Alzheimer's disease and neuronal loss; a new paradigm using human neural stem cell transplantation* (July 17, 2012), available online at www.stemcellsinc.com/LiteratureRetrieve.aspx?ID=142864.

[37] *See, e.g.,* California Institute for Regenerative Medicine, *Grant Abstract: Developmental Candidates for Cell-Based Therapies for Parkinson's Disease*, available online at

greater mental augmentation in chimeric animals, which could lead to these creatures approaching the cognitive capacity of humans in the near future.

C. Ethical Implications of Using Human-Animal Chimeras

In the wake of this emerging chimera research, Jason Robert and Francoise Baylis sparked a wave of scholarly literature about the ethical implications[38] with their 2003 article *Crossing Species Boundaries*.[39] That article critically examined the question of whether it is unethical to mix human cells or genes with animals.[40] It focused largely on the issue of moral confusion caused by upsetting traditional moral categories of species and suggested that moral confusion did not justify a prohibition on such research.[41]

Much of the subsequent literature has focused not on moral confusion but rather on the welfare of the chimera being. In 2005, a team led by Mark Greene considered whether grafting human brain cells into nonhuman primates could cause a change in cognitive capacity and moral status in the recipients.[42] Greene acknowledged that it would be difficult to anticipate the effects of neural grafting and to observe such effects if they occurred.[43] The group unanimously concluded

---

http://www.cirm.ca.gov/our-funding/awards/developmental-candidates-cell-based-therapies-parkinsons-disease-pd-0 (last accessed Nov. 27, 2013); *see also* Greely et al., *supra* n.21 (citing research involving the transplantation of human neural cells in green vervet monkey brains).

[38] *See Greene* et al., supra n.23; Streiffer, *At the Edge of Humanity: Human Stem Cells, Chimeras, and Moral Status*, KENNEDY INSTITUTE OF ETHICS JOURNAL, vol. 15, no. 4, 347 (Dec. 2005), available online at http://ntp.neuroscience.wisc.edu/NPP/ PTreading/Streiffer%20-%202005%20HS%20Cells%20Chimeras%20and%20Moral%20Status.pdf; *see also* Boyle, *supra* n.30; Hagglund, *Patentability of Human-Animal Chimeras*, 25 SANTA CLARA COMP. & HIGH TECH. L. J. 51 (2008); Bennett, *Comment: Chimera and the Continuum of Humanity*, 55 EMORY L.J. 347, 379-380 (2008).

[39] Robert & Baylis, *Crossing Species Boundaries*, THE AM. JOURNAL OF BIOETHICS, vol. 3, no. 3 (Summer 2003).

[40] *Id.* at 1.

[41] *Id.* at 9-10.

[42] Greene et al., *supra* n.23.

[43] *Id.*

that it was "unable to rule out the possibility of effects on cognition of the sort that matter to moral status."[44] It recommended oversight throughout the research process and proposed the following factors to consider as a starting framework: "(i) proportion of engrafted human cells, (ii) neural development, (iii) NHP species, (iv) brain size, (v) site of integration, and (vi) brain pathology."[45] It also recommended monitoring and reporting any changes in brain function resulting from chimerization.[46]

In 2007, a group of ethicists led by Hank Greely published an article about the ethics of a hypothetical experiment proposed by scientist Irving Weissman's involving a mouse with all human neurons.[47] Greely et al. made recommendations similar to Greene's, but in the context of the hypothetical human neuron mouse protocol.[48] Greely acknowledged that "conferring humanity" was the focus of most ethical discussions over the use of human-animal chimeras.[49] While the Greely team found it unlikely that the particular hypothetical experiment would confer "some aspects of human consciousness" or "some distinctively human cognitive abilities" on the chimera, they also could not rule out the possibility.[50] Accordingly, they recommended that such experiments be carried out in stages and carefully monitored.[51] They recommended that if observers found any evidence of human brain structures or behaviors, the experiment be stopped and reconsidered, but they did not specify what that reconsideration should entail. [52]

---

[44] *Id.*

[45] *Id.*

[46] *Id.*

[47] Greely et al., *supra* n.21.

[48] *Id.*

[49] *Id.*

[50] *Id.*

[51] *Id.*

[52] *Id.*

Greene and Greely both looked at research involving the grafting of human cells into animal brains.[53] Both agreed that such experiments should be subject to careful oversight because these procedures could possibly confer increased cognitive capacity that would be morally significant.[54] However, neither group indicated precisely what this increased capacity signified and at what level it became significant. [55]

Robert Streiffer addressed this very question and suggested that "the introduction of human stem cells into a nonhuman animal that would substantially enhance its moral status is wrong [because] subsequent treatment of the subject likely will fall far below what its new moral status demands."[56] Streiffer notes that chimeric research has the potential to imbue the chimera recipient with "high level cognitive capacities" similar to what normal adult humans possess,[57] and that a chimera who obtained that high level cognitive capacity may have the moral status of a human.[58] Streiffer expresses concern that the research would be unethical unless the chimera was guaranteed enhanced protection in line with her new moral status.

What exactly constitutes high level cognitive capacity? Streiffer cites the work of another group of ethicists, suggesting this capacity entails many dimensions:

> It is not only the capacities for reasoning, choosing freely, and acting for moral reasons, as Kant argues, or for entertaining and acting on the basis of self-chosen purposes, as Gewirth holds, that are at the core of what we mean by human dignity. The notion also encompasses such capacities as those for engaging in sophisticated forms of communication and language, participating in interweaving social relations, developing a secular or religious world view, and displaying sympathy and empathy in emotionally complex ways.[59]

---

[53] Greely et al., *supra* n.21; Greene et al, *supra* n.23.

[54] *Ibid.*

[55] *See ibid.*

[56] Streiffer, *supra* n.38 at 348.

[57] *Id.* at 352-354.

[58] *Id.* at 354.

[59] *Id.* at 354-355, *citing* Karpowicz et al., *Developing Human-Nonhuman Chimeras in Human Stem Cell Research: Ethical Issues and Boundaries*, KENNEDY INSTITUTE OF ETHICS JOURNAL vol. 15, 107-34 at 120 (2005).

Streiffer cautions that this list looks "excessively demanding" and indicates that a chimera research subject might attain enhanced moral status even if he fell some degree short of attaining all the requirements on this list. Others concur that it is difficult to determine exactly when enhanced cognitive capacities warranting extra protection exist, with one commentator invoking Justice Stewart's famous standard defining pornography: "I know it when I see it."[60]

In sum, there is consensus among bioethicists that conferring some degree of improved cognition to a nonhuman animal via human cells raises serious ethical issues. Research involving the introduction of human cells into animal chimera recipients should require careful planning and oversight since it is possible such research will result in improved cognition and because it is difficult to predict whether an introduction of human cells will have that result. While it remains unsettled precisely how much cognitive improvement is significant and what difference in treatment is warranted, it seems clear that, *at a minimum*, human-animal chimeras with cognitive capacity similar to normal adult humans should be afforded the same protection that humans routinely enjoy.

D. National Academy of Science Guidelines

The National Academy of Science's National Research Council published its Guidelines for Human Embryonic Stem Cell Research (NAS Guidelines) around the same time as the previously discussed articles and made similar recommendations.[61] The NAS Guidelines agree

[60] Bennett, *supra* n.40 at 374, *citing Jacobellis v. Ohio*, 378 U.S. 184, 197 (1964) (Stewart, J., concurring). Indeed, it may be that some animals already meet this standard without any enhancement owing to human brain cells. Greene et al. noted that many members of the team expressed reservations about using non-chimeric primates for invasive research. The NIH has recently effectively ended federal funding for chimpanzee research. And as stated earlier, a group of prominent neuroscientists recently acknowledged that all mammals and birds are conscious with emotions, sensitivity to reward and punishment, and self-recognition in the Cambridge Declaration. *See* CAMBRIDGE DECLARATION, *supra* n.6.

[61] *Guidelines for Human Embryonic Stem Cell Research*, NAT'L ACADEMY OF SCIENCES, 39-40 (2005), available online at http://www.nap.edu/catalog.php?record_id=11278. The NAS Guidelines were amended in 2007, 2008, and 2010 and these later versions can be found at the same url. *See 2007 Amendments to the National Academies' Guidelines for Human Embryonic Stem Cell Research*, NAT'L ACADEMY OF SCIENCES (2007), available online at

that "the idea that human neuronal cells might participate in 'higher-order' brain functions in a nonhuman animal, however likely that may be, raises concerns that need to be considered."[62] They go on to state that the effect of grafting human cells in a chimera can be predicted to some degree by considering "the number of [human embryonic stem cells][63] to be transferred, what areas of the animal body would be involved, and whether the cells might migrate through the animal's body."[64]

The NAS Guidelines ultimately recommend review by an Embryonic Stem Cell Oversight (ESCO) committee or an equivalent body.[65] This committee is similar to an IRB or an Institutional Animal Care and Use Committee (IACUC), but tasked with ensuring careful planning and monitoring of research using *human stem cells*.[66] The ESCO committee ensures adherence to the special ethical considerations presented by such research.[67]

Regarding human-animal chimera research specifically, the NAS Guidelines require additional review and approval of an ESCO committee or its equivalent:

> Research involving the introduction of [human embryonic stem cells] into nonhuman animals at any stage of embryonic, fetal, or postnatal development [is permissible only after additional review and approval by an ESCO committee or equivalent]. Particular attention should be paid to the probable pattern and effects

---

http://www.nap.edu/catalog.php?record_id=11871; *2008 Amendments to the National Academies' Guidelines for Human Embryonic Stem Cell Research*, NAT'L ACADEMY OF SCIENCES (2008), available online at http://www.nap.edu/catalog.php?record_id=12260; *Final Report of the National Academies' Human Embryonic Stem Cell Research Advisory Committee and 2010 Amendments to The National Academies' Guidelines for Human Embryonic Stem Cell Research*, NAT'L ACADEMY OF SCIENCES (2010), available online at http://www.nap.edu/catalog.php?record_id=12923.

[62] NAS Guidelines (2005), *supra* n.61 at 39-40.

[63] Although the scope of the NAS Guidelines is limited to the use of embryonic stem cells, adult stem cells can also be used in chimeric research and the same considerations should apply there as well.

[64] NAS Guidelines (2005), *supra* n.61 at 50.

[65] NAS Guidelines (2010 amendments), *supra* n.61 at Appendix C § 2.0.

[66] *Id.*

[67] *Id.*

> of differentiation and integration of the human cells into nonhuman animal tissues.[68]

In addition to this special review process, NAS Guidelines *prohibit* the introduction of human embryonic stem cells in a nonhuman primate blastocyst because of concerns that the human stem cells might have an especially strong influence on the resulting chimera in that kind of research.[69] They also prohibit breeding animals who have received human embryonic stem cells due largely to concern that such stem cells may alter the animal's reproductive germline.[70]

The NAS Guidelines are subject to at least two criticisms. First, the prohibitions are too narrow to protect the type of humanized chimeras, those with high level cognitive capacity, contemplated by Streiffer.[71] The Guidelines are under-inclusive because a chimera with high level cognitive capacity could be produced by means other than by introducing human embryonic stem cells into a nonhuman primate blastocyst or allowing two human-animal chimeras to breed.[72] Streiffer offers this point in his article:

> If one introduces enough pluripotent human stem cells into an animal embryo, *primate or otherwise*, one could, in principle at least, end up with a human inner cell mass surrounded by a nonhuman trophectoderm, affecting both its species and its potential to develop robust cognitive capacities. Furthermore, because brain development occurs after the blastocyst stage, it seems likely that even a transplant that occurred after the blastocyst stage still could affect the characteristics relevant to the individual's cognitive capacities.[73]

If a humanized chimera were to arise by other means, the NAS Guidelines only require, vaguely, that the ESCO's protocols "include ethically sensitive plans to manage [that possibility]."[74]

---

[68] NAS Guidelines (2010 amendments), *supra* n.61 at Appendix C § 1.3(b)(ii).

[69] *Id.* at Appendix C § 1.3(c)(ii).

[70] *Id.* at Appendix C § 1.3(c)(iii); NAS Guidelines (2005), *supra* n. 61 at 50.

[71] *See* NAS Guidelines, *supra* n.61; Streiffer, *supra* n.38 at 364-65.

[72] *See* Streiffer, *supra* n.38 at 364-65.

[73] *Id.* at 365 (emphasis added).

[74] NAS Guidelines (2005), *supra* n.61 at 50.

Second, the NAS Guidelines are merely recommendations to researchers by a private institution and do not carry the force of law or even the force of requirements attached to the receipt of funding from a government agency. Greely notes that it is unknown how many research facilities are actually in compliance with the NAS Guidelines.[75] However, as shown below, some principles of the NAS Guidelines have made their way into law and researchers must be in compliance with those requirements.

IV. LEGAL BACKGROUND ON RESEARCH INVOLVING HUMAN SUBJECTS

HHS is tasked with the duty of protecting human subjects in federally supported research. While HHS has adopted some of the NAS Guidelines through the National Institutes of Health Guidelines for Human Stem Cell Research (NIH Guidelines), it fails to provide comprehensive measures protecting humanized chimeras who obtain high level cognitive capacity. HHS can and should protect such chimeras through its powers to regulate the recipients of federally funded research grants and assure research institutions protect the rights of human subjects.

A. The PHSA and the Belmont Report

The PHSA and the Belmont Report are important background authorities setting the stage for modern regulation of research involving human subjects. The PHSA establishes the statutory basis for such regulation by HHS and imposes a duty on the agency to protect human research subjects. HHS's general statutory powers and responsibilities are heavily influenced by the Belmont Report, which is a set of ethical principles and guidelines that calls for careful forward-looking consideration of research proposals to ensure respect for persons, beneficence, and justice.

1. PHSA Duty for HHS to Protect Human Research Subjects

The PHSA imposes a duty on HHS to protect human research subjects in federally-funded research.[76] It does so by establishing NIH, which is responsible for administering

[75] Greely et al, *supra* n.21.

[76] 42 U.S.C. § 289(a).

government funding of medical research.[77] Thus, NIH is part of HHS and many powers and obligations associated with NIH's activity therefore fall to HHS.[78]

Due to this interagency relationship, the PHSA imposes a statutory duty on HHS to regulate research supported by the federal government to ensure protection of human research subjects:

> The [HHS] Secretary *shall* by regulation require that each entity which applies for a grant, contract, or cooperative agreement under this Act for any project or program which involves the conduct of biomedical or behavioral research involving human subjects submit [ ] with its application for such grant, contract, or cooperative agreement assurances satisfactory to the Secretary that it has established (in accordance with regulations which the Secretary shall prescribe) a[n] [IRB] to review biomedical and behavioral research involving human subjects conducted at or supported by such entity in order to protect the rights of the human subjects of such research.[79]

Additionally, the statute requires that the Secretary establish a program to provide clarification and guidance to researchers using human subjects[80] and include a process within the NIH to handle reported violations.[81]

2. The Belmont Report

The Belmont Report serves as the modern cornerstone for the ethical principles and guidelines in research involving human subjects. Congress commissioned The Belmont Report in the National Research Act of 1974. That act created the National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research, and tasked the commission with drafting a report that "identif[ied] the basic ethical principles that should underlie the conduct of [ ]

[77] 42 U.S.C. §§ 281, *et seq.*

[78] 42 U.S.C. § 281.

[79] 42 U.S.C. § 289(a) (emphasis added).

[80] 42 U.S.C. § 289(b)(1).

[81] 42 U.S.C. § 289(b)(2).

research involving human subjects and to develop guidelines [ ] in accordance with those principles."[82]

It is widely acknowledged that a major motivation behind the National Research Act was the infamous Tuskegee syphilis study.[83] The federally-funded study, which began in 1932, sought to learn more about syphilis by studying infected individuals.[84] However, the subjects were never told that they had the disease, were not given important information about the study, and were denied adequate treatment for the disease even after penicillin was identified as a cure as early as 1947.[85] Worse yet, the subjects were disadvantaged racial minorities.[86] The unethical methodology of the study came to light in a 1972 Associated Press story that prompted widespread public outcry.[87]

This outcry led to the passage of the National Research Act, which commissioned the Belmont Report to help ensure that horrors like the Tuskegee study never happened again.[88] It identified three ethical principles that should govern research involving human subjects and also provided three sets of applied guidelines to make sure these principles are respected.[89] The principles and guidelines have remained a bedrock of United States bioethics ever since.

---

[82] *Belmont Report*, The Nat'l Comm'n for the Protection of Human Subjects of Biomedical and Behavioral Research, Ethical Principles and Guidelines for the Protection of Human Subjects of Research (Apr. 18, 1979), available online at http://archive.hhs.gov/ohrp/humansubjects/guidance/belmont.htm#xselect.

[83] *How Tuskegee Changed Research Practices*, Center for Disease Control, available at http://www.cdc.gov/tuskegee/after.htm (last updated June 15, 2011).

[84] *The Tuskegee Timeline*, Centers for Disease Control, available at http://www.cdc.gov/tuskegee/timeline.htm (last updated June 15, 2011).

[85] *Id.*

[86] *Id.*

[87] *Id.*

[88] *How Tuskegee Changed Research Practices*, *supra* n.83.

[89] *Belmont Report*, *supra* n.82.

The three ethical principles are: (1) respect for persons, (2) beneficence, and (3) justice.[90] Respect for persons involves respect for individuals as autonomous agents and recognition that persons with diminished autonomy are entitled to protection.[91] Beneficence requires protecting individuals from harm in addition to respecting their autonomous decisions; it is encapsulated by the Hippocratic maxim "do no harm."[92] Justice requires fair distribution of benefits and burdens – the unfair selection of disadvantaged racial minorities in the Tuskegee syphilis study was specially referenced as a violation of this principle.[93]

The Belmont Report took these ethical principles and created three applied guidelines for conducting research: (1) informed consent, (2) assessment of risks and benefits, and (3) selection of subjects.[94] Informed consent stems from respect for persons; it requires that subjects be given sufficient information about the research, comprehend that information, and voluntarily agree to participate.[95] Assessment of risks and benefits, which derives from beneficence, requires careful analysis of possible consequences and prohibits research that contains unbalanced risks or is otherwise inhumane.[96] Selection of subjects, which originates from justice, requires fairness in the selection of research subjects and is especially salient in research involving vulnerable subjects.[97]

The Belmont Report's ethical principles and applied guidelines have proven to be immensely influential since their publication in 1979, serving as a vital touchstone for researchers and prompting issuance of the prevailing federal regulatory regime on the topic.

---

[90] *Id.*

[91] *Id.*

[92] *Id.*

[93] *Id.*

[94] *Id.*

[95] *Id.*

[96] *Id.*

[97] *Id.*

B. Basic HHS Policy for Protection of Human Research Subjects

The statutory duty of the HHS to protect human research subjects under the PHSA, in tandem with the Belmont Report, led to the promulgation of a codified Basic HHS Policy for Protection of Human Research Subjects in 1991.[98] This regulation is sometimes referred to as the Common Rule because other federal agencies have adopted it verbatim. It lays out specific rules designed to protect human subjects through IRB oversight.[99] The IRB is required to approve research proposals initially and conduct continuing review.[100] In accordance with the Belmont Report, an IRB may only approve research if, among other requirements, it finds that: informed consent is given,[101] confidentiality is protected,[102] risks to the research subjects are minimized, [103] and selection of subjects is equitable.[104] Limited exceptions to the informed consent requirement exist only where the research involves no more than minimal risk to the subjects and the research could not practicably be carried out without the waiver.[105]

C. NIH Guidelines for Human Stem Cell Research

Despite its mandate to protect human subjects, HHS has done little to ensure that researchers recognize the risk that humanized chimeras may develop high level cognitive capacity, or that such individuals must receive appropriate treatment as human research subjects.[106] The only regulatory activity regarding human-animal chimera research is the 2009 NIH Guidelines for

[98] 45 C.F.R. §§ 46.101, *et seq.*

[99] 45 C.F.R. §§ 46.107-125.

[100] 45 C.F.R. § 46.109.

[101] 45 C.F.R. §§ 46.109(b) & 46.116.

[102] 45 C.F.R. § 46.102(f)(2).

[103] 45 C.F.R. § 46.111(a)(1)-(2).

[104] 45 C.F.R. § 46.111(a)(3).

[105] *See* 45 C.F.R. §§ 46.116(c) & (d).

[106] Nat'l Institutes of Health, *Guidelines for Human Stem Cell Research*, 74 Fed. Reg. 32,170 (July 7, 2009).

Human Stem Cell Research (NIH Guidelines).[107] In line with the NAS Guidelines,[108] the NIH Guidelines deny federal funding to research involving the transplant of human embryonic or induced pluripotent stem cells in nonhuman primate blastocysts.[109] The NIH Guidelines also deny federal support for research involving the breeding of human-animal chimeras where human embryonic or induced pluripotent stem cells may contribute to the germ line.[110]

The NIH Guidelines are notable for what they omit. As discussed previously, the narrow prohibition on research involving nonhuman primate blastocysts and breeding chimeras will not necessarily prevent the creation of humanized individuals.[111] In addition to being under-inclusive, the NIH Guidelines do not afford protection to the type of human-animal chimera most deserving of rights, i.e., ones with the cognitive capacity of a normal adult human,[112] and they do not prohibit research with a relatively high risk of creating such a humanized chimera.[113] The NIH Guidelines do not even require recognition or oversight of the risk of creating an ethically ambiguous creature posed by human-animal chimera research, despite the recommendation of ethicists and the NAS Guidelines previously discussed.[114]

While some chimeras are protected as animals under the Animal Welfare Act,[115] the AWA does not protect rats, mice or birds, which are most frequently used in research. Furthermore, invasive research involving intentional harm or more than minimal risk to animals is subject to only a few substantive limitations under the AWA.[116] Such invasive research would be

[107] *Id.*

[108] *See* NAS Guidelines, *supra* n.61.

[109] NIH Guidelines, *supra* n.106 at 32,175.

[110] *Id.*

[111] *See* NIH Guidelines, *supra* n.106; discussion, *supra* III.B, C, and D.

[112] *See* NIH Guidelines, *supra* n.106; discussion, *supra* III.C.

[113] *See* NIH Guidelines, *supra* n.106; discussion, *supra* III.B and III.C.

[114] *See* NIH Guidelines, *supra* n.106; discussion, *supra* III.C.

[115] 7 U.S.C. §§ 2131, *et seq.*

[116] *See id.*

unequivocally prohibited if conducted on human research subjects.[117] At a minimum, such research should likewise be prohibited on human-animal chimeras with the cognitive capacity of a normal adult human.[118]

V. PROPOSED RULE FOR THE COMPREHENSIVE PROTECTION OF CHIMERAS WHO QUALIFY AS HUMAN SUBJECTS UNDER THE PHSA

HHS has ample authority to promulgate comprehensive regulations that would protect humanized chimeras pursuant to the Administrative Procedures Act,[119] the HHS implementing regulations,[120] and its PHSA duty to protect human subjects.[121] Accordingly, ALDF submits the following rule to be codified at 42 C.F.R. Pt. 45 Sub. E:

§ 1 Scope.

This subpart applies to all research involving human-animal chimeras that is conducted or otherwise supported by the federal government.

§ 2 Definitions.

(a) "High level cognitive capacity" means mental ability that is substantially similar to a normal adult human considering the individual's:

(i) linguistic ability;

(ii) degree of self-awareness;

(iii) sense of past and future self;

(iv) moral agency; and

(v) rational agency.

---

[117] 42 U.S.C. § 289.

[118] *See* discussion, *supra* III.C.

[119] 5 U.S.C. § 553(e).

[120] 45 C.F.R. §§ 160.101, *et seq.*

[121] 42 U.S.C. §§ 201, *et seq.*; 42 U.S.C. § 289(a).

(b) "Human-animal chimera" means a nonhuman animal implanted with human stem cells, or cells derived therefrom, during any stage of life.

(c) "Humanized chimera" means a human-animal chimera with high level cognitive capacity.

§ 3 IRB review.

(a) An IRB shall conduct a preliminary review of all research proposals involving human-animal chimeras to determine if there is a substantial risk of the subject becoming a humanized chimera.

(b) In determining whether there is a substantial risk of a human-animal chimera becoming a humanized chimera, the IRB shall consider:

(i) proportion of engrafted human cells;

(ii) stage of neural development;

(iii) species of animal;

(iv) brain size;

(v) degree of integration; and

(vi) brain pathology.[122]

§ 4 Humanized chimeras protected as research subjects.

(a) Any individual who has high level cognitive capacity under Section 2(a) and is a human-animal chimera as defined by Section 2(b) is a "humanized chimera" and shall have the same protection as other human research subjects under this part.

(b) If at any point in the review process described in Section 3 the IRB finds there is a substantial risk that the subject will become or has obtained humanized chimera status, it shall require the researchers to reduce the risks to a non-substantial level. If the risks cannot be reduced to a non-substantial level then the IRB shall ensure that the individual is protected as a research subject under this part.

[122] Greene et al., *supra* n.23.

(c) If the IRB determines there is no substantial risk of a human-animal chimera becoming a humanized chimera, it shall nonetheless continue to monitor the research and ensure the individual's protection as a human research subject upon plausible evidence that high level cognitive capacity has been obtained.

VI. Legal Justification to Promulgate Proposed Rule

A. Scope

The Proposed Rule protects humanized chimeras but affects "all research involving human-animal chimeras that is conducted or supported by the federal government."[123] The basis for this broad scope is that the effect of chimerization is inherently unpredictable and there is always a risk, however small, of creating a humanized chimera.[124] HHS thus has authority to issue the Proposed Rule to at least require special consideration of research that risks the creation of humanized chimeras since those research subjects would fit within the definition of human subject under the PHSA[125] and accompanying regulations.[126]

B. Definitions

The Proposed Rule defines a humanized chimera as being a "human-animal chimera with high level cognitive capacity."[127] High level cognitive capacity is in turn defined as:

> mental ability that is substantially similar to a normal human considering the individual's (i) linguistic ability; (ii) degree of self-awareness; (iii) sense of past and future self; (iv) moral agency; and / or (v) rational agency.[128]

The Proposed Rule focuses on high level cognitive capacity in keeping with the view of high moral status described by Streiffer.[129] The factors are ones commonly connected with adult

[123] *See* discussion, *supra* V (Proposed Rule § 1).

[124] *See* discussion, *supra* III.B; Greely et al., *supra* n.21; Greene et al., *surpa* n.23.

[125] 42 U.S.C. § 289(a).

[126] 45 C.F.R. §§ 46.101, *et seq.*

[127] *See* discussion, *supra* V (Proposed Rule § 2(c)).

[128] *See* discussion, *supra* V (Proposed Rule § 2(a)).

human cognitive ability.[130] The use of several factors rather than one bright-line test reduces definitional clarity but is nonetheless prudent in light of the difficulty of isolating a single trait as warranting additional protections. The standard that a chimera's cognitive capacity be "substantially similar" to a normal adult human's[131] acknowledges the difficulty in defining a normal adult human's cognitive capacity and allows for flexibility in making a judgment.

The definition also limits humanized chimera status to those individuals with at least some human cells. This limitation satisfies any interpretation of the PHSA that requires that human subjects have some human biology.[132] However, the rule intentionally imposes no minimum quantity of human cells because it is problematic to morally justify disparate treatment of two beings with similarly high level cognitive capacity based on the species of cells in their respective bodies.

C. IRB Review

Committee oversight of research involving human-animal chimeras to ensure adequate protection of individuals obtaining high level cognitive capacity is in line with the recommendation of ethicists and the National Academy of Sciences.[133] As previously discussed, most ethicists encourage review of the special context posed by human-animal chimera research.[134] Their primary ethical concern is the possibility of conferring humanity on human-animal chimeras[135] and the Proposed Rule ensures appropriate oversight of just that.

---

[129] Streiffer, *supra* n.38 at 354-56; discussion, *supra* III.C.

[130] *Ibid.*

[131] *See* discussion, *supra* V (Proposed Rule Rule § 2(a)).

[132] *See* Streiffer, *supra* n.38 at 356-58 (describing a biologically anthropocentric view of human moral status).

[133] *See* discussion, *supra* III.C & III.D.

[134] *Id.*

[135] *Id.*

The oversight required by the Proposed Rule differs from other authorities in at least one significant respect: by placing the review task with an IRB instead of an ESCRO or its equivalent. Although the NAS Guidelines recommend creation of an ESCRO,[136] there is no such infrastructure required by federal law or regulations. A federal ESCRO requirement on top of the IRB and IACUC processes would be burdensome and unnecessary, especially in light of the Proposed Rule's relatively limited scope.[137] Nonetheless, IRB consultation with an established ESCRO committee will typically be desirable when evaluating the risks associated with research proposals involving human-animal chimeras.

In addition to providing a framework for review, the Proposed Rule also suggests several factors that an IRB should consider in deciding whether a proposed research project carries with it substantial risk of producing a humanized chimera. These factors are borrowed almost verbatim from the Greene working group and should be fairly generalizable.[138]

D. Humanized Chimeras Protected As Research Subjects

The Proposed Rule makes clear that humanized chimeras are human subjects under the PHSA and deserve full protection in accord with that status. Because current laws do not explicitly make this connection, there is considerable risk that a humanized chimera, should one arise, would not be afforded her due treatment under the law. While this rule should be easily applied in situations where researchers are confronted with an unambiguously humanized chimera, there remains a question about how to handle the risk posed by research that has the possibility of creating such a being.

---

[136] NAS Guidelines (2010 amendments), *supra* n.61 at Appendix C § 2.0.

[137] *See* Dolgin, *Time to ditch stand-alone stem cell oversight panels, experts say*, NATURE MEDICINE vol. 19 no. 250 (Mar. 2013), available online at http://www.nature.com/nm/journal/v19/n3/full/nm0313-250.html, *discussing* Greely, *Assessing ESCROs: Yesterday and Tomorrow*, AM. J. BIOETHICS vol. 13 issue 1, 44-52 (Jan. 11, 2013), abstract available online at http://www.tandfonline.com/doi/abs/10.1080/15265161.2013.747340.

[138] Greene et al., *supra* n.23.

The Proposed Rule resolves this issue by creating standards that govern situations where there is a substantial risk of creating a humanized chimera, or plausible (but not necessarily conclusive) evidence that a humanized chimera has been created. Where there is a substantial risk of creating a humanized chimera, the Proposed Rule requires that researchers treat the chimera as a human subject according to the PHSA. Although the chimera may not in fact be humanized, the presumption of this status is necessary in light of the PHSA's extra requirements for research involving a significant risk of harm to human subjects.[139] Some of those requirements may require ending experimentation if, for example, selection of the subject is not equitable[140] or satisfactory informed consent cannot be not obtained.[141] For similar reasons, researchers should treat a chimera as a human subject upon plausible evidence that the individual has obtained high level cognitive capacity.[142]

VII. PROMULGATION OF THE PROPOSED RULE IS TIMELY WITH RESPECT TO THE PRESENT STATE OF SCIENCE

HHS's promulgation of the Proposed Rule protecting humanized chimeras is timely notwithstanding the lack of evidence that the creation of such a humanized chimera is imminent. The fact remains that experiments generating human-animal chimeras presently carry a risk of imbuing an individual with high level cognitive capacity with an attendant risk of harm and the scientific literature carries calls for the creation of chimeras who exhibit a greater degree of human

[139] The PHSA regulations to protect human subjects include a list of criteria for IRB approval that requires minimization of harm, the reasonableness of risks in light of anticipated benefits, and informed consent, among others. In essence, the risk of harm is that the chimera has the legal status of a human but is not treated accordingly for some time. *See* 45 C.F.R. § 46.111.

[140] 45 C.F.R. §46.111(a)(3).

[141] 45 C.F.R. § 46.111(a)(4)-(5).

[142] Although it should be readily apparent what cognitive capacities an individual possesses, it may be difficult to make such a determination if, for example, differences in physiology make it difficult to communicate. *See* Greene et al., *supra* n.21 (acknowledging the challenges in assessing cognitive capacity of non-chimeric nonhuman animals).

chimerism.[143] The failures of HHS and IRBs to simply consider such risks constitute an abdication of responsibility under the principles espoused by the Belmont Report and reflected in the PHSA.

Present research involving the creation of human-animal chimeras implicates all three of the Belmont Report principles: (1) respect for person, (2) beneficence, and (3) justice.[144] As stated previously, beneficence involves protecting individuals from harm and requires careful analysis of risks and benefits to ensure a positive balance.[145] Experiments like Polzin et al. (geep chimeras had both goat and sheep physical traits)[146] and Han et al. (mice with human brain cells had augmented mental capacity)[147] demonstrate the potential of chimerization to dramatically alter an individual's traits. Furthermore, the type and degree of changes chimerization will cause are unpredictable and at least deserve consideration by HHS.[148] If such chimerization does generate a humanized chimera with high level cognitive capacity, then there is a risk of harm to the subject stemming from, for example, research techniques, changes in biology, and housing accommodations. The principle of beneficence thus mandates consideration by a review board of both the risk of generating a humanized chimera and the risk that she will be harmed during the course of the experiment.

---

[143] James et al., *Contribution of Human Embryonic Stem Cells to Mouse Blastocysts*, DEVELOPMENTAL BIOLOGY vol. 295 no. 1, p. 100 (2006), available online at http://www.sciencedirect.com/science/article/pii/S0012160606002260.

[144] *Belmont Report*, *supra* n.82.

[145] *Id.*

[146] Polzin et al., *supra* n.18.

[147] Han et al., *supra* n.34.

[148] *See* discussion, *supra* III.C; Greely et al., *supra* n.21 (declining to rule-out the possibility that a mouse with human neurons could obtain some aspects of human consciousness or disctively human cognitive abilities); Greene et al., *supra* n.23 (acknowledging the difficulty in anticipating the effects of grafting human neurons onto an animal).

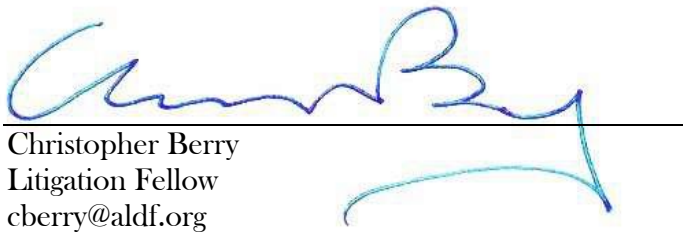
The principles of respect for person and justice likewise compel timely passage of the Proposed Rule.[149] A humanized chimera who obtains high level capacity during the course of an experiment demands respect for her autonomy. The special vulnerability of the individual – who is subject to the mercy of her experimenter and without clear legal rights under the status quo – further demands clarification from HHS that IRB review is necessary in experiments involving the introduction of human cells into animals.

VIII. CONCLUSION

Petitioner ALDF submits that the Secretary should initiate rulemaking to clarify that protection for human subjects under the PHSA and accompanying regulations applies, at a minimum, to human-animal chimeras with cognitive capacity substantially similar to a normal adult human. The risk of creating such a humanized chimera is significant, and HHS must make clear that those individuals are fully protected in accordance with the ethical principles, guidelines, and laws governing research on human subjects.

Dated: December 3, 2013

Respectfully submitted,



Christopher Berry
Litigation Fellow
cberry@aldf.org
ANIMAL LEGAL DEFENSE FUND
170 E. Cotati Ave.
Cotati, CA 94931
Tel: (707) 795-2533
Fax: (707) 795-7280

[149] *Belmont Report*, *supra* n.82.

BEFORE THE UNITED STATES DEPARTMENT OF HEALTH AND HUMAN SERVICES

APPENDIX VOLUME 1 TO THE CITIZEN PETITION FOR RULEMAKING TO PROTECT HUMANIZED CHIMERAS UNDER THE PUBLIC HEALTH SERVICES ACT

ANIMAL LEGAL DEFENSE FUND,

Citizen petitioner,

170 E. Cotati Ave.

Cotati, CA 94931

Filing with:

KATHLEEN SEBELIUS,

In her official capacity as Secretary of the

United States Department of Health and Human Services,

200 Independence Avenue S.W.

Washington, D.C. 20201

APPENDIX VOLUME 1 TAB

THE CAMBRIDGE DECLARATION ON CONSCIOUSNESS (2012) n.6

King et al., *Vocal copying of individually distinctive signature whistles in bottlenose dolphins*, PROCEEDINGS OF THE ROYAL SOCIETY B, vol. 280 no. 1757 (Apr. 22, 2013) n.9

Osvath and Karvonen, *Spontaneous Innovation for Future Deception in Male Chimpanzees*, PLOS ONE vol. 7 no. 5 (May 9, 2012) n.10

Bartal et al., *Empathy and Pro-Social Behavior in Rats*, SCIENCE, vol. 335, pp. 1427-1430 (Dec. 9, 2011) n.12

Populin et al., *Rhesus Monkeys (Macaca mulatta) Do Recognize Themselves in the Mirror: Implication for the Evolution of Self-Recognition*, PLOS ONE, vol. 5 (Sept. 29, 2010) n.13

Chimera, THEFREEDICTIONARY, available online at http://www.thefreedictionary.com/ Chimera (last accessed Nov. 25, 2013) n.15-1

Greely, *Defining Chimeras . . . and Chimeric Concerns*, AM. J. BIOETHICS, vol. 3, no. 3 (Summer 2003) n.15-2

Boklage, *Embryogenesis of chimeras, twins and anterior midline asymmetries*, HUMAN REPRODUCTION, vol. 21, no. 3 (2006) n.16

Polzin et al., *Production of Sheep-Goat Chimeras by Inner Cell Mass Transplantation*, J. OF ANIMAL. SCI., vol. 65, pp. 325-330 (1987) n.18-1

Science: It's a Geep, TIME MAGAZINE (Feb. 27, 1984) n.18-2

Greely et al., *Thinking About the Human Neuron Mouse*, AM. J. BIOTECH, vol. 7, no. 5 (May 2007) n.21

Greene et al., *ETHICS: Moral Issues of Human-Non-Human Primate Neural Grafting*, SCIENCE, vol. 309, no. 5733 (July 15, 2005) n.23

Knowles, *Ethics of Research Using Hybrids, Chimeras and Cytoplasmic Hybrids*, STEM CELL NETWORK (2003) n.26

Hunter, *Chimeras: The Ethics of Creating Human-Animal Interspecifics*, Dissertation (Jan. 20, 2009) n.27

Vince, *Pig-human chimeras contain cell surprise*, NEW SCIENTIST (Jan. 13, 2004) n.29

Boyle, E*ndowed by Their Creator? The Future of Constitutional Personhood*, BROOKINGS INSTITUTE (Mar. 9, 2011) n.30

Weiss, *Of Mice, Men and In-Between*, WASH. POST (Nov. 20, 2004) n.33-1

Mott, *Animal-Human Hybrids Spark Controversy*, NAT'L GEO. NEWS (Jan. 25, 2005) n.33-2

Han et al., *Forebrain Engraftment by Human Glial Progenitor Cells Enhances Synaptic Plasticity and Learning in Adult Mice*, CELL STEM CELL, vol. 12, pp. 342-353 (Mar. 7, 2013).....n.34

Blurton-Jones et al., Presentation,*Restoration of memory in mouse models of Alzheimer's disease and neuronal loss; a new paradigm using human neural stem cell transplantation* (July 17, 2012).....n.36

California Institute for Regenerative Medicine, *Grant Abstract: Developmental Candidates for Cell-Based Therapies for Parkinson's Disease* (last accessed Nov. 27, 2013).....n.37

Streiffer, *At the Edge of Humanity: Human Stem Cells, Chimeras, and Moral Status*, KENNEDY INSTITUTE OF ETHICS JOURNAL, vol. 15, no. 4 (Dec. 2005).....n.38-1

Hagglund, *Patentability of Human-Animal Chimeras*, 25 SANTA CLARA COMP. & HIGH TECH. L. J. 51 (2008).....n.38-2

Bennett, *Comment: Chimera and the Continuum of Humanity*, 55 EMORY L. J. 347 (2008).....n.38-3

Robert and Baylis, *Crossing Species Boundaries*, THE AM. JOURNAL OF BIOETHICS, vol. 3, no. 3 (Summer 2003).....n.39

Karpowicz et al., *Developing Human-Nonhuman Chimeras in Human Stem Cell Research: Ethical Issues and Boundaries*, KENNEDY INSTITUTE OF ETHICS JOURNAL, vol. 15 , no. 2 (2005).....n.59

THE CAMBRIDGE DECLARATION ON CONSCIOUSNESS (2012)

The Cambridge Declaration on Consciousness[*]

On this day of July 7, 2012, a prominent international group of cognitive neuroscientists, neuropharmacologists, neurophysiologists, neuroanatomists and computational neuroscientists gathered at The University of Cambridge to reassess the neurobiological substrates of conscious experience and related behaviors in human and non-human animals. While comparative research on this topic is naturally hampered by the inability of non-human animals, and often humans, to clearly and readily communicate about their internal states, the following observations can be stated unequivocally:

- The field of Consciousness research is rapidly evolving. Abundant new techniques and strategies for human and non-human animal research have been developed. Consequently, more data is becoming readily available, and this calls for a periodic reevaluation of previously held preconceptions in this field. Studies of non-human animals have shown that homologous brain circuits correlated with conscious experience and perception can be selectively facilitated and disrupted to assess whether they are in fact necessary for those experiences. Moreover, in humans, new non-invasive techniques are readily available to survey the correlates of consciousness.

- The neural substrates of emotions do not appear to be confined to cortical structures. In fact, subcortical neural networks aroused during affective states in humans are also critically important for generating emotional behaviors in animals. Artificial arousal of the same brain regions generates corresponding behavior and feeling states in both humans and non-human animals. Wherever in the brain one evokes instinctual emotional behaviors in non-human animals, many of the ensuing behaviors are consistent with experienced feeling states, including those internal states that are rewarding and punishing. Deep brain stimulation of these systems in humans can also generate similar affective states. Systems associated with affect are concentrated in subcortical regions where neural homologies abound. Young human and non-human animals without neocortices retain these brain-mind functions. Furthermore, neural circuits supporting behavioral/electrophysiological states of attentiveness, sleep and decision making appear to have arisen in evolution as early as the invertebrate radiation, being evident in insects and cephalopod mollusks (e.g., octopus).

- Birds appear to offer, in their behavior, neurophysiology, and neuroanatomy a striking case of parallel evolution of consciousness. Evidence of near human-like levels of consciousness has been most dramatically observed in African grey parrots. Mammalian and avian emotional networks and cognitive microcircuitries appear to be far more homologous than previously thought. Moreover, certain species of birds have been found to exhibit neural sleep patterns similar to those of mammals, including REM sleep and, as was demonstrated in zebra finches, neurophysiological patterns, previously thought to require a mammalian neocortex. Magpies in

particular have been shown to exhibit striking similarities to humans, great apes, dolphins, and elephants in studies of mirror self-recognition.

- In humans, the effect of certain hallucinogens appears to be associated with a disruption in cortical feedforward and feedback processing. Pharmacological interventions in non-human animals with compounds known to affect conscious behavior in humans can lead to similar perturbations in behavior in non-human animals. In humans, there is evidence to suggest that awareness is correlated with cortical activity, which does not exclude possible contributions by subcortical or early cortical processing, as in visual awareness. Evidence that human and non-human animal emotional feelings arise from homologous subcortical brain networks provide compelling evidence for evolutionarily shared primal affective qualia.

We declare the following: "*The absence of a neocortex does not appear to preclude an organism from experiencing affective states. Convergent evidence indicates that non-human animals have the neuroanatomical, neurochemical, and neurophysiological substrates of conscious states along with the capacity to exhibit intentional behaviors. Consequently, the weight of evidence indicates that humans are not unique in possessing the neurological substrates that generate consciousness. Non-human animals, including all mammals and birds, and many other creatures, including octopuses, also possess these neurological substrates."*

***** The Cambridge Declaration on Consciousness was written by Philip Low and edited by Jaak Panksepp, Diana Reiss, David Edelman, Bruno Van Swinderen, Philip Low and Christof Koch. The Declaration was publicly proclaimed in Cambridge, UK, on July 7, 2012, at the Francis Crick Memorial Conference on Consciousness in Human and non-Human Animals, at Churchill College, University of Cambridge, by Low, Edelman and Koch. The Declaration was signed by the conference participants that very evening, in the presence of Stephen Hawking, in the Balfour Room at the Hotel du Vin in Cambridge, UK. The signing ceremony was memorialized by CBS 60 Minutes.

King et al.,

Vocal copying of individually distinctive signature whistles in bottlenose dolphins,

PROCEEDINGS OF THE ROYAL SOCIETY B, vol. 280, no. 1757 (Apr. 22, 2013)

PROCEEDINGS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Vocal copying of individually distinctive signature whistles in bottlenose dolphins

Stephanie L. King, Laela S. Sayigh, Randall S. Wells, Wendi Fellner and Vincent M. Janik

Proc. R. Soc. B 2013 **280**, 20130053, published 20 February 2013

PROCEEDINGS OF THE ROYAL SOCIETY B

rspb.royalsocietypublishing.org

Research

Cite this article: King SL, Sayigh LS, Wells RS, Fellner W, Janik VM. 2013 Vocal copying of individually distinctive signature whistles in bottlenose dolphins. Proc R Soc B 280: 20130053.
http://dx.doi.org/10.1098/rspb.2013.0053

Received: 8 January 2013
Accepted: 24 January 2013

Subject Areas:
behaviour, cognition

Keywords:
vocal learning, *Tursiops*, imitation, communication

Author for correspondence:
Vincent M. Janik
e-mail: vj@st-andrews.ac.uk

Electronic supplementary material is available at http://dx.doi.org/10.1098/rspb.2013.0053 or via http://rspb.royalsocietypublishing.org.

Royal Society Publishing
Informing the science of the future

Vocal copying of individually distinctive signature whistles in bottlenose dolphins

Stephanie L. King[1], Laela S. Sayigh[2], Randall S. Wells[3], Wendi Fellner[4] and Vincent M. Janik[1]

[1]Sea Mammal Research Unit, School of Biology, University of St Andrews, St Andrews, Fife KY16 8LB, UK
[2]Biology Department, Woods Hole Oceanographic Institution, Woods Hole, MA 02543, USA
[3]Chicago Zoological Society, c/o Mote Marine Laboratory, 1600 Ken Thompson Parkway, Sarasota, FL 34236, USA
[4]The Seas, Epcot, Walt Disney World Resort, 2016 Avenue of the Stars, EC Trl. W-251, Lake Buena Vista, FL 32830, USA

Vocal learning is relatively common in birds but less so in mammals. Sexual selection and individual or group recognition have been identified as major forces in its evolution. While important in the development of vocal displays, vocal learning also allows signal copying in social interactions. Such copying can function in addressing or labelling selected conspecifics. Most examples of addressing in non-humans come from bird song, where matching occurs in an aggressive context. However, in other animals, addressing with learned signals is very much an affiliative signal. We studied the function of vocal copying in a mammal that shows vocal learning as well as complex cognitive and social behaviour, the bottlenose dolphin (*Tursiops truncatus*). Copying occurred almost exclusively between close associates such as mother–calf pairs and male alliances during separation and was not followed by aggression. All copies were clearly recognizable as such because copiers consistently modified some acoustic parameters of a signal when copying it. We found no evidence for the use of copying in aggression or deception. This use of vocal copying is similar to its use in human language, where the maintenance of social bonds appears to be more important than the immediate defence of resources.

1. Introduction

Vocal production learning enables animals to copy novel sounds in their environment or to develop their own distinctive calls, avoiding overlap with those heard before [1]. Most commonly, vocal learning leads to convergence in sound parameters between individuals. A good example of this can be found in bird song dialects [2] or in the development of group-specific contact calls [3–7]. The exchange of such shared calls between individuals can be aggressive or affiliative in nature. While contact calls are known to be affiliative [7], song type matching in song birds tends to have an aggressive connotation [8]. Song sparrows, for example, use song type matching when defending their territory against an unknown male, but avoid it when interacting with known neighbours with whom they use more subtle repertoire matching [9,10]. Repertoire matching, i.e. the use of a shared song type while avoiding a reply with the same song type, may allow the addressing of a neighbour in a more affiliative or neutral way.

In most instances, these interactions occur with calls that are shared by more than one individual. In the case of contact calls, the common call belongs either to a group or a pair of animals. In bird song, animals have individual repertoires where each song type is shared with other individuals, but the overall composition of the repertoire may be unique. Production rates for each shared call or song type are usually similar across the individuals that share it. Individual call or song types survive in populations as cultural traditions that can outlive the animals that produce them at any one time [11].

The signature whistle of the bottlenose dolphin stands out from these examples in that it seems to be more individually specific. Bottlenose dolphins produce a large variety of narrow-band frequency-modulated whistles and pulsed sounds for communication [12]. As part of their repertoire, each individual also develops an individually distinctive signature whistle [13,14] that develops under the influence of vocal learning [15–17]. Individuals listen to their acoustic environment early in life and then develop their own novel frequency modulation pattern or contour for their signature whistle [15]. The result is a novel and unique modulation pattern that identifies the individual even in the absence of general voice cues [18]. Interindividual variation in signature whistles is much larger than that found in recognition signals of other species [19].

Bottlenose dolphins live in fluid fission–fusion societies with animals forming a variety of different social relationships [20]. This social organization, coupled with restrictions in underwater vision and olfaction, has led to natural selection favouring designed individual signature whistles [12,14] instead of relying on the by-product distinctiveness of voice features [19]. The signature whistle tends to be the most commonly used whistle in each individual's repertoire accounting for around 50 per cent of all whistles produced by animals in the wild [21]. Bottlenose dolphins are, however, able to learn new sounds throughout their lives [22], and conspecifics occasionally imitate the signature whistles of others [23]. Thus, one animal's signature whistle can form a minor part of another animal's vocal repertoire as a result of copying [17,23,24]. Signature whistle copying is, however, rare [23,25–27], albeit significantly more common than expected by chance [25]. As such, each signature whistle forms only a major part of one animal's repertoire, allowing it to be a label for that particular individual when copied.

Nevertheless, the function of copying events remains unclear. It has been argued that copying of signature whistle types is equivalent to addressing other individuals. Such addressing can be affiliative or aggressive. Unlike songbirds, delphinids are not territorial and do not sing. Instead, they use their acoustic signals in the context of social interactions and group cohesion [12]. Bottlenose dolphins have low rates of aggression towards close associates and higher ones towards social competitors, for example among male alliances [20]. Investigating who is copying who can therefore give us information on the signal value of copying. In addition to affiliative and aggressive functions, a third hypothesis for whistle copying is that it is used as a deceptive form of signalling [28]. For example, deceptive signature whistle copying by male dolphins could allow them to gain access to females guarded by other males or to avoid directed aggression from a male alliance [29]. It appears that copies are sufficiently rare to allow for such a use without jeopardizing the reliability of signature whistles as identity signals.

To investigate these three hypotheses, the occurrence of signature whistle copying was studied in captive and briefly captured and subsequently released wild bottlenose dolphins. We hypothesized that if signature whistle copying is affiliative it should only occur between close associates. Alternatively, copying in an aggressive context should be more common between animals that are less closely associated. Furthermore, copies used in a deceptive way should ideally not be recognizable as copies, whereas in affiliative or aggressive contexts, they could be recognizable as such. We also investigated the temporal aspects of whistle copying given the importance of signal type matching in other species.

2. Material and methods

(a) Social and acoustic data from the wild

Data were collected from wild bottlenose dolphins around Sarasota Bay, FL, USA between 1984 and 2009. The amount of time animals are sighted together can be used to give a measure of their association. The half-weight ratio coefficients of association (CoA) [30] is defined as $\mathrm{CoA} = 2N_{ab}/N_a + N_{b,}$ in which N_{ab} is the number of times individuals A and B have been seen together, N_a is the number of times individual A has been seen without B, and N_b is the number of times individual B has been seen without A. CoAs were calculated for all study animals from data gained during regular, systematic photographic identification surveys of dolphins. CoAs given for each pair of animals caught together are from the year the recordings were taken. Wild bottlenose dolphin acoustic recordings were collected during capture–release events for health assessments and life-history studies in Sarasota Bay [31]. One such event takes on average 108 min from the time the net is set to the time the individual is released. During these events, animals were physically restrained and frequently out of visual sight, but not acoustic range, of one another. The signature whistle of an individual is the most common whistle type emitted in such isolation conditions [14]. The Sarasota Dolphin Research Programme has now accumulated a catalogue of whistles from over 250 individual dolphins from the resident community in Sarasota Bay since 1975 [14], many of which were recorded in multiple capture–release sessions. We compared all whistles produced by an individual with the signature whistles of all others in the same capture set in order to identify copying events. Ages of animals were known from long-term observations [32] or from analysing growth rings in teeth [33].

The vocalizations of each individual were recorded via a suction cup hydrophone, allowing the identification of the caller for each recorded call. Either custom-built or SSQ94 hydrophones were used (High Tech Inc.). Between 1984 and 2004, the acoustic recordings were taken with either Marantz PMD-430 or Sony TC-D5M stereo-cassette recorders (frequency response of recording system: 0.02–18 kHz $\pm$ 5 dB) or Panasonic AG-6400 or AG-7400 video-cassette recorders (frequency response of recording system: 0.02–25 kHz $\pm$ 3 dB). For recordings taken from 2005 onwards, a Sound Devices 744T digital recorder was used (sampled at 96 kHz, 24-bit, frequency response of recording system: 0.02–48 kHz $\pm$ 1 dB).

The first step of analysis consisted of visual comparisons of spectrograms of 205 h and 23 min of acoustic recordings of temporarily caught and released, wild bottlenose dolphins by one observer in order to identify copying events within each capture set. The total recording time inspected in this way was 110 h and 55 min for pairs of animals caught together with low association levels ($\mathrm{CoA} < 0.5$) and 94 h and 28 min for pairs of animals caught together with high association levels ($\mathrm{CoA} > 0.5$). The second step involved a detailed analysis of 32 h and 12 min (table 1) of recordings where vocal copying had been found. These contained a total of 10 219 whistles, which is the dataset on which this in-depth analysis is based.

(b) Social and acoustic data from captivity

To investigate the social context of copying, four captive adult males were recorded at The Seas Aquarium, Lake Buena Vista, FL, USA, during May–June 2009. One male, Ranier, was estimated to be 28 years old and was collected at approximately 3 years of age in the northern Gulf of Mexico. The other males were Calvin (15 years old), Khyber (18 years old) and Malabar (8 years old), who were all captive born. All four animals had been together for 3.5 years at the start of the study; Ranier and Calvin had been together for 6 years. Vocalizations of these dolphins

Table 1. Pairs of animals involved in signature whistle copying events, with the animal producing copies in bold. The mean similarity values are given for each animal's signature whistle when compared with the vocal copy. The copier's own signature whistles had low similarity scores with the copy while the signature whistles of the copied animals had high similarity scores with the copies (see the electronic supplementary material, figure S1).

pair	sex	relationship	CoA	age	recording time (min)	no. of vocal copies	average similarity values
1. Calvin	M	associates	1[a]	15	70	13	1.5
Ranier	M			28	70	—	4.5
2a. FB26	M	alliance	0.8	31	93	38	1.0/3.2[b]
2b. FB48	M	partners		29	101	5	1.0/3.5[b]
3. FB114	M	associates	0.07	16	51	4	2.4
FB20	M			15	95	—	3.3
4. FB90	F	mother	0.98	25	92	17	1.3
FB122	M	calf		4	92	—	3.3
5. FB65	F	calf	0.67	6	70	1	1.2
FB67	F	mother		21	70	—	3.6
6. FB228	M	calf	0.95	5	106	8	1.1
FB65	F	mother		21	106	—	3.5
7. FB5	F	mother	1.0	29	85	17	1.3
FB55	F	calf		3	85	—	3.3
8a. FB35	F	mother	0.9	32	92	2	1.7/3.7[b]
8b. FB93	F	calf		3	92	4	2.5/3.2[b]
9. FB71	F	mother	1.0	28	97	13	1.0
FB95	F	calf		1	97	—	3.3
10. FB5	F	mother	0.56	29	79	40	1.0
FB155	F	calf		2	79	—	3.5
11. FB9	F	mother	0.9	20	105	9	1.2
FB177	F	calf			105	—	3.4

[a]These animals were permanent residents in a captive facility.

[b]Where both animals copied one another the average similarity value for that animal's own signature with the copy it produced of the other animal's signature whistle is given first (low number) followed by the average similarity value for that animal's own signature whistle with the copy produced by the other animal in the pair (larger number).

were recorded with two HTI-96 MIN hydrophones (frequency response: 0.002–30 kHz ± 1 dB) and two CRT hydrophones (C54 series; frequency response: 0.016–44 kHz ± 3 dB) onto a Toshiba Satellite Pro laptop using a four channel Avisoft v. 416 UltrasoundGate recording device (sampled at 50 kHz, 8 bit).

A total recording time of 16 h for the four males was analysed. The length of recording time when copying between pairs could be identified (as determined by their positions in the pool system) was as follows: 16 h (100%) for Ranier and Calvin, Ranier and Malabar, Khyber and Calvin and Khyber and Malabar; 14 h (87.3%) for Ranier and Khyber and 2 h (12.7%) for Calvin and Malabar. The caller was identified, using passive acoustic localization [23]. The social association of male pairs at The Seas was evaluated by measuring synchrony in their swimming patterns [34]. A focal animal instantaneous sampling method was used with an observation period of 7.5 min and a 15 s interval. At each 15 s interval, the focal animal's synchrony status was assessed relative to each other animal in the group. Observations took place 5 days per week between 08.00 and 18.00, and each animal served as the focal animal once each day in an order determined by a balanced, randomly ordered schedule. Observations were made between January 2009 and June 2009 when all four dolphins were together in the same pool.

(c) Identifying copying events

Initially, one observer (S.L.K.) compared all whistles in a given captured or captive group with each other, and identified all occurrences where the same whistle type was being produced by more than one animal by inspecting spectrograms (fast Fourier transform (FFT) length 512, overlap 100%, Blackmann–Harris window) in Adobe Audition v. 2.0 (Adobe Systems). Five naive human observers, blind to context and animal identity, were then used to rate the similarity of each copy of a signature whistle to the original signature whistle (the whistle as produced by its owner) and to the copier's own signature whistle. Visual classification was used as it is more reliable than computer-based methods in dolphin whistle classification [14,35] and is frequently used in animal communication studies [2,36]. The five observers were given the extracted contours (frequency modulation pattern) of the whistles as plots of frequency versus time and were asked to rate whistle similarity using a five-point similarity index ranging from 1 (dissimilar) to 5 (similar). Only copied whistles that reached a mean similarity score of more than 3 with the original signature whistle and less than 3 with the copier's own signature whistle were deemed copies and included in the analysis. A value of 3 indicates a relatively high similarity as indicated in previous studies [25,29,37].

(d) Acoustic analysis

The whistle contours of every copy as well as of randomly chosen exemplars of signature whistles of both interacting individuals were extracted using a supervised contour extraction programme [38], with a time resolution of 5 ms. From the contours, the following parameters were measured: start frequency, end frequency, minimum frequency, maximum frequency, frequency range, duration and mean frequency. One further parameter, number of loops, was read directly from the spectrogram where applicable. A loop was defined as a repeated modulation pattern within a signature whistle that could be separated by periods of stereotyped, discrete segments of silence. These periods of silence were taken to be 250 ms or less, which is the maximum inter-loop interval found in this population [39].

(e) Statistical analysis

All statistical procedures were conducted in R (R project for statistical computing; GNU project). Acoustic parameters were analysed by first testing for normality using the Lilliefors (Kolmogorov–Smirnov) test. Depending upon the outcome, either the Mann–Whitney test or a Welch's *t*-test was used to compare differences between parameters of the copies with the original signature whistles and the copier's own signature whistle. A sampling statistic was then created by multiplying these test statistics together, which created a combined test statistic for all parameters. This allowed comparisons of overall difference between two whistle types. A permutation test was used to shuffle the acoustic parameter measurements of the copies with those of the original signature whistles within each pair of animals. This was carried out to test whether the combined acoustic parameter statistic was significantly different from a random distribution. Ten thousand permutations were performed to calculate the distribution of the test statistic under the null hypothesis (random distribution), and the observed test statistic was then compared with this random distribution. A two-tailed test was used with a Bonferroni-adjusted significance level of $p < 0.002$. In addition, all parameters were used in a non-metric multi-dimensional scaling analysis with a good STRESS fit of 0.04.

A permutation test was also used to test whether signal copying only occurred between affiliated pairs of animals. This involved shuffling the CoAs of the pairs of animals who produced vocal copies ($n = 11$) with those that did not ($n = 191$). Many of the individuals who copied were also in pairs with other animals where copying was not present. The sampling statistic of interest was the mean CoA for the pairs involved in signal copying. Ten thousand permutations were performed to calculate the distribution of the test statistic under the null hypothesis that the CoAs of copiers were randomly distributed. The observed test statistic was then compared with the random distribution.

Permutation tests were also performed on the timing of copies after the original signature whistle. The times of copies ($n = 108$) were shuffled with the times of the copier's own signature whistles given in response to the copied signature whistles ($n = 1651$). The random distribution was calculated from 10 000 permutations under the null hypothesis that there was no difference between the timing of copies of signature whistles after the occurrence of the template whistle and the timing of the copier's own signature whistle after the occurrence of the template whistle. The observed test statistic (mean time between original signature whistle and copy) was compared with the random distribution.

3. Results

(a) Who copies whom?

In total, 85 different capture–release events of wild dolphins were analysed, comprising 121 individuals in different group compositions. Of these individuals, 48 were sampled on more than one occasion (range: 2–7). Of the 85 capture–release events analysed, 11 consisted of single male–male pairs, 31 consisted of single mother–calf pairs and the remaining 43 consisted of groups of different compositions. These compositions included two or more adults of the same or both sexes, mother–calf pairs with other adults and groups of mother–calf pairs.

As in previous studies [14,40], each bottlenose dolphin almost exclusively used its own, individually distinctive signature whistle during capture–release events. Whistle rates were generally high at these events, with a mean of 5.3 whistles per minute per individual. In 10 of 85 different capture–release sets, however, individuals were found occasionally copying the signature whistle of another animal in the set (mean rate in sets with copying: 0.18 copies per minute per individual). This occurred in 10 of 179 pairs of animals recorded from 1988 through 2004, consisting of two of the 11 male–male pairings and eight of the 31 mother–calf pairs. In some instances, both members of a pair copied one another (figure 1 and table 1; electronic supplementary material, figure S1). The total number of individuals who produced vocal copies was therefore 12. The five human judges who viewed frequency contour plots to quantify similarity of the copies with both the originals and the copier's own signature whistles showed statistically significant agreement ($\kappa = 0.42$, $z = 29.9$, $p < 0.0001$) [41]. Similarity values for all copies are given in table 1.

The results of a permutation test clearly showed that signature whistle copying occurred between closely affiliated pairs of animals ($p = 0.0006$). The mean half-weight coefficient of association (CoA; which can range from 0 to 1) for the 10 pairs of animals that copied was 0.8, whereas the mean CoA for non-copiers was 0.4 (figure 2). Interestingly, there were also three instances of copying of whistles that were not signatures between two adult, wild females of low association (see the electronic supplementary material, figure S2). These animals also produced their own signature whistles but no signature whistle copies.

In recordings of four aquarium housed males (forming six possible pairs) at The Seas, one pair also engaged in signature whistle copying. These two individuals showed high levels of synchronous behaviour (23% of 285 min of observation time) in the pool. Synchrony is a sign of social bonding in male bottlenose dolphins [34]. One exchange of signature whistle copying between these males was 30 s in duration: both males emitted the signature whistle of one of them in an interactive sequence consisting of 13 and 11 renditions respectively (see the electronic supplementary material, figure S3). Copying in these individuals was not accompanied by aggressive behaviour (total observation time 16 h with 13 copies produced). The synchrony of the other male pairs was generally lower (7–13% of the observation time). One other pair, however, had a high level of synchrony (26%) but did not engage in whistle copying. Thus, copying does not necessarily occur in bonded males.

(b) How accurate are vocal copies?

Frequency parameter measurements of copies produced by 11 animals (one captive and 10 wild animals; two wild copiers were excluded owing to small sample sizes) revealed consistent differences between signature whistle copies and the original, copied signature whistle (table 2 and figure 3).

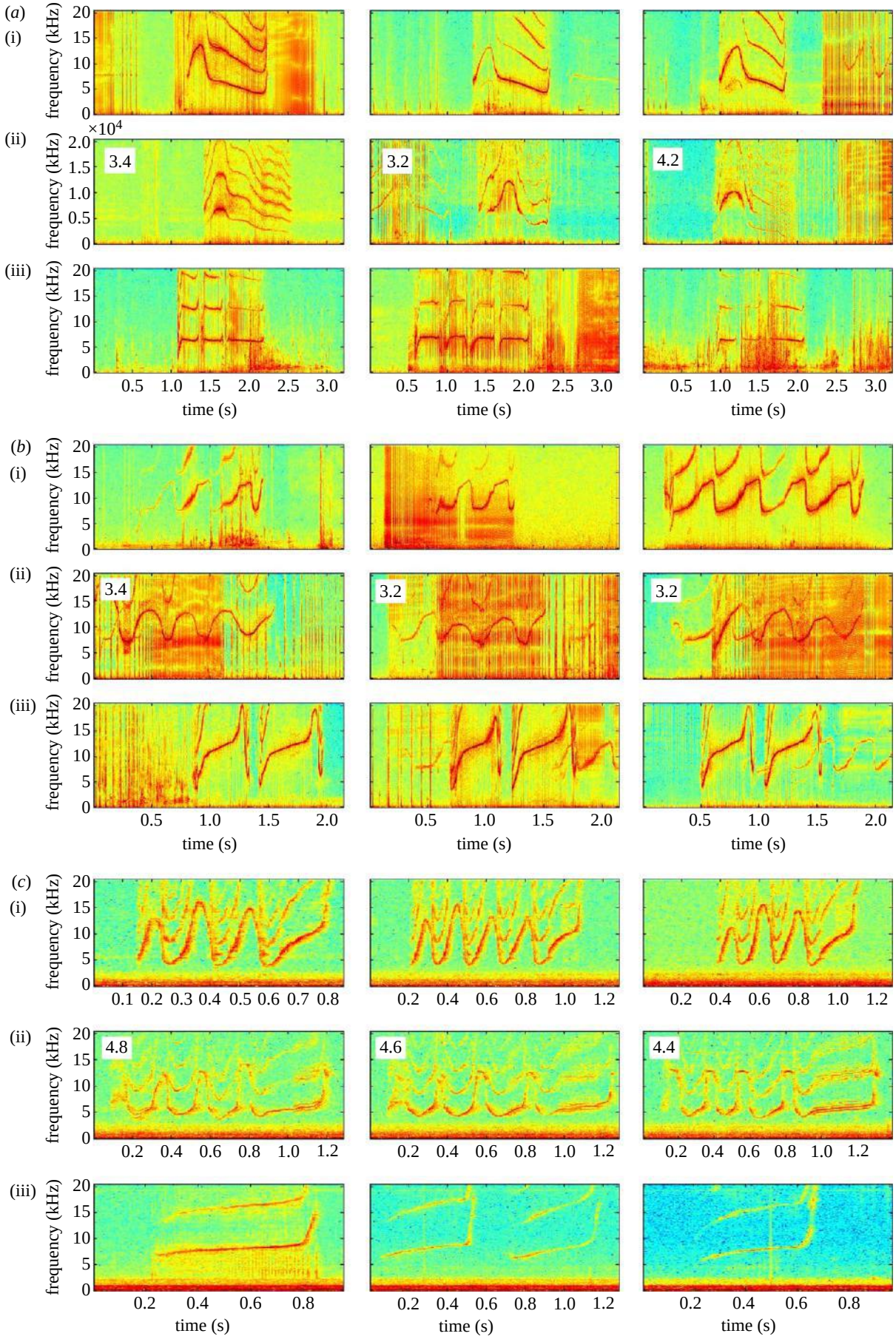


Figure 1. Spectrograms showing three examples each of the (i) signature whistle of the animal being copied, (ii) signature whistle copies and (iii) the signature whistle of the copier; sampling rate: 40 000 Hz, FFT length: 1024, Hanning window function. Numbers on the middle spectrograms give the mean human observer similarity scores between the original and the copy for each pair of whistles on a scale from 1 (not similar) to 5 (very similar). (*a*) Vocal interaction of a mother–calf pair. The mother, FB65, was the signature whistle owner (i) and the male calf, FB228, was the copier (iii). The male produced copies are in row *a* ii. (*b*) Vocal interaction of another mother–calf pair. The male calf, FB122, was the signature whistle owner (i) and the mother, FB90, was the copier (iii). The copies she produced are in row *b* ii. (*c*) Vocal interaction of a male–male pair from The Seas. The first adult male, Ranier, was the signature whistle owner (i) and the second adult male, Calvin, was the copier (iii). The copies he produced are in row *c* ii. (Online version in colour.)

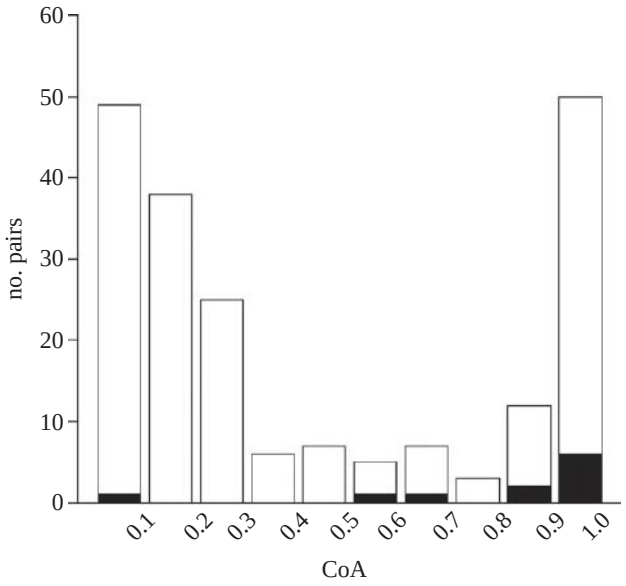


Figure 2. Coefficients of associations (CoA) of the pairs of animals that copied (black) and did not copy (white). The *y*-axis is the number of pairs of animals ($n = 202$), and the *x*-axis is the CoA in the year prior to the recording.

While the overall frequency modulation pattern of the copied whistle showed high similarity to the original (figure 1), copiers introduced consistent variation in single acoustic parameters such as the start or end frequency (see the electronic supplementary material, table S1). In these parameters, copies were often closer to other whistle contours than to the copied signature whistle (figure 3). Individuals varied in the parameters modified; on average 4.4 parameters (range: 1–6) differed significantly between the copy and the original signature whistle. Copies most frequently differed from the original (for 10 of 11 copiers) in mean frequency and maximum frequency (see the electronic supplementary material, table S1). Over half of the copiers also produced copies that differed significantly from the original signature whistle in end frequency (six of 11 copiers) and frequency range (seven of 11 copiers). The copies were equally likely to be higher or lower in frequency than the original. In addition to frequency parameters, one adult male, FB26, altered the number of loops in a multi-looped whistle in his copies of the signature whistle of his alliance partner, adult male FB48. Although FB48 varied his number of loops (range: 3–6), FB26 almost always produced a three-looped copy. The number of loops in FB26's copies and FB48's originals differed significantly (Mann–Whitney: $W = 152.5$, $N_1 = 38$, $N_2 = 35$, $p < 0.0001$). All of the signature whistle copies also differed significantly from those of the copiers' own signature whistles in some parameters (mean number of parameters different = 3.54; range: 1–7), whereas other parameters of a copy resembled those of the copier's own signature whistle (mean = 2; range: 0–5).

(c) Vocal matching

To further investigate whether copies were emitted in response to the identified model (referred to as the original signature whistle), we investigated whether they were temporally correlated and thus occurred in vocal matching interactions. Vocal matching can be described as a receiver responding to a signal by changing some features of its own vocal behaviour in order to imitate the preceding signal. Bottlenose dolphins had very high vocalization rates during these capture–release events, so it was difficult to judge whether whistles were produced in response to those of other animals. An investigation into the timing of signature whistle copies, however, revealed that the mean time between an original signature whistle and its copy was significantly less than the mean time between an original

Table 2. Test statistics for all acoustic parameter measurements combined for each copy and original signature whistle comparison. Shown are the sampling statistic of actual combined parameter measurements (observed), and the mean test statistic of combined parameter measurements under the null hypothesis based on 10 000 permutations (expected). Differences between acoustic parameter measurements of vocal copies and original signature whistles are significant at a level of $p < 0.002$.

	observed test statistic	expected test statistic	*p*
Ranier versus copy of Ranier	−7.52	−0.002	0.002
FB48 versus copy of FB48	0.19	−0.007	0.12
FB26 versus copy of FB26	559	0.025	<0.0001
FB20 versus copy of FB20	166	0.43	0.0031
FB122 versus copy of FB122	0.27	0.003	0.1
FB65 versus copy of FB65	1004	0.03	<0.0001
FB55 versus copy of FB55	24 000	0.016	<0.0001
FB35 versus copy of FB35	125	−0.01	<0.0001
FB95 versus copy of FB95	−1439	−0.01	<0.0001
FB155 versus copy of FB155	3 071 589	1.85a	<0.0001
FB177 versus copy of FB177	−2646	−0.0003	<0.0001

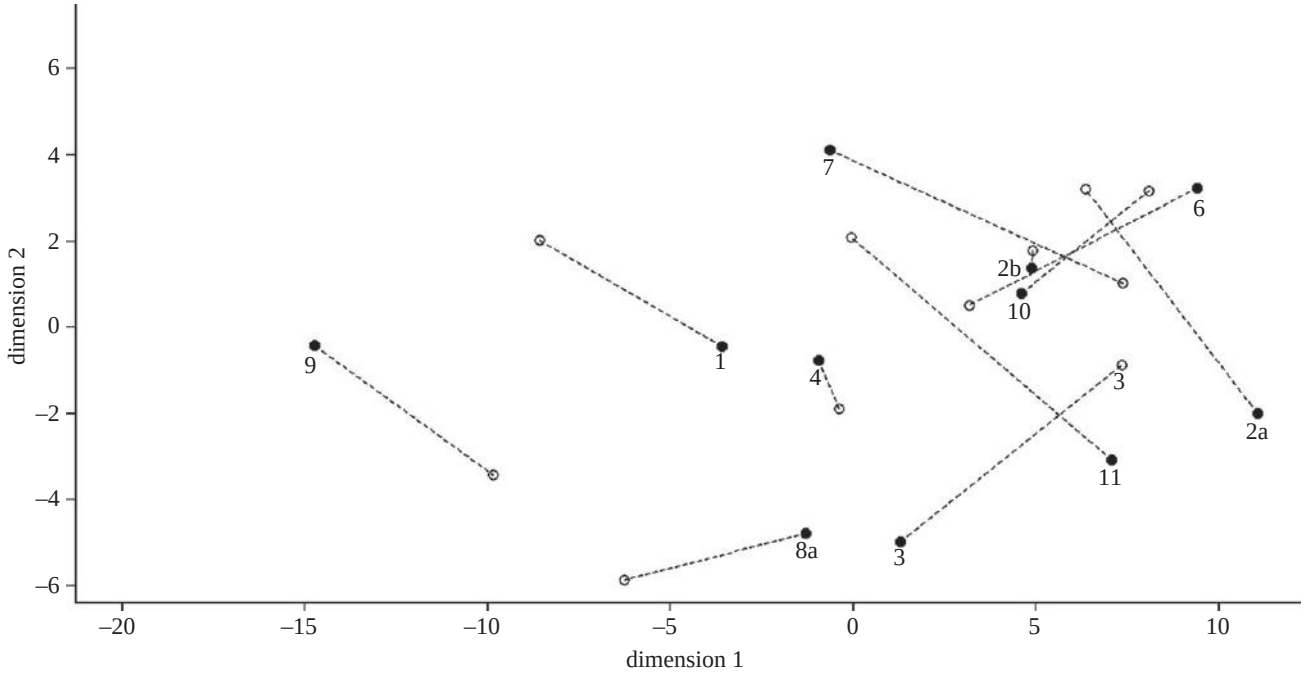


Figure 3. Multi-dimensional scaling plot based on all acoustic parameter measurements. The dotted lines join signature whistle copies (black circles) with the original signature whistles (open circles). Numbers correspond to pairs of animals as given in table 1 (see the electronic supplementary material, table S1). While originals and copies differed significantly in parameters such as start and end frequency as shown here, the overall frequency modulation pattern of the whistle was copied accurately as shown in figure 1.

signature whistle and a copier's own signature whistle (0.94 versus 2.55 s; permutation, $p < 0.0001$). In the long-term captive males, vocal rates were lower, and the matching pattern was clearer: almost all copying events occurred within 1 s after the emission of the original signature whistle by its owner, indicating copies were directed towards the owner of the original signature whistle.

4. Discussion

We conducted a large-scale analysis on the occurrence of vocal copying in wild bottlenose dolphins that were briefly caught, sampled and released. This dataset offered a unique opportunity to study the vocal interactions between individuals whose vocal repertoires [14,40] and association patterns had been well documented over decades in the wild [32,42]. In line with previous studies [23,25,26], we found whistle copying to be rare. This is consistent with the idea that signature whistles are used to indicate identity, because such a system would not be sustainable with high copying rates. While a copy could be recognizable as such if it occurred only in specific contexts, aquatic organisms usually have only limited contextual information with the acoustic signals they receive. Frequent copying of signature whistles would therefore render the identity information of the whistle unreliable. The rare copying of signature whistles may, however, be particularly suited to addressing close associates [23–25].

We found that copying occurred primarily in matching interactions between animals with high CoAs outside aggressive contexts, demonstrating that it is an affiliative signal. All pairs of animals that produced signature whistle copies were close associates, with only one pair having a low CoA for the year prior to recording. However, these two males were each other's closest male associate in the 4 year period prior to the recording. Many of the copiers were mother–calf pairs, with both mothers and calves likely to copy one another. While most female calves' signature whistles are distinct from their mothers', males sometimes do sound like their mothers [37]. The signature whistles of the male calves in this study, however, did not resemble those of their mothers (see figure 1 and electronic supplementary material, figure S1). Signature whistles of male alliance partners also tend to become more alike over time [43]. In this study, however, males continued producing their own, non-identical, signature whistles as well as copying the finer details of each other's preferred whistle type. Thus, age, sex and relatedness were not significant factors for the results presented here.

We found no evidence for a deceptive function of signature whistle copies. In animals that are capable of vocal learning, variations can be introduced into a copied signal, allowing encoding of additional information. Bottlenose dolphins produced accurate copies of the frequency modulation pattern of a whistle (figure 1), but introduced fine-scale differences in some acoustic parameters (table 2 and figure 3). As a result, signature whistle copies were clearly recognizable as such. Copies may even carry identity information of the copier, as some individuals maintained some frequency parameters of their own signature whistles in their copies (see the electronic supplementary material, table S1). While these variations may appear subtle, they were generally outside the acoustic variations used by the signature whistle owner itself. Dolphins are clearly capable of detecting such differences in the fundamental frequency as well as the upper harmonics [44,45]. Hence, these copies cannot function in a deceptive manner. Only animals that are familiar with the whistle of the owner would, however, be able to recognize copies. In encounters with unknown animals, a high rate of copying would still lead to confusion, arguing for low rates of copying overall. In fact, wild bottlenose dolphins do not copy signature whistles when encountering other groups of dolphins at sea [46].

Three lines of evidence suggest that active selection may have resulted in the variation found in signature whistle copies. First, bottlenose dolphins are capable of producing almost perfect copies of model sounds [22], suggesting that the variation is not due to limits on copying performance. Second, in experimental copying studies, bottlenose dolphins sometimes alter parameters of copies from one session to the next, and subsequently only produce copies with these novel parameter values [47]. Third, it has been shown that some dolphins introduce novel components such as sidebands to whistle copies, while they are perfectly capable of producing whistles without sidebands at these frequencies [24]. Thus, it is unlikely that variations introduced to copies are merely errors or reflect limitations in copying performance.

A role of vocal learning in the development of signals used in group cohesion and the maintenance of social bonds can be found in a number of social species [3–7,48,49]. The bottlenose dolphin signature whistle stands out in that it is invented by its main producer and can only be shared by animals who had experience with the inventor. Besides humans, bottlenose dolphins appear to be the other main example of affiliative copying with such individually specific learned signals, although some parrot species do use vocal learning to develop labels for social companions [50–52] and therefore deserve further investigation in this context. Further studies are also needed to elucidate whether copying such signals is different from sharing learned contact calls or adjusting acoustic parameters in communal displays as found in other birds and primates. Bottlenose dolphins can be trained to use vocal copies of novel, arbitrary sounds to refer to objects [22]. It is not yet known whether they use learned signals in this way in their own communication system. However, bottlenose dolphins have been found to copy signature whistles of animals that are not present in their group [27]. It is possible that signature whistle copying represents a rare case of referential communication with learned signals in a communication system other than human language [12]. Future studies should look closely at the exact context, flexibility and role of copying in a wider selection of species to assess its significance as a potential stepping stone towards referential communication.

We thank Ana Catarina Alves, Aasta Eik-Nes, Thomas Götz, Teresa Gridley, Mike Lonergan, Silvana Neves, Cornelia Oedekoven, Peter McGregor and Peter Tyack for help and advice during this study. Mike Beecher and three anonymous reviewers have provided helpful comments on the manuscript. We thank Walt Disney World Animals, Science and Environment and The Seas, Epcot, Walt Disney World Resorts, Lake Buena Vista, FL, USA for letting us work with their animals, especially Heidi Harley, Andy Stamper, Patrick Berry and Jane Davis. Fieldwork in Sarasota Bay would not have been possible without the efforts of a large team of biologists, veterinarians and dolphin handlers, including the staff of the Sarasota Dolphin Research Programme, Blair Irvine, Michael Scott, Jay Sweeney and a team of experienced volunteers. Ages of some of the Sarasota Bay dolphins were provided by Aleta Hohn. This work was supported by a BBSRC Doctoral Training Grant, Dolphin Quest, the Chicago Zoological Society, the National Oceanic and Atmospheric Administration (NOAA) Fisheries Service, Disney's Animals, Science and Environment, Dolphin Biology Research Institute, Mote Marine Laboratory, Harbor Branch Oceanographic Institute and a Royal Society University Research Fellowship and a Fellowship of the Wissenschaftskolleg zu Berlin to V.M.J. Work was conducted under NOAA Fisheries Service Scientific Research permit nos 417, 655, 945, 522–1569 and 522-1785 (to R.S.W.), IACUC approval through Mote Marine Laboratory, and approval of Disney's Animal Care and Welfare Committee. All data are archived and accessible at the Sea Mammal Research Unit, University of St Andrews.

References

1. Janik VM, Slater PJB. 2000 The different roles of social learning in vocal communication. *Anim. Behav.* **60**, 1–11. (doi:10.1006/anbe.2000.1410)
2. Catchpole CK, Slater PJB. 2008 *Bird song: biological themes and variations*, 2nd edn. Cambridge, UK: Cambridge University Press.
3. Boughman JW. 1998 Vocal learning by greater spear-nosed bats. *Proc. R. Soc. Lond. B* **265**, 227–233. (doi:10.1098/rspb.1998.0286)
4. Hile AG, Plummer TK, Striedter GF. 2000 Male vocal imitation produces call convergence during pair bonding in budgerigars. *Anim. Behav.* **59**, 1209–1218. (doi:10.1006/anbe.1999.1438)
5. Mammen DL, Nowicki S. 1981 Individual differences and within-flock convergence in chickadee calls. *Behav. Ecol. Sociobiol.* **9**, 179–186. (doi:10.1007/BF00302935)
6. Mundinger PC. 1970 Vocal imitation and individual recognition of finch calls. *Science* **168**, 480–482. (doi:10.1126/science.168.3930.480)
7. Brown ED, Farabaugh SM. 1997 What birds with complex social relationships can tell us about vocal learning: vocal sharing in avian groups. In *Social influences on vocal development* (eds CT Snowdon, M Hausberger), pp. 98–127. Cambridge, UK: Cambridge University Press.
8. Searcy WA, Beecher MD. 2009 Song as an aggressive signal in songbirds. *Anim. Behav.* **78**, 1281–1292. (doi:10.1016/j.anbehav.2009.08.011)
9. Beecher MD, Stoddard PK, Campbell SE, Horning CL. 1996 Repertoire matching between neighbouring song sparrows. *Anim. Behav.* **51**, 917–923. (doi:10.1006/anbe.1996.0095)
10. Beecher MD, Campbell SE. 2005 The role of unshared songs in singing interactions between neighbouring song sparrows. *Anim. Behav.* **70**, 1297–1304. (doi:10.1016/j.anbehav.2005.03.008)
11. Janik VM, Slater PJB. 2003 Traditions in mammalian and avian vocal communication. In *The biology of traditions: models and evidence* (eds DM Fragaszy, S Perry), pp. 213–235. Cambridge, UK: Cambridge University Press.
12. Janik VM. 2009 Acoustic communication in delphinids. *Adv. Study Behav.* **40**, 123–157. (doi:10.1016/S0065-3454(09)40004-4)
13. Caldwell MC, Caldwell DK. 1965 Individualized whistle contours in bottlenose dolphins (*Tursiops truncatus*). *Nature* **207**, 434–435. (doi:10.1038/207434a0)
14. Sayigh LS, Esch HC, Wells RS, Janik VM. 2007 Facts about signature whistles of bottlenose dolphins (*Tursiops truncatus*). *Anim. Behav.* **74**, 1631–1642. (doi:10.1016/j.anbehav.2007.02.018)
15. Fripp D, Owen C, Quintana-Rizzo E, Shapiro A, Buckstaff K, Jankowski K, Wells R, Tyack P. 2005 Bottlenose dolphin (*Tursiops truncatus*) calves appear to model their signature whistles on the signature whistles of community members. *Anim. Cogn.* **8**, 17–26. (doi:10.1007/s10071-004-0225-z)
16. Miksis JL, Tyack PL, Buck JR. 2002 Captive dolphins, *Tursiops truncatus*, develop signature whistles that match acoustic features of human-made model sounds. *J. Acoust. Soc. Am.* **112**, 728–739. (doi:10.1121/1.1496079)
17. Tyack PL, Sayigh LS. 1997 Vocal learning in cetaceans. In *Social influences on vocal development* (eds CT Snowdon, M Hausberger), pp. 208–233. Cambridge, UK: Cambridge University Press.
18. Janik VM, Sayigh LS, Wells RS. 2006 Signature whistle contour shape conveys identity information to bottlenose dolphins. *Proc. Natl Acad. Sci. USA* **103**, 8293–8297. (doi:10.1073/pnas.0509918103)
19. Boughman JW, Moss CF. 2003 Social sounds: vocal learning and development of mammal and bird calls. In *Acoustic communication* (eds AM Simmons, AN Popper, RR Fay), pp. 138–224. New York, NY: Springer.

20. Connor RC, Wells RS, Mann J, Read AJ. 2000 The bottlenose dolphin: social relationships in a fission-fusion society. In *Cetacean societies: field studies of dolphins and whales* (eds J Mann, RC Connor, PL Tyack, H Whitehead), pp. 91–126. Chicago, IL: University of Chicago Press.
21. Cook MLH, Sayigh LS, Blum JE, Wells RS. 2004 Signature-whistle production in undisturbed free-ranging bottlenose dolphins (*Tursiops truncatus*). *Proc. R. Soc. Lond. B* **271**, 1043–1049. (doi:10.1098/rspb.2003.2610)
22. Richards DG, Wolz JP, Herman LM. 1984 Vocal mimicry of computer-generated sounds and vocal labeling of objects by a bottlenosed dolphin, *Tursiops truncatus*. *J. Comp. Psychol.* **98**, 10–28. (doi:10.1037/0735-7036.98.1.10)
23. Janik VM, Slater PJB. 1998 Context-specific use suggests that bottlenose dolphin signature whistles are cohesion calls. *Anim. Behav.* **56**, 829–838. (doi:10.1006/anbe.1998.0881)
24. Tyack P. 1991 Use of a telemetry device to identify which dolphin produces a sound. In *Dolphin societies: discoveries and puzzles* (eds K Pryor, KS Norris), pp. 319–344. Berkeley, CA: University of California Press.
25. Janik VM. 2000 Whistle matching in wild bottlenose dolphins (*Tursiops truncatus*). *Science* **289**, 1355–1357. (doi:10.1126/science.289.5483.1355)
26. Nakahara F, Miyazaki N. 2011 Vocal exchanges of signature whistles in bottlenose dolphins (*Tursiops truncatus*). *J. Ethol.* **29**, 309–320. (doi:10.1007/s10164-010-0259-4)
27. Watwood SL, Owen ECG, Tyack PL, Wells RS. 2005 Signature whistle use by temporarily restrained and free-swimming bottlenose dolphins, *Tursiops truncatus*. *Anim. Behav.* **69**, 1373–1386. (doi:10.1016/j.anbehav.2004.08.019)
28. Janik VM. 1999 Origins and implications of vocal learning in bottlenose dolphins. In *Mammalian social learning: comparative and ecological perspectives* (eds HO Box, KR Gibson), pp. 308–326. Cambridge, UK: Cambridge University Press.
29. Watwood SL, Tyack PL, Wells RS. 2004 Whistle sharing in paired male bottlenose dolphins, *Tursiops truncatus*. *Behav. Ecol. Sociobiol.* **55**, 531–543. (doi:10.1007/s00265-003-0724-y)
30. Ginsberg JR, Young TP. 1992 Measuring association between individuals or groups in behavioural studies. *Anim. Behav.* **44**, 377–379. (doi:10.1016/0003-3472(92)90042-8)
31. Wells RS *et al*. 2004 Bottlenose dolphins as marine ecosystem sentinels: developing a health monitoring system. *EcoHealth* **1**, 246–254.
32. Wells RS. 2003 Dolphin social complexity: lessons from long-term study and life history. In *Animal social complexity: intelligence, culture, and individualized societies* (eds FBM de Waal, PL Tyack), pp. 32–56. Cambridge, MA: Harvard University Press.
33. Hohn AA, Scott MD, Wells RS, Sweeney JC, Irvine AB. 1989 Growth layers in teeth from known-age, free-ranging bottlenose dolphins. *Mar. Mammal Sci.* **5**, 315–342. (doi:10.1111/j.1748-7692.1989.tb00346.x)
34. Connor RC, Smolker R, Bejder L. 2006 Synchrony, social behaviour and alliance affiliation in Indian Ocean bottlenose dolphins, *Tursiops aduncus*. *Anim. Behav.* **72**, 1371–1378. (doi:10.1016/j.anbehav.2006.03.014)
35. Janik VM. 1999 Pitfalls in the categorization of behaviour: a comparison of dolphin whistle classification methods. *Anim. Behav.* **57**, 133–143. (doi:10.1006/anbe.1998.0923)
36. Garland EC, Goldizen AW, Rekdahl ML, Constantine R, Garrigue C, Hauser ND, Poole MM, Robbins J, Noad MJ. 2011 Dynamic horizontal cultural transmission of humpback whale song at the ocean basin scale. *Curr. Biol.* **21**, 687–691. (doi:10.1016/j.cub.2011.03.019)
37. Sayigh LS, Tyack PL, Wells RS, Scott MD, Irvine AB. 1995 Sex differences in signature whistle production of free-ranging bottlenose dolphins, *Tursiops truncatus*. *Behav. Ecol. Sociobiol.* **36**, 171–177. (doi:10.1007/BF00177793)
38. Deecke VB, Janik VM. 2006 Automated categorization of bioacoustic signals: avoiding perceptual pitfalls. *J. Acoust. Soc. Am.* **119**, 645–653. (doi:10.1121/1.2139067)
39. Esch HC, Sayigh LS, Wells RS. 2009 Quantifying parameters of bottlenose dolphin signature whistles. *Mar. Mammal Sci.* **24**, 976–986. (doi:10.1111/j.1748-7692.2009.00289.x)
40. Sayigh LS, Tyack PL, Wells RS, Scott MD. 1990 Signature whistles of free-ranging bottlenose dolphins, *Tursiops truncatus*: mother-offspring comparisons. *Behav. Ecol. Sociobiol.* **26**, 247–260. (doi:10.1007/BF00178318)
41. Siegel S, Castellan Jr NJ. 1988 *Nonparametric statistics for the behavioral sciences*, 2nd edn. New York, NY: McGraw-Hill.
42. Wells RS, Scott MD, Irvine AB. 1987 The social structure of free-ranging bottlenose dolphins. In *Current mammalogy* (ed. HH Genoways), pp. 247–305. New York, NY: Plenum Press.
43. Smolker R, Pepper JW. 1999 Whistle convergence among allied male bottlenose dolphins (Delphinidae, *Tursiops* sp.). *Ethology* **105**, 595–617. (doi:10.1046/j.1439-0310.1999.00441.x)
44. Harley HE. 2008 Whistle discrimination and categorization by the Atlantic bottlenose dolphin (*Tursiops truncatus*): a review of the signature whistle framework and a perceptual test. *Behav. Process.* **77**, 243–268. (doi:10.1016/j.beproc.2007.11.002)
45. Thompson RKR, Herman LM. 1975 Underwater frequency discrimination in the bottlenosed dolphin (1–140 kHz) and the human (1–8 kHz). *J. Acoust. Soc. Am.* **57**, 943–948. (doi:10.1121/1.380513)
46. Quick NJ, Janik VM. 2012 Bottlenose dolphins exchange signature whistles when meeting at sea. *Proc. R. Soc. B* **279**, 2539–2545. (doi:10.1098/rspb.2011.2537)
47. Sigurdson J. 1993 Frequency-modulated whistles as a medium for communication with the bottlenose dolphin (*Tursiops truncatus*). In *Language and communication: comparative perspectives* (eds HL Roitblat, LM Herman, PE Nachtigall), pp. 153–173. Hillsdale, NJ: Lawrence Erlbaum Associates.
48. Janik VM, Slater PJB. 1997 Vocal learning in mammals. *Adv. Study Behav.* **26**, 59–99. (doi:10.1016/S0065-3454(08)60377-0)
49. Mitani JC, Gros-Louis J. 1998 Chorusing and call convergence in chimpanzees: tests of three hypotheses. *Behaviour* **135**, 1041–1064. (doi:10.1163/156853998792913483)
50. Balsby TJS, Bradbury JW. 2009 Vocal matching by orange-fronted conures (*Aratinga canicularis*). *Behav. Process.* **82**, 133–139. (doi:10.1016/j.beproc.2009.05.005)
51. Balsby TJS, Scarl JC. 2008 Sex-specific responses to vocal convergence and divergence of contact calls in orange-fronted conures (*Aratinga canicularis*). *Proc. R. Soc. B* **275**, 2147–2154. (doi:10.1098/rspb.2008.0517)
52. Wanker R, Sugama Y, Prinage S. 2005 Vocal labelling of family members in spectacled parrotlets, *Forpus conspicillatus*. *Anim. Behav.* **70**, 111–118. (doi:10.1016/j.anbehav.2004.09.022)

Osvath and Karvonen,

Spontaneous Innovation for Future Deception in Male Chimpanzees,

PLoS ONE, vol. 7, no. 5 (May 9, 2012)

OPEN ACCESS Freely available online

PLoS one

Spontaneous Innovation for Future Deception in a Male Chimpanzee

Mathias Osvath[1]*, **Elin Karvonen**[2]

1 Department of Cognitive Science, Lund University, Lund, Sweden, **2** Lund University Primate Research Station Furuvik, Furuvik, Sweden

Abstract

Background: The ability to invent means to deceive others, where the deception lies in the perceptually or contextually detached future, appears to require the coordination of sophisticated cognitive skills toward a single goal. Meanwhile innovation for a current situation has been observed in a wide range of species. Planning, on the one hand, and the social cognition required for deception on the other, have been linked to one another, both from a co-evolutionary and a neuroanatomical perspective. Innovation and deception have also been suggested to be connected in their nature of relying on novelty.

Methodology/Principal Findings: We report on systematic observations suggesting innovation for future deception by a captive male chimpanzee (*Pan troglodytes*). As an extension of previously described behaviour – caching projectiles for later throwing at zoo visitors – the chimpanzee, again in advance, manufactured concealments from hay, as well as used naturally occurring concealments. All were placed near the visitors' observation area, allowing the chimpanzee to make throws before the crowd could back off. We observed what was likely the first instance of this innovation. Further observations showed that the creation of future-oriented concealments became the significantly preferred strategy. What is more, the chimpanzee appeared consistently to combine two deceptive strategies: hiding projectiles and inhibiting dominance display behaviour.

Conclusions/Significance: The findings suggest that chimpanzees can represent the future behaviours of others while those others are not present, as well as take actions in the current situation towards such potential future behaviours. Importantly, the behaviour of the chimpanzee produced a future event, rather than merely prepared for an event that had been reliably re-occurring in the past. These findings might indicate that the chimpanzee recombined episodic memories in perceptual simulations.

Citation: Osvath M, Karvonen E (2012) Spontaneous Innovation for Future Deception in a Male Chimpanzee. PLoS ONE 7(5): e36782. doi:10.1371/journal.pone.0036782

Editor: Georges Chapouthier, Université Pierre et Marie Curie, France

Received March 19, 2012; **Accepted** April 13, 2012; **Published** May 9, 2012

Funding: The work was supported by the Swedish Science Foundation, grant number 2009–2332. Link: http://www.vr.se/inenglish.4.12fff4451215cbd83e4800015152.html. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

* E-mail: mathias.osvath@lucs.lu.se

Introduction

We present systematic observations of a male chimpanzee who appears to have invented the use of concealments – both manufactured and naturally occurring ones – to be used for projectiles for future throwing at zoo visitors. That is, planning behaviours that produced a possibly desired outcome in the future, instead of relying on mere preparation for an upcoming situation that has been experienced before.

It has been suggested that human planning skills evolved in response to an increasingly complex social environment [1,2]. Undoubtedly, thinking about how one's current actions will affect others' future behaviours often steers one's choices. Our long-term social predictions are arguably important in both cooperative and competitive contexts. Planning for how to deceive prey or opponents before encountering them is an effective low-cost strategy.

The ability to solve new problems or to come up with novel solutions to old problems has often been associated with innovation. Innovations for deception are prime examples of social innovations [3].

Foresight

The theoretical roots of cognitive foresight research lie in the field of memory studies. In 1972, Tulving proposed a distinction between semantic and episodic memory [4], creating an essential framework for current animal research on foresight and memory. An easy way to distinguish them is to regard the first as *knowing*, the latter as *remembering*.

The semantic system represents general knowledge about the world. By contrast, the episodic system involves perceptual simulations from a first-person perspective. Knowing that Budapest is the capital of Hungary comes from the semantic system, but remembering the sight and smell of the fig tree in the back yard of the city's royal palace comes from the episodic system.

Tulving made a notable addition to his initial theory by making a type of consciousness – *autonoetic* (self-knowing) consciousness –

a necessary correlate of the episodic system [5]. At the same time Tulving was introducing autonoetic consciousness, another hypothesis was being put forward: the episodic system provides not only memories of past events but also mental constructs of possible future ones. This hypothesis has now been confirmed in several areas, from neurocognition to child development (for review see e.g. [6,7]). It appears as though episodic memory contributes previously experiences that are recombined into a novel construct, representing a possible future event.

To elucidate the distinctively subjective, first-person-perspective of autonoetic consciousness, Tulving used the phrase *mental time travel*: autonoetic consciousness makes it possible to travel in time cognitively and phenomenally, to revisit or pre-visit events. Metaphorically, autonoetic consciousness provides the "inner eye" by which one "sees" past or future, perceptually simulated, events.

Animal studies face a problem: it is problematic methodologically to rely on a terminology that presupposes phenomenal consciousness. This has caused considerable quandaries over how to parsimoniously interpret the results of certain studies on planning and memory in corvines and primates [8–15]. Is it ever possible to know whether an animal uses an episodic system given that one has no way to probe subjective experiences? Is it therefore also valid to deny the existence of an episodic system even if behavioural and neurobiological data suggest one, just because of the lack of phenomenal insight?

It is in fact not known whether the phenomenal experience that accompanies human foresight is functional or merely an epiphenomenal byproduct of other processes. It is however roughly known which brain areas are involved in episodic operations in humans, and that those operations seem to rely partly on reorganising stored perceptual inputs (for review see e.g. [7]). In principle, those operations are empirically testable in non-humans – indeed, they have partly been studied [16]. One way to avoid arguments dependent on phenomenological access is to distinguish sensations from perceptions: sensations describe the subjective experience of events, perceptions their physical interpretation [17]. An episodic system relying on perceptual simulation does not logically entail subjective experience. However, it does presuppose (re-)organization of perceptually detached information. This is a somewhat different way to avoid the problem of subjective experience than the one taken by Clayton and colleagues [18]: instead of returning to the initial definition of episodic memories – which did not include consciousness or simulation – we propose a more neurobiologically based, but also non-phenomenal, approach, where perceptual simulations are central.

An important empirical challenge is to show whether the future-oriented behaviour in question relies on something more than mere cognitive repetition of an entire previous experience. That is, whether the animal under study can prepare for novel situations that require mentally recombining perceptual elements into new configurations, as the human episodic system allows. Such a finding for a non-human species would strongly suggest the existence of an episodic system. Many investigations and much debate have concerned the so-called Bischof-Köhler hypothesis [19–23]. Suddendorf and Corballis [24] first offered the hypothesis, stating that "…animals other than humans cannot anticipate future needs or drive states and are therefore bound to a present that is defined by their current motivational state". It does seem that an episodic system facilitates such anticipation; however, passing or failing the Bischof-Köhler "test" is not necessary, and perhaps not even sufficient, for establishing or rejecting episodic foresight in non-human animals: a certain flexibility appears just as important. (For similar ideas, see [25])

Deception

Numerous reports of deceptive primate behaviours exist [26,27]. Some exist for corvines as well [28–31]. Byrne and Whiten [32] introduced the concept of *tactical deception*, which they later elaborated on [33]. Tactical deception is a type of behavioural deception, not a morphological one as for example mimicking the colour pattern of a venomous snake. Under normal circumstances, the behaviour in question is presented "honestly"; however, in this case it is used tactically, to mislead. Consider a raven that appears to make a cache in the presence of onlookers, even though it does not empty the contents of its beak.

Of course, in many instances tactical deception can occur without the deceiver having any representation of the false knowledge states of the deceived. Such representations require that one have a so-called *Theory of Mind* [34]: an understanding of that the other's psychological state lies behind the behaviour. That skill is sometimes called *mind reading*. Theory of mind or mind reading is not required where the "reader" has associatively learned

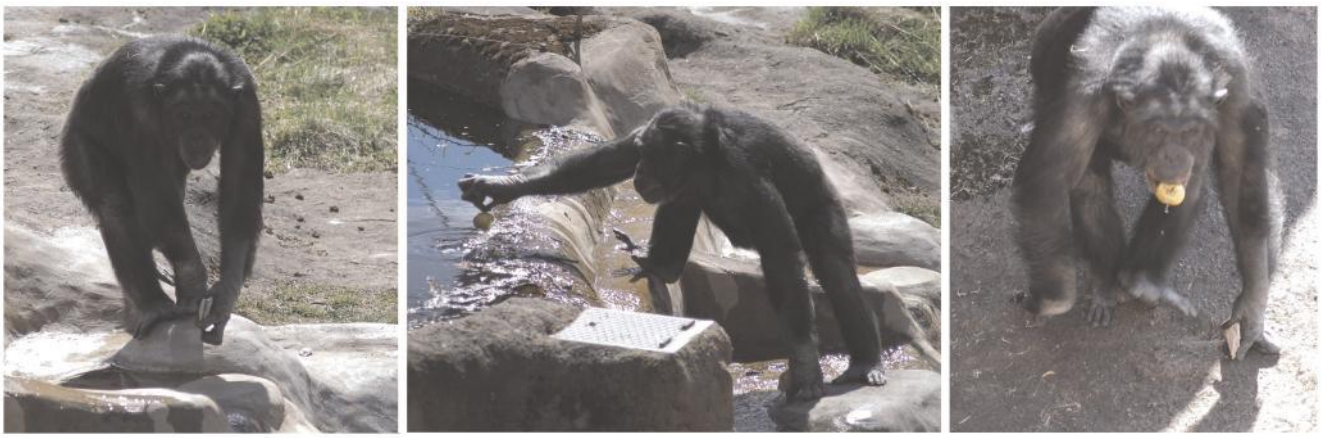


Figure 1. The deceptive approach. The series shows the chimpanzee when he slowly moved towards the group of visitors before releasing his projectiles. Note the two projectiles in his left hand. The picture on the left was taken 31 seconds before the throw; the central picture, where he picks up an apple from the water moat, was taken 15 seconds before; the right picture was taken 1 second before the throw. (The times are estimated from a video footage recorded at the same occasion). (Photo: Tomas Persson).
doi:10.1371/journal.pone.0036782.g001

relationships of others' behavioural responses to different circumstances – or even where one can reason what one *would* have done in the situation the other is in, without assuming anything about the other's state of mind. Such exceptions to mind reading could include one's generalized experience of others' direct line of gaze, with no conceptual understanding of them as "seeing". An example would be that when food is and has been outside the other's direct line of gaze, the other makes no attempt to take it [35]. This broader category of behaviour-predicting skills is often referred to as *behaviour reading*.

Although no single study has provided unequivocal evidence for mindreading in non-human animals, some argue that the combined weight of studies imply that at least chimpanzees and some corvines take into account the goals and perceptual perspectives of others – although maybe not their beliefs [36]. Those who reject this often argue that the studies are methodologically flawed and unable even in principle to infer mental state attribution: the results could be interpreted as reflecting no more than behaviour reading [35,37].

Innovation

Innovations in animals have been observed in a wide range of species [38–40]. Such innovation has received most attention from ecological approaches and from the perspective of its role in cultural transmission. However, it remains under-studied from a cognitive perspective, so that the underlying proximate mechanisms are neither well identified nor understood. The difficulty pinpointing the cognitive mechanisms underlying innovation is partly related to the difficulty of defining it. Innovation can be viewed either as the product (i.e., a novel behaviour pattern [39]) of or the process that results in novel behaviour [41]. Given these two perspectives, Reader and Laland [42] argue that innovations (the product) are learned behaviour patterns. It follows that innovation (the process) requires learning. This excludes from the definition mere chance behaviour or innate behavioural expressions. Reader and Laland recognize that general learning alone cannot explain innovation. They suggest a number of broad cognitive mechanisms – or behavioural processes – underlying innovation (facilitating the necessary learning): e.g., exploration, insight, creativity, and behavioural flexibility. Unfortunately, these labels are all more or less poorly understood. The cognition behind innovation remains largely uncharted.

What is interesting given the scope of the current study is the way that innovation and deception have been linked in the context of primates' social life [3,39]. The two skills do seem closely related: innovation can be said to occur when an existing signal or other behaviour is used in a novel way [39]; tactical deception occurs when a familiar and normally honest signal is used in a new and misleading way [33].

Previous report on the chimpanzee of this study

In 2009 one of us (MO) reported on the projectile related behaviour of the male chimpanzee, who is also the subject in this study [23]. In 1997 the chimpanzee started to gather stones from the water moat surrounding the outside compound and storing them hours before he threw them in dominance displays at the arriving zoo visitors. The behaviour was detected after some days of unusually high number of projectiles being thrown. When cleaning the island compound, the zookeepers found five stone caches placed at the shoreline facing the visitors' area. Following days a zookeeper placed herself in a blind to observe the chimpanzee behaviour during the morning hours. He was found to retrieve stones from the moat and place them in piles. In 1998, the chimpanzee started to manufacture projectiles by breaking off loose pieces from the compound's concrete surface, and then placing them in the caches. The behaviour was observed a high number of times during the decade covered by the report. The key findings were not only that the ape prepared for future throwing when the visitors was outside his field of perception, but also that there appeared to be a dissociation between his emotional states: calm during the gathering process, agitated during the throwing sessions. These behaviours indicate foresight based on the episodic system.

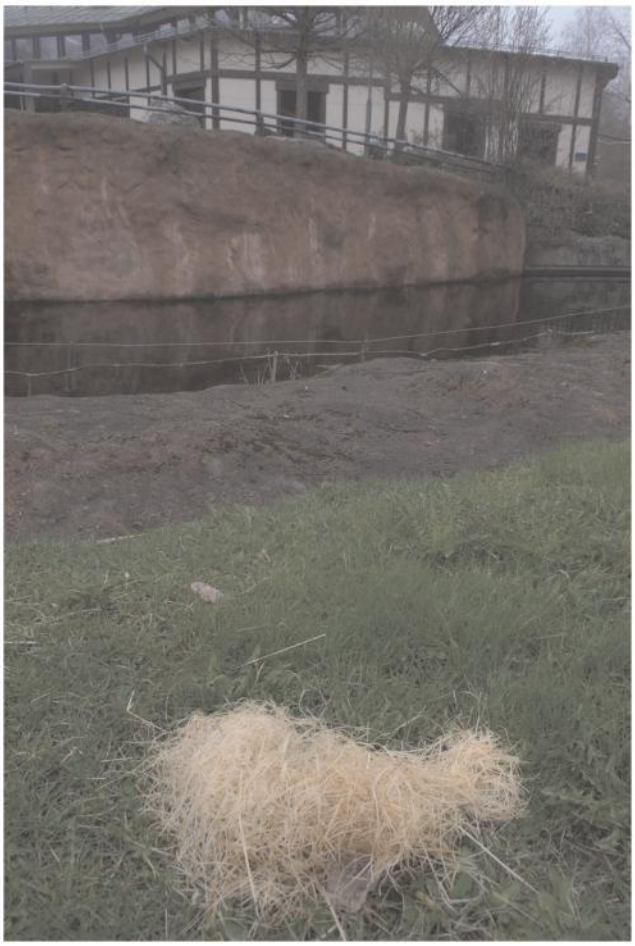


Figure 2. The first hay concealment made by the chimpanzee. Note the projectile in the lower part under the heap. The visible projectile above the heap was not present during the first throws. The picture was taken at the end of the day.
doi:10.1371/journal.pone.0036782.g002

Nonetheless, concerns have been raised over how the findings should be interpreted – because no detailed data is available on the chimpanzee's behaviour and circumstances at the moment when the first caches were made [43,44]. Such information would have been valuable for the understanding of the underlying factors behind the behaviour. That said, explanations based solely on associative learning mechanisms are difficult to motivate. Even if the behaviour did start out by chance, or if initially, the chimpanzee took the stones from the water and cached them along the shore for some purpose other than throwing them later – i.e., even if he only came later to realise that they could be thrown – one still needs an explanation for the complexity of the resulting behaviour, including the time spans and the manufacturing of projectiles. One also needs to take into account the experimental

results on foresighted behaviours in chimpanzees, which suggest that associative learning alone cannot explain such behaviour. It has been experimentally controlled for that chimpanzees do not merely rely on conditioning in tasks of future tool use [22,45]. And, on the other side of the coin, it has been suggested that chimpanzees are unable to learn to bring an item intended for future exchange for food from a human, despite extensive prior reinforcement training on the item [46]. These different findings suggest that associative learning cannot *on its own* explain foresighted behaviour in chimpanzees.

To gain more detailed information we systematically studied how the projectile related behaviour starts at the beginning of a zoo visitors' season. This does not address the problem with lack of data from the behaviour's initial inception; however, it complements the earlier work and offers potential for more fine-grained insights. During the 2010 season, previously unobserved behaviours were documented, comprising both deception and innovation in relation to the chimpanzee's projectile planning activities.

Methods

Ethics statement

The work was carried out under the Uppsala regional ethics committee approval No C199/9. The Swedish Agricultural board (No. 31-2599/09) has approved Furuvik Zoo as a cognitive research facility on chimpanzees.

Subject

The male chimpanzee, Santino, was born in 1978 at Munich Zoo in West Germany. At the age of five, he was transferred to Furuvik Zoo, Sweden, where he has lived ever since. Over the years, the composition of Santino's group varied, ranging between four and seven individuals of mixed sexes and ages. When Santino became the dominant male at the age of 16, there was only one other male in the group. This male died within the first year of Santino's dominance, leaving Santino as the sole male, as he has remained until the date of this study. When this study was conducted, apart from the male, the group consisted of five females, two adults, two sub-adults and one infant.

Methodological premises

Furuvik Zoo is only open to the general public for a short season: typically June to August. The general season is in some years preceded by a shorter pre-season – usually in May – during which the only visitors are guided educational groups. This study was carried out in 2010 and the pre-season and general season followed this pattern. The division of pre- and general season governed the methods used.

Conducting a study where human bystanders are involved presents challenges: in particular, the ethics of studying a potentially dangerous behaviour. Ethically, the observer, aware of Santino's projectile-throwing behaviour, could not fail to intervene upon observing preparations for impending throws.

During the pre-season, a zoo ethologist guided the groups, and each visitor was informed about the chimpanzee's throwing behaviour. Given this, it was ethically appropriate to observe the chimpanzee's preparation of the projectiles without interference. The pre-season afforded a well-controlled setting compared to the general season, when a large number of visitors is moving around. Among other things, it was possible to make accurate observations on whether visitors were out of the chimpanzee's view. Two principal, complementary methods were used: (i) direct behavioural observations and (ii) recovery of projectiles from the compound at the end of a day. During the general season, only the latter method could be used.

Figure 3. A visitor's view of the chimpanzee island. The X in the left of the picture marks the position of the first hay heap. The arrow on the left points at the protruding rock structure that was used as concealment. The other two arrows point at the two logs that also served as concealing obstacles.
doi:10.1371/journal.pone.0036782.g003

Behavioural observations

The primary goal was to address how the chimpanzee initiates his projectile-throwing behaviour at the start of the visitors' season. Therefore, behaviour sampling with continuous recording was used from the moment visitors were present during the pre-season. An observation session began the moment a visitors' group entered the vicinity of the chimpanzee compound. The session ended 30 minutes after the visitors left. Two central observational codes requires some elaboration:

Throws and *throw attempts* were recorded according to the position from which they were executed. It was not always possible to reliably observe the number of projectiles per throw, given the speed of the throws and the frequency with which multiple projectiles were thrown at once. Likewise it was not possible to reliably retrieve thrown projectiles, due to the dense vegetation around the compound.

A *hiding* was recorded if the observer clearly saw at least one projectile being placed behind or underneath something that would block the view. No hidings were recorded where the chimpanzee was simply active in areas that were later found to contain projectiles. This was a conservative coding, given the difficulty of seeing projectiles in the chimpanzees' closed hand. (Obviously, this code was not incorporated immediately, but only after the first observation of a hiding).

The observer needed to be out of the chimpanzee's view, during the periods when he did not have visitors. In consequence, the observer did not have an unobstructed view of the entire island: that would only have been possible with three simultaneous observers, who would have been visible to the chimpanzee. However, none of these restrictions proved problematic for recording of the essential initial behaviours.

Recovery of projectiles

At the end of each day, remaining projectiles and concealments were documented and removed. This was the only method deployed once the general season began, and the monitoring continued for 114 days. However, Santino only engaged in projectile-related behaviour on two days of the general season.

Although none of the projectiles concealed by the hay piles originated at the place of concealment, that possibility did arise for those projectiles placed behind one of two logs, where in each case potentially loose concrete was present. The position of the projectiles might in this case then be a result of chance, rather than from intentional concealment. Therefore two types of controls were used. First, two observers independently scanned all concrete areas of the island, both visually and by probing the concrete with the side of the fist (similar to Santino's own behaviour). Second, the two observers independently examined the colour and structure of the projectiles, to judge whether they matched the pattern of the adjacent concrete.

Results

Initial behaviours

The primary aim of this study was to document how the projectile behaviour was initiated in a zoo season, and it turned out that the first observations yielded findings indicating intentional deception and innovation. Therefore the initial behaviours were essential and are described in detail.

The first attempt to throw projectiles in 2010 involved the first visitors of the pre-season. The attempt was preceded by typical male chimpanzee dominance display behaviour: aggressive bipedal locomotion, pilo-erection and vocalization. The projectiles were chipped off the surface layer of the concrete in the outdoor compound island immediately before they were used. The guiding zoo ethologist backed the group away before the ape could release the projectile. He consequently desisted from throwing. This pattern repeated three times in a row. When the group returned, 190 minutes later, the male made no aggressive displays. Instead he walked from the centre of the compound island toward the group, with two concrete projectiles in his hand. To the guide, his appearance did not suggest intentions of throwing. The chimpanzee even stopped and picked up an apple floating in the water from which he took a bite as he continued approaching the visitors. Just within range, he made a sudden throw at the group (see Figure 1). This behaviour fits with a category of deception referred to as *creating a neutral image*. In this case, inhibiting an aggressive intent in order to secure a close approach [3].

Following day, the chimpanzee made two further attempts, preceded by aggressive display. In both cases, the group backed away, and he desisted. When the group left, the chimpanzee were observed being active in the area of one of the logs, thereafter he brought a melon-sized heap of hay from the inside enclosure (see Figure 2). This was placed on the island, close (8 metres) to the visitors' area. Subsequently he put an unknown number of projectiles under the hay that were carried in his hand. When the group returned to the compound 60 minutes later, the chimpanzee sat beside the hay. As the group approached, without preceding display, he threw a projectile stored under the heap. Shortly after, the chimpanzee positioned himself behind the log close to another part of the visitors' area (7 metres). When the group moved into this area, he threw two stored projectiles from behind the log. No display preceded the throws. When the group left the compound again the chimpanzee was observed to cache two more projectiles under the hay pile. These were thrown, with no preceding display, 20 minutes later when the group returned to the compound. In the evening the observers recovered twelve remaining projectiles from the island, all from concrete. Out of these, seven were found in hides: one under the hay pile facing the moat, five behind the log and one under the hay outside the door to the indoor enclosure.

Date (2010)	Visible	Log	Rock	Hay
May 12 *pre-season*	5	5		2
May 17 *pre-season*		10		
May 21 *pre-season*	1			9
May 22 *pre-season*		3	2	2
June 2 *general season*				1
June 6 *general season*	5			1

Figure 4. The distribution of recovered projectiles during the season. The numbers in the blocks represent the number of projectiles in each category.
doi:10.1371/journal.pone.0036782.g004

A hay pile on the island, or any concealing behaviour, had not been observed previously, either by the authors of the current study or by the zookeepers. Due to the close monitoring and documentation of the chimpanzee's projectile caches since its beginning, it is close to certain that the hay hide was a first case of innovation for deception. The chimpanzee did however sometimes use hay as resting material directly outside the door to the enclosure, in a sheltered area approximately 22 meters, and out of view, from centre of the actual island. On the time of the first hay concealment the chimpanzee had taken out no such resting material, only afterwards. Although later that day this resting material also served as concealment.

The whole zoo season

Through the course of the zoo season four hidings were directly observed as they took place (i.e. the actual projectiles were seen), always with an observer outside of the chimpanzees view. In two cases the hay was transported from the inside enclosure and placed over the projectiles, and at two occasions the projectiles were placed under the hay. In these instances the chimpanzee had first encountered a group, and cached immediately after they left. In one of these occasions he did not throw the concealed projectiles, as the group did not return. In turned out to be problematic to directly observe any unambiguous hidings behind the logs and the rock structure. Projectile oriented behaviour occurred in seven days in a period of 27 days. In all, 46 projectiles were recovered, of which 35 came from concealments. Three types of concealments were used: hay, logs (two different) and a protruding rock structure (see Figure 3 for the perspective from the visitors side on the different concealments).

Hay concealments were never placed behind the logs or the rock structure. The concealments from naturally occurring obstacles were visible to the chimpanzee but not to the visitors.

Out of the 35 concealed recovered projectiles, 15 were placed under hay heaps (under 6 heaps; 2 "empty" heaps were also recovered), 18 were placed behind logs and 2 were placed behind a protruding rock structure (see Figure 4 for the distribution of projectiles on different dates). The non-visible projectiles were significantly more than expected by chance (binomial test, $P<0{,}001$) (see Figure 5). Chance level was set at 50% which is much conservative for three reasons: (1) the number of places with naturally occurring obstacles on the island is far less than 50% of the island's area; (2) the number of potential behaviours the ape

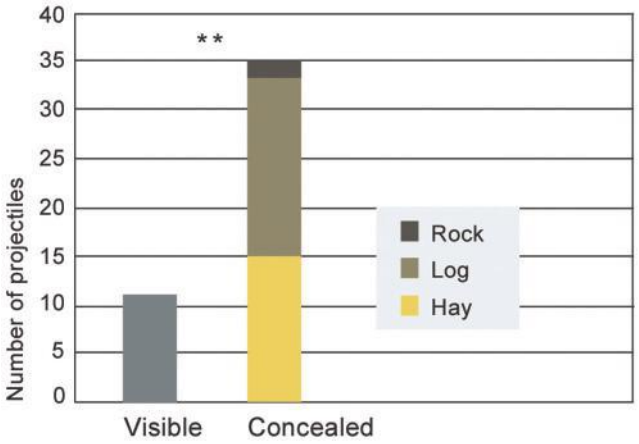


Figure 5. The number of visible and concealed projectiles. Throughout the season the amount of recovered concealed projectiles was significantly higher than that of the visible ones (binomial test, $P = 0{,}00054$).
doi:10.1371/journal.pone.0036782.g005

can perform instead of manufacturing a hide from hay is far more than one; (3) a majority of the observed throws were made from hides, i.e. the remaining projectiles recovered from hides were fewer than the number that was actually hidden as compared to the visible caches.

The controls of the areas with loose concrete, which did not yield concealments or visible caches, revealed eight such areas in both of the independently performed controls. The distribution of the 18 projectiles behind two (the logs) out of ten possible areas with loose concrete significantly deviates from chance distribution (binomial test, $P<0{,}001$). The controls of colour and structure showed that at least four projectiles almost certainly did not originate from the vicinity of the concealments (only applicable for the logs; the other concealment areas did not have loose concrete). This is a highly conservative measure as the concrete is quite similar throughout the compound, and it should be understood as only a complementary control and not as the true numbers of transported projectiles.

They way the chimpanzee coupled display behaviour with throws and throwing attempts further hints at the intent with hiding the projectiles. Out of the observed throws and throwing attempts 8 were done with projectiles from hides and 8 with visible projectiles. The chimpanzee did not display a single time before throwing from a concealment, while he displayed 7 out of 8 times when the projectiles were visible; the difference between the conditions is significant (Fisher's exact test, $P = 0{,}00139$). The only time the chimpanzee did not display in the visible condition was in the event described in the above section. These figures should be treated with some caution, as they also include throwing *attempts* in the visible condition.

Discussion

The likely explanation for the concealments is that they were intended for deception. Several factors converge on such an assumption: no hay concealments were ever placed behind concealing obstacles; most projectiles behind or under the concealments had been transported there; when concealed, the projectiles could be seen only from the viewpoint of the chimpanzee or not by anyone; observed throws from the concealments were never preceded by any display (suggesting the combining of two deceptive strategies); concealing behaviours were never observed when anyone was in the chimpanzee's view.

It is less clear what prompted these deceptive behaviours and the use of hay as the concealing material. One could speculate about the chimpanzee's initial throwing experience of the season, watching the people backing away. Perhaps this led him to take deceptive action, so he could release the projectiles at closer range. The first time the chimpanzee – atypically for him – was observed slowly approaching the visitors, displaying no obvious aggressive intent, before suddenly throwing projectiles at them fits well with a documented deceptive category in primates. There is no way to tell whether this was the first time he ever used this strategy. The strategy might occasionally have been used in the past. What is close to certain, however, is that there had never before been a hay concealment on the chimpanzee island, nor had projectiles ever previously been found behind naturally occurring obstacles, only as completely visible and close to the shore line.

The day the first concealments were made began as the day before, with the onlookers backing away. Those first concealments included both manufactured and naturally occurring ones. The chimpanzee was quite familiar with hay, giving him plenty of opportunities to learn its effect of blocking the view of objects; he was similarly familiar with logs. He also occasionally transported hay to a resting place just outside the door to the indoor enclosure, giving him experience of bringing hay from the inside. That said, any answer why and how he came up with the new strategy on his second day of visitors would be speculative. Interestingly, he did not start out on that second day using the deceptive strategy; his initial encounter with the visitors played out as before, and only on the second encounter did the aggression inhibition and use of concealment occur. One obvious gain from the new strategy is that the chimpanzee could use more projectiles in short succession. By combining his old strategy of gathering projectiles in advance with his new strategy of concealment and behavioural inhibition, he could extend his ability to throw stones at visitors from close range. Although, there is no way to tell whether this really was his motivation.

Both the manufacture and use of the concealments were likely premeditated. The behaviour never occurred when anyone was within the chimpanzee's view, but only after a group had been present and left: i.e., prior to their possible return. That is, it appears to have been prompted by the prior presence of visitors on those days when it occurred: the chimpanzee prepared no concealments on days when he had not previously seen visitors. This departs from the chimpanzee's previously reported behaviour, by which he typically collected projectiles in the morning before the zoo opened, on days when the zoo had visitors. That said, the earlier observations were based mainly on the general season, not on the (rare) pre-season. During the general season, visitors come every day, while during the pre-season, they arrive sporadically, several days apart (see Figure 4 for the dates of the pre-season in 2010). Taken together, the results suggest that the chimpanzee crafted a desired outcome in a perceptually detached future by acting innovatively in his current situation. Such activity *produces* a specific future event, in contrast to activity that merely prepares for a future situation as repetition of a previously experienced event. That is why the most critical finding of this study is the observation of the first instance of the concealment behaviour. This is indication of the existence of that type of perceptual simulation used by humans in certain planning tasks: a recombining of components of previously experienced events. The data further show that chimpanzees are able to plan for social situations – at least for deception – and that social planning in general is not out of reach for chimpanzees, as was suggested in

a study where chimpanzees were unable to plan for future exchange with humans [46].

Do the results imply that the chimpanzee possesses a theory of mind? *Sensu stricto*, it appears as the results do not: however elaborate, the concealments could be based on the chimpanzee's understanding of line of gaze. What the behaviour does appear to show is that the chimpanzee is able to predict the behavioural responses of others not present at the time of the prediction. Mind reading is characterized as reasoning about what is not overt in behaviour: i.e., mental states. What the chimpanzee appears to be reading is likewise not overt in any behaviour (the visitors are not present). That said, the performance is possible without representing anyone else's mental states. What does seem to be a possibility is detached perceptual constructs of others' behaviours.

One means by which this might be achieved is again the episodic system, allowing the agent to simulate others in the context of a potential future situation. It has been suggested that in humans, foresight, memory, and the taking of others' viewpoints all seem to be supported by a common brain network [47]. The relevant brain structures appear to be largely shared with chimpanzees [33]. In the context of theory of mind and planning, it has been suggested that the meta-representational ability required for representing others' deviating psychological states is a prerequisite for representing *one's own* future deviating mental states and hence planning for them. The alleged lack of such an ability in non-human animals is one reason their planning is often taken to be highly restricted [1]. However, such an assumption is not necessary. When it comes to planning for your own deviating mental states it has been suggested that the perceptual construct of a potential situation plays a trick on the phylogenetically older parts of the brain: the structures governing motivation treat the construct more or less as true perceptions [48]. So, the potential future mental state, or motivation, is brought to the present and might act as a break on the motivations directed towards the current situation. When planning for potential future behaviours of others, we suggest that this could in principle also be solved by detached perceptual construct of behaviours priorly experienced under different circumstances. Then there is no need for theory-like reasoning about other's mental states, the behaviour could be "read" from the perceptual simulations (it is not necessary to represent other's mental states even for creating the constructs; a learned behavioural catalogue would suffice). What underlies the perceptual simulations of potential futures, what makes them to form, is a highly interesting question beyond the scope of speculations of this study.

The present report should be followed up by experimental investigations whether chimpanzees – and other great apes – are in general capable of planning for future deception; and whether they have the ability to form representations of future behaviours of others who are not present, given different situations. Such experiments would provide an interesting avenue for advancing the study of social cognition.

As an endnote: when observations were continued in the 2011 season, the chimpanzee did not cache or throw a single projectile. He had suffered a hip injury at the beginning of the season and was both generally slowed down and reluctant to leave his indoor enclosure. By the middle of the season, at which point he had healed, he showed no inclination to throw stones. This is consistent with the pattern in the present and previous study, in which his projectile-related behaviour was found to stop sometime before the middle of the season.

Acknowledgments

We are grateful to the visitors of the pre-season allowing us to observe whole sequences of a potentially dangerous behaviour. We are especially grateful to Furuvik Zoo for granting Lund University access to the apes without in any way restricting or influencing the research. We thank Ing-Marie Persson, Ellinor Sandström, Ida Engström, Helena Osvath and Tomas Persson.

Author Contributions

Conceived and designed the experiments: MO EK. Performed the experiments: EK. Analyzed the data: MO. Wrote the paper: MO.

References

1. Suddendorf T, Corballis MC (2007) The evolution of foresight: What is mental time travel and is it unique to humans? Behav Brain Sci 30: 299–351.
2. Barrett L, Henzi P, Dunbar R (2003) Primate cognition: from 'what now?' to 'what if?'. Trends Cogn Sci 7: 494–497.
3. Byrne RW (2003) Novelty in deceit. In: Reader SM, Laland KN, eds. Animal Innovation. Oxford University Press: New York. pp 237–259.
4. Tulving E (1972) Episodic and semantic memory. In: Tulving E, Donaldson W (eds) Organization of memory Academic Press, New York.
5. Tulving E (1985) Memory and consciousness. Canadian Psych 26: 1–12.
6. Suddendorf T (2010) Episodic memory verus episodic foresight: similarities and differences. Cogn Sci 1: 99–107.
7. Szpunar KK (2010) Episodic future thought: An emerging concept. Perspect Psychol Sci 5: 142–162.
8. Clayton NS, Bussey TJ, Emery NJ, Dickinson A (2003) Prometheus to Proust: the case for behavioural criteria for 'mental time travel'. Trends Cogn Sci 7: 436–437.
9. Suddendorf T, Busby J (2003) Like it or not? The mental time travel debate: reply to Clayton et al. Trends Cogn Sci 7: 437–438.
10. Suddendorf T, Corballis MC (2008) New evidence for animal foresight? Anim Behav 75: e1–e3.
11. Clayton NS, Correia SPC, Raby CR, Alexis DM, Emery NJ, Dickinson A (2008) Response to Suddendorf & Corballis (2008): in defence of animal foresight. Anim Behav 76: e9–e11.
12. Suddendorf T, Corballis MC, Collier-Baker E (2009) How great is great ape foresight? Anim Cogn 12: 751–4.
13. Osvath M (2010) Great ape foresight is looking great. Anim. Cogn 13: 777–781.
14. Osvath M, Raby CR, Clayton NS (2010) What should be compared in comparative mental time travel? Trends Cogn Sci 14: 51–52.
15. Roberts WA, Feeney MC (2010) Temporal sequencing is essential to future planning: response to Osvath, Raby and Clayton. Trends Cogn Sci 14: 52–53.
16. Rilling JK, Barks SK, Parr LA, Preuss TM, Faber TL, et al. (2007) A comparison of resting-state brain activity in humans and chimpanzees. Proc Natl Acad Sci USA 104: 17146–17151.
17. Humphrey N (1992) A history of the mind. Springer Verlag Copernicus, New York.
18. Clayton NS, Bussey TJ, Dickinson A (2003) Can animals recall the past and plan for the future? Nat Rev Neurosci 4: 685–691.
19. Naqshbandi M, Roberts WA (2006) Anticipation of future events in squirrel monkeys (Saimiri sciureus) and rats (Rattus norvegicus): tests of the Bischof-Kohler hypothesis. J Comp Psychol 120: 345–357.
20. Raby CR, Alexis DM, Clayton NS (2007) Planning for the future by western scrub-jays. Nature 445: 919–921.
21. Correia SPC, Dickinson A, Clayton NS (2007) Western scrub-jays anticipate future needs independently of their current motivational state. Curr Biol 17: 856–861.
22. Osvath M, Osvath H (2008) Chimpanzee (*Pan troglodytes*) and orangutan (*Pongo abelii*) forethought: self-control and pre-experience in the face of future tool use. Anim Cogn 11: 661–674.
23. Osvath M (2009) Spontaneous planning for future stone throwing by a male chimpanzee. Curr Biol 19: 190–192.
24. Suddendorf T, Corballis MC (1997) Mental time travel and the evolution of the human mind. Genet Soc Gen Psychol Monogr 123: 133–167.
25. Raby CR, Clayton NS (2009) Prospective cognition in animals. Behav Proc 80: 314–324.
26. Byrne RW, Whiten A (1990) Tactical deception in primates: the 1990 database. Primate Rep 27: 1–101.
27. de Waal FBM (1992) Intentional deception in primates. Evol Anthropol 1: 86–92.
28. Bugnyar T, Kotrschal K (2002) Observational learning and the raiding of food caches in ravens, *Covus Corax*: is it 'tactical' deception? Anim Behav 64: 185–195.
29. Bugnyar T, Kotrschal K (2004) Leading a conspecific away from food in ravens (*Corvus corax*)? Anim Cogn 7: 69–76.

30. Bugnyar T, Heinrich B (2006) Pilfering ravens, *Corvus corax*, adjust their behaviour to social context and identity of competitors. Anim Cogn 9: 369–376.
31. Dally JM, Emery NJ, Clayton NS (2005) Cache protection strategies by western scrub-jays, *Aphelocoma californica*: implications for social cognition. Anim Behav 70: 1251–1263.
32. Byrne RW, Whiten A (1985) Tactical deception of familiar individuals in baboons (*Papio ursinus*). Anim Behav 33: 669–673.
33. Whiten A, Byrne RW (1988) Tactical deception in primates. Behav Brain Sci 11: 233–244.
34. Premack D, Woodruff G (1978) Does the chimpanzee have a theory of mind? Behav Brain Sci 4: 515–526.
35. Lurz RW (2011) Mindreading animals. MIT Press, Cambridge.
36. Call J, Tomasello M (2008) Does the chimpanzee have a theory of mind? 30 years later. Trends Cogn Sci 12: 187–192.
37. Penn DC, Povinelli DJ (2007) On the lack of evidence that non-human animals possess anything remotely resembling a "theory of mind". Phil Trans R Soc B 362: 731–744.
38. Kawai M (1965) Newly-aquired pre-cultural behavior of the natural troop of Japanese monkeys on Koshima Islet. Primates 6: 1–30.
39. Kummer H, Goodall J (1985) Conditions of innovative behaviour in primates. Phil Trans R Soc B 308: 203–214.
40. Lefebvre L, Whittle P, Lascaris E, Finkelstein A (1997) Feeding innovations and forebrain size in birds. Anim Behav 53: 549–560.
41. Lee P (1991) Adaptations to environmental change: An evolutionary perspective. In: Box HO. Primate responses to environmental change. Chapman & Hall: London. pp 39–56.
42. Reader SM, Laland KN (2003) Animal innovation: an introduction. In: Reader SM, Laland KN. Animal innovation. Oxford University Press: New York. pp 3–35.
43. Shettleworth SJ (2010) Clever animals and killjoy explanations in comparative psychology. Trends Cog Sci 14: 477–481.
44. Suddendorf T, Corballis MC (2010) Behavioural evidence for mental time travel in nonhuman animals. Behav Brain Res 215: 292–298.
45. Mulcahy NJ, Call J (2006) Apes save tools for future use. Science 312: 1038–1040.
46. Dufour V, Sterck EHM (2008) Chimpanzees fail to plan in an exchange task but succeed in a tool-using procedure. Behav Proc 79: 19–27.
47. Buckner RL, Carroll DC (2007) Self-projection and the brain. Trends Cogn Sci 11: 49–57.
48. Boyer P (2008) Evolutionary economics of mental time travel? Trends Cog Sci 12: 219–224.

Bartal et al.,

Empathy and Pro-Social Behavior in Rats,

SCIENCE, vol. 335, pp. 1427-1430 (Dec. 9, 2011)

Empathy and Pro-Social Behavior in Rats

Inbal Ben-Ami Bartal *et al.*

Science **334**, 1427 (2011);
DOI: 10.1126/science.1210789

Science (print ISSN 0036-8075; online ISSN 1095-9203) is published weekly, except the last week in December, by the American Association for the Advancement of Science, 1200 New York Avenue NW, Washington, DC 20005.

As with any method, there are limitations to the use of nighttime satellite imagery; the exact association between brightness and population density varies between locations and is affected by environmental (*15*) and economic factors (*25*–*27*). Additionally, images must be selected carefully to avoid contamination from solar and lunar illumination and cloud cover (SOM part 1).

Measuring the drivers of seasonal variability in transmission rates, particularly in areas with sparse disease surveillance and strong epidemic nonlinearities (*2*), is critical for improving the design of epidemiological control measures. It is now possible to improve outbreak response strategies based on fluctuations in population density and disease transmission, as we have shown for a recent measles outbreak in Niamey. This would be particularly useful in areas with repetitive seasonal fluctuations in density where targeted campaigns could maximize the number of individuals present during vaccinations. It is also possible that this method could be adapted for near–real-time analyses, as images are uploaded from the satellite within ~48 hours (although the usability of individual images is sensitive to environmental conditions).

The advantages of understanding changes in population density are broadly applicable. This information can aid in estimating population changes caused by large-scale human movements—i.e., displacement due to conflict (*17*) or recurring movements such as the Hajj. Measurements of fluctuations in population density provide important information to guide decisions on disease control strategies, international aid and humanitarian responses, and assessments of economic development.

References and Notes

1. M. J. Ferrari *et al.*, *Proc. Biol. Sci.* **277**, 2775 (2010).
2. M. J. Ferrari *et al.*, *Nature* **451**, 679 (2008).
3. W. P. London, J. A. Yorke, *Am. J. Epidemiol.* **98**, 468 (1973).
4. D. P. Word, J. K. Young, D. A. T. Cummings, D. C. Laird, paper presented at the 20th European Symposium on Computer Aided Process Engineering— ESCAPE20, Ischia, Naples, Italy, 6 to 9 June 2010.
5. S. S. Hutchins *et al.*, *Am. J. Epidemiol.* **132**, 157 (1990).
6. S. Altizer *et al.*, *Ecol. Lett.* **9**, 467 (2006).
7. N. C. Grassly, C. Fraser, *Proc. Biol. Sci.* **273**, 2541 (2006).
8. R. M. Anderson, R. M. May, *Infectious Diseases of Humans: Dynamics and Control* (Oxford Univ. Press, New York, 1991).
9. M. Keeling, P. Rohani, *Modeling Infectious Diseases in Human and Animals* (Princeton Univ. Press, Princeton, NJ, 2008).
10. R. M. Prothero, *Glob. Change Hum. Health* **3**, 20 (2002).
11. J. L. Aron, I. B. Schwartz, *J. Theor. Biol.* **110**, 665 (1984).
12. M. J. Keeling, B. T. Grenfell, *Science* **275**, 65 (1997).
13. R. H. Faulkingham, P. F. Thorbahn, *Popul. Stud.* **29**, 463 (1975).
14. D. Rain, *Eaters of the Dry Season: Circular Labor Migration in the West African Sahel* (Westview Press, Boulder, CO, 1999).
15. C. D. Elvidge, K. E. Baugh, E. A. Kihn, H. W. Kroehl, E. R. Davis, *Photogramm. Eng. Remote Sensing* **63**, 734 (1997).
16. P. Sutton, D. Roberts, C. D. Elvidge, K. E. Baugh, *Int. J. Remote Sens.* **22**, 3061 (2001).
17. J. Agnew, T. W. Gillespie, J. Gonzalez, B. Min, *Environ. Plan. A* **40**, 2285 (2008).
18. R. F. Grais *et al.*, *Trans. R. Soc. Trop. Med. Hyg.* **100**, 867 (2006).
19. M. Begon *et al.*, *Epidemiol. Infect.* **129**, 147 (2002).
20. K. R. Yaméogo *et al.*, *Int. J. Epidemiol.* **34**, 556 (2005).
21. M. C. C. Camargo, J. C. de Moraes, V. A. U. F. Souza, M. R. Matos, C. S. Pannuti, *Rev. Panam. Salud Publica* **7**, 359 (2000).
22. D. Balk *et al.*, *Adv. Parasitol.* **62**, 119 (2006).
23. J. Dobson, E. Bright, P. R. Coleman, R. Durfee, B. Worley, *Photogramm. Eng. Remote Sensing* **66**, 849 (2000).
24. M. C. González, C. A. Hidalgo, A. L. Barabási, *Nature* **453**, 779 (2008).
25. A. Noor, V. Alegana, P. Gething, A. Tatem, R. Snow, *Popul. Health Metr.* **6**, 5 (2008).
26. S. Ebener, C. Murray, A. Tandon, C. C. Elvidge, *Int. J. Health Geogr.* **4**, 5 (2005).
27. C. D. Elvidge *et al.*, *Comput. Geosci.* **35**, 1652 (2009).

Acknowledgments: This study was supported by the Bill and Melinda Gates Foundation. A.J.T. is supported by a grant from the Bill and Melinda Gates Foundation (49446). A.J.T., M.J.F., and B.T.G. are also supported by the Research and Policy for Infectious Disease Dynamics (RAPIDD) program of the Science and Technology Directorate, Department of Homeland Security and the Fogarty International Center, NIH. All DMSP OLS imagery is available from the Space Physics Interactive Data Resource (http://spidr.ngdc.noaa.gov/spidr/).

Supporting Online Material

www.sciencemag.org/cgi/content/full/334/6061/1424/DC1
Materials and Methods
SOM Text
Figs. S1 to S3
Tables S1 to S4
References (*28*–*40*)

1 July 2011; accepted 21 October 2011
10.1126/science.1210554

Empathy and Pro-Social Behavior in Rats

Inbal Ben-Ami Bartal,[1] Jean Decety,[1,2,4] Peggy Mason[3,4]

Whereas human pro-social behavior is often driven by empathic concern for another, it is unclear whether nonprimate mammals experience a similar motivational state. To test for empathically motivated pro-social behavior in rodents, we placed a free rat in an arena with a cagemate trapped in a restrainer. After several sessions, the free rat learned to intentionally and quickly open the restrainer and free the cagemate. Rats did not open empty or object-containing restrainers. They freed cagemates even when social contact was prevented. When liberating a cagemate was pitted against chocolate contained within a second restrainer, rats opened both restrainers and typically shared the chocolate. Thus, rats behave pro-socially in response to a conspecific's distress, providing strong evidence for biological roots of empathically motivated helping behavior.

Pro-social behavior refers to actions that are intended to benefit another. One common motivator of pro-social behavior in humans is empathic concern: an other-oriented emotional response elicited by and congruent with the perceived welfare of an individual in distress (*1*, *2*). Sharing another's distress via emotional contagion can result in overwhelming fear and immobility unless one's own distress is down-regulated, thus allowing empathically driven pro-social behavior (*3*, *4*). Building on observations of emotional contagion in rodents (*5*–*10*), we sought to determine whether rats are capable of empathically motivated helping behavior. We tested whether the presence of a trapped cagemate induces a pro-social motivational state in rats, leading them to open the restrainer door and liberate the cagemate.

Rats were housed in pairs for 2 weeks before the start of testing. In each session, a rat (the free rat) was placed in an arena with a centrally located restrainer in which a cagemate was trapped (trapped condition, $n = 30$ rats, 6 females). The free rat could liberate the trapped rat by applying enough force to tip over the restrainer door (Fig. 1A). If a free rat failed to open the door, the experimenter opened it halfway, allowing the trapped rat to escape and preventing learned helplessness. Rats remained in the arena together for the final third of the session. Door-opening only counted as such if the free rat opened the door before the experimenter opened it halfway. Sessions were repeated for 12 days. Control conditions included testing a free rat with an empty restrainer (empty condition, $n = 20$ rats, 6 females) or toy rat–containing restrainer (object condition, $n = 8$ males). As an additional control, for the number of rats present, we tested a free rat with an empty restrainer and an unrestrained cagemate located across a perforated divide (2+empty condition, $n = 12$ males). Free rats' heads were marked and their movements were recorded with a top-mounted camera for offline analysis (*11*).

Free rats circled the restrainer, digging at it and biting it, and contacted the trapped rat through holes in the restrainer (Fig. 1B and movie S1). They learned to open the door and liberate the trapped cagemate within a mean of 6.9 ± 2.9 days. Free rats spent more time near the restrainer in

[1]Department of Psychology, University of Chicago, Chicago, IL, USA. [2]Department of Psychiatry and Behavioral Neuroscience, University of Chicago, Chicago, IL, USA. [3]Department of Neurobiology, University of Chicago, Chicago, IL, USA. [4]Committee on Neurobiology, University of Chicago, Chicago, IL, USA.

the arena center [$P < 0.001$, mixed model analysis (MMA), Fig. 1C] and showed greater movement speed (hereafter termed activity, $P < 0.001$, MMA, Fig. 1D) than did control rats. Before learning to open the restrainer door, free rats in the trapped condition stayed significantly more active in the second half of sessions relative to the first half than did rats in control conditions [$P < 0.001$, MMA, protected least significant difference (PLSD) test, Fig. 1E]. Thus, rats were motivated to move and act specifically in the presence of a trapped cagemate.

In the trapped condition, the proportion of rats that opened the door increased (Fig. 2A), and the latency to door-opening decreased (Fig. 2B and movie S2) across sessions, which is evidence of learning. Significantly more rats in the trapped [23 out of 30 (23/30)] than control (5/40) conditions were classified as "openers" by the end of the experiment ($P < 0.001$, χ-square test), opening the door within minutes of placement in the arena (*11*). A sharp increase in the free rat's activity was observed at the time of door-opening (Fig. 2C), suggesting that the liberation of a trapped cagemate is a salient event.

Initially, rats in the trapped condition opened the door in any of three ways: tipping the door over from the side or top or pushing it up with their heads. However, on days 6 to 12, they consistently opened the door with their heads (Fig. 2D). Furthermore, whereas rats initially froze after the door fell over, later on they did not freeze (Fig. 2E), demonstrating that door-opening was the expected outcome of a deliberate, goal-directed action.

Ultrasonic (~23 kHz) vocalizations were collected from multiple testing arenas with a bat-detector and were analyzed to determine whether rats emitted alarm calls. Significantly more alarm calls were recorded during the trapped condition (13%) than during the empty and object conditions [3 to 5%, $P < 0.05$ analysis of variance (ANOVA), PLSD < 0.05, Fig. 2F] in randomly sampled files from all days of testing. Alarm calls occurred more frequently (20 to 27%) on days 1 to 3, when door-opening was rare. In 90% of files containing alarm calls on day 1, the trapped rat was identified as the source; in the remaining samples, we were not able to identify the caller. These data suggest that trapped rats were indeed stressed.

A greater proportion of female rats (6/6) than male rats (17/24) in the trapped condition became door-openers ($P < 0.05$, χ-square), which is consistent with suggestions that females are more empathic than males (*7*, *12*, *13*). Further, female rats in the trapped condition opened the restrainer door at a shorter latency than males on days 7 to 12 ($P < 0.01$, MMA, Fig. 3A). Female rats were also more active than males in the trapped condition ($P < 0.001$, ANOVA) but not in the empty condition (Fig. 3B).

To examine whether individual differences in boldness influenced door-opening, we tested the latency for approach to the ledge of a half-opened

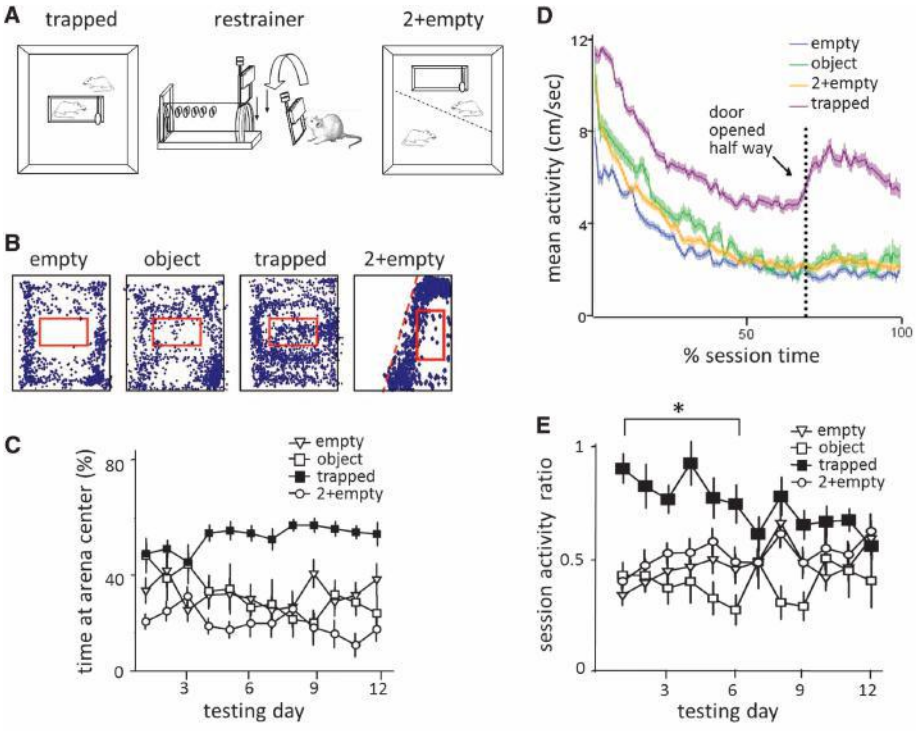


Fig. 1. (**A**) Top views of the trapped and 2+empty conditions and side views of the restrainer and door. (**B**) The locations (0.5 frames per second) of representative free rats with respect to the restrainer (red box) are plotted for each condition on day 1 of testing. (**C**) Rats in the trapped condition spent more time (mean ± SEM) in the arena center (>5 cm away from the wall) than did rats in control conditions. (**D**) The velocity (mean ± SEM) of rats in the trapped condition was greater than that of control rats throughout the session. (**E**) The ratio of the average activity during the second half of sessions relative to the average activity during the first half (mean ± SEM) was greater for rats in the trapped condition on days 1 to 6 than for rats in control conditions.

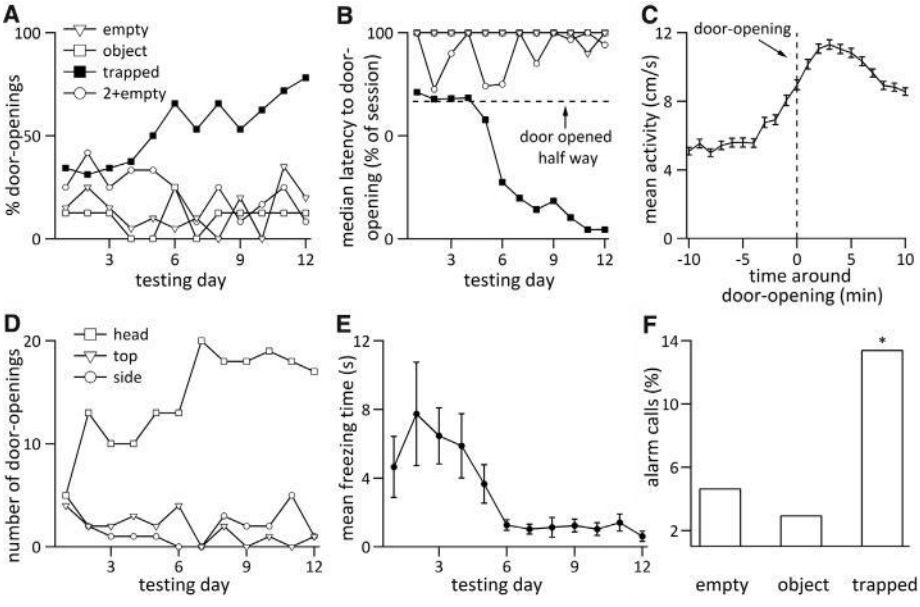


Fig. 2. (**A**) The proportion of rats in the trapped condition that opened the door increased across the days of testing. (**B**) Only rats in the trapped condition opened the door at decreasing latencies across days of testing. (**C**) Rats in the trapped condition showed a sharp increase in activity when the restrainer door was opened (time 0). (**D**) Across days, free rats in the trapped condition developed a consistent opening style, lifting the door up with their heads. (**E**) As rats learned to open the door, they stopped freezing in response to door-opening. (**F**) More alarm calls were recorded in the trapped condition ($n = 67$ sample files) than in empty ($n = 64$) or object ($n = 67$) conditions.

cage before the experiment (*11*). Animals who became openers had lower approach latencies than nonopeners ($P < 0.01$, t test), suggesting that successful opening behavior correlates with boldness scores (fig. S1). This demonstrates that individual trait differences may factor into the expression of pro-social behavior.

To determine whether anticipation of social interaction is necessary to motivate door-opening, we tested rats in a modified setup in which the trapped animal could only exit into a separate arena (separated condition, Fig. 4, A and B). Rats (12 pairs) were first exposed to the trapped condition (12 days); three rats did not open the door on any of the last 3 days and were not tested further. Next, rats were placed in the separated setup with a restrainer that was either empty (separated empty) or contained a cagemate (separated cagemate) for 29 days of testing. Finally, conditions were reversed so that rats previously in the separated cagemate condition were tested in the separated empty condition and vice versa, for 27 days. Thus, all nine rats were tested in counterbalanced order with both an empty and a full restrainer. Rats placed in the separated cagemate condition either continued or returned to opening the door at short latency as they had in the trapped condition. In contrast, when rats were placed in the separated empty condition, they stopped opening the door of the empty restrainer ($P < 0.001$, MMA, PLSD, Fig. 4, A and B). Thus, rats opened the door of a cagemate-containing restrainer but not of an empty restrainer, indicating that the expectation of social contact is not necessary for eliciting pro-social behavior.

In order to examine the relative value of liberating a trapped cagemate, we tested a cohort of rats in a cagemate versus chocolate paradigm. When given a choice, these non–food-deprived rats ate an average of >7 chocolate chips and no rat chow, indicating that they found chocolate highly palatable. The free rat was placed in an arena with two restrainers, one containing the trapped cagemate and the other containing five chocolate chips (chocolate cagemate condition, Fig. 4, C and D). As a control, one restrainer was empty while the other contained chocolate (chocolate empty condition). For rats in the chocolate cagemate condition, there was no difference in the door-opening latencies for the two restrainers during days 6 to 12 (Fig. 4C). In contrast, rats in the chocolate empty condition opened the chocolate-containing restrainer more quickly than the empty one ($P < 0.01$, t test, Fig. 4D). These results show that the value of freeing a trapped cagemate is on par with that of accessing chocolate chips. Like rats in the trapped condition, rats needed several days (5.8 ± 2.1) to learn to open the chocolate restrainer, which is evidence that door-opening was neither easy nor instinctual.

Although free rats in the chocolate cagemate condition could potentially eat all five

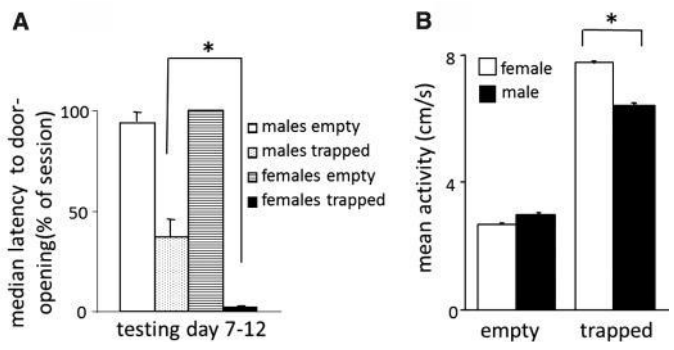


Fig. 3. (**A**) Females in the trapped, but not empty, condition opened the door at consistently shorter latencies than did males on days 7 to 12. (**B**) Activity was greater for females than males in the trapped, but not empty, condition.

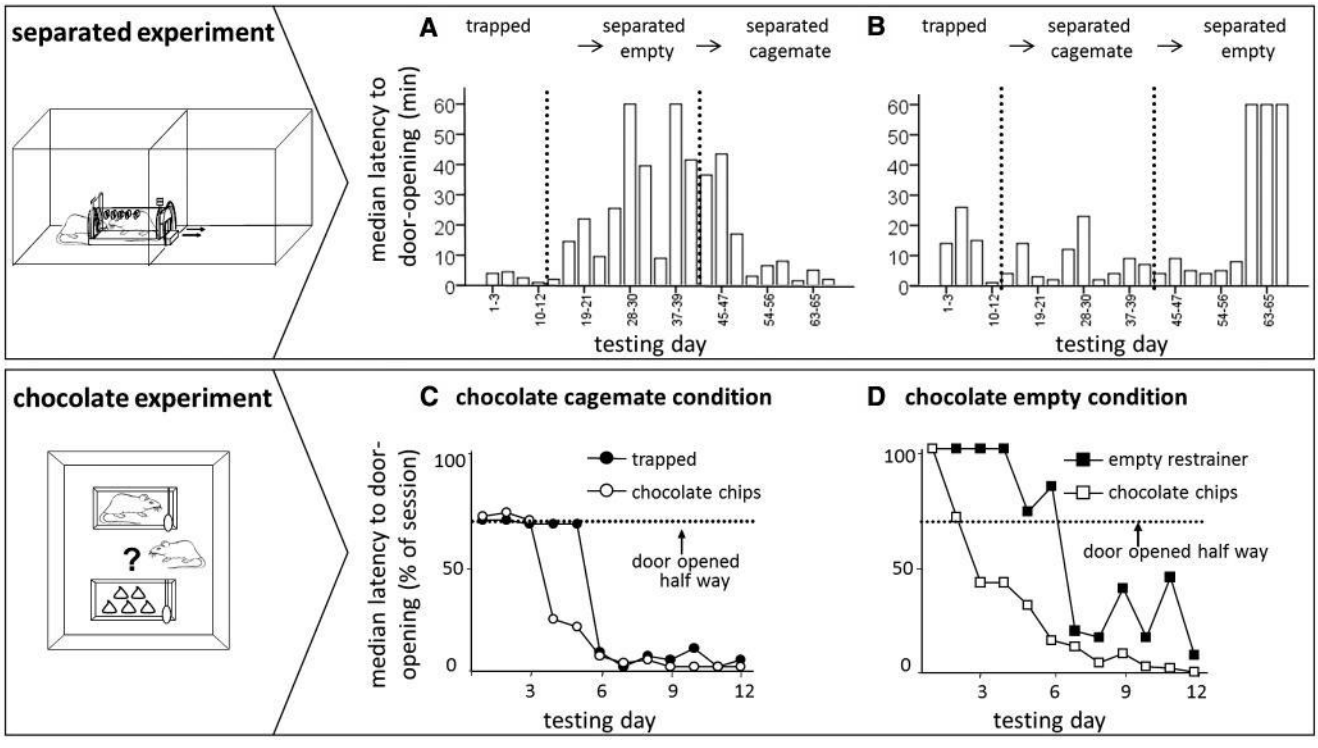


Fig. 4. (**A** and **B**) Rats opened the door for a trapped cagemate even when no social interaction was possible between the two animals after door-opening. Door-opening was extinguished when the restrainer was empty but either resumed (A) or persisted (B) when the restrainer contained a cagemate, regardless of the order of testing [$n = 4$ rats, (A); $n = 5$, (B)]. (**C**) On days 6 to 12, the latencies at which rats opened a restrainer containing a trapped cagemate and one containing chocolate chips were not different. (**D**) Rats in the chocolate empty condition opened the empty restrainer at significantly longer latencies than the chocolate restrainer.

chocolate chips, they shared them in half of all trials (52%) and in 61% of trials on days 6 to 12. Rats in the chocolate empty condition ate virtually all the chips (4.8 ± 0.7), whereas free rats in the chocolate cagemate condition ate fewer chips (3.5 ± 1.5, $P < 0.01$, t test), which allowed trapped rats to eat the remaining chips (1.5 ± 1.4).

Our study demonstrates that rats behave pro-socially when they perceive a conspecific experiencing nonpainful psychological restraint stress (*14*, *15*), acting to end that distress through deliberate action. In contrast to previous work (*5*, *9*, *16*, *17*), the present study shows pro-social behavior accomplished by the deliberate action of a rat. Moreover, this behavior occurred in the absence of training or social reward, and even when in competition with highly palatable food.

Our observations could have alternative explanations. Rats may have acted to stop the alarm calls of the trapped rats (*18*). Yet alarm calls occurred too infrequently to support this explanation. Alternatively, rats may have been attracted to the trapped cagemate by curiosity. However, door-opening in the separated cagemate condition persisted for over a month, a time period over which curiosity extinguishes (*19*). Finally, door-opening could be a coincidental effect of high activity levels. This is unlikely because once rats learned to open the door, they did so at short latency, using a consistent style, and were unsurprised by door-opening. Additionally, door-opening is not easy, rendering accidental openings unlikely. Thus, the most parsimonious interpretation of the observed helping behavior is that rats free their cagemate in order to end distress, either their own or that of the trapped rat, that is associated with the circumstances of the trapped cagemate. This emotional motivation, arguably the rodent homolog of empathy, appears to drive the pro-social behavior observed in the present study.

The presence of empathy in nonhuman animals is gaining support in the scientific community (*20*–*26*), although skeptics remain (*27*). In the current study, the free rat was not simply empathically sensitive to another rat's distress but acted intentionally to liberate a trapped conspecific. The ability to understand and actively respond to the affective state of a conspecific is crucial for an animal's successful navigation in the social arena (*4*) and ultimately benefits group survival.

References and Notes

1. D. C. Batson, in *The Social Neuroscience of Empathy*, J. Decety, W. J. Ickes, Eds. (MIT Press, Cambridge, MA, 2009), pp. 3–15.
2. J. Decety, P. L. Jackson, *Behav. Cogn. Neurosci. Rev.* **3**, 71 (2004).
3. N. Eisenberg *et al.*, *J. Pers. Soc. Psychol.* **57**, 55 (1989).
4. J. Decety, M. Svetlova, *Devel. Cogn. Neurosci.*, 10.1016/j.dcn.2011.05.003 (2011).
5. R. M. Church, *J. Comp. Physiol. Psychol.* **52**, 132 (1959).
6. D. Jeon *et al.*, *Nat. Neurosci.* **13**, 482 (2010).
7. D. J. Langford *et al.*, *Soc. Neurosci.* **5**, 163 (2010).
8. C. Zalaquett, D. Thiessen, *Physiol. Behav.* **50**, 221 (1991).
9. G. E. Rice, P. Gainer, *J. Comp. Physiol. Psychol.* **55**, 123 (1962).
10. D. J. Langford *et al.*, *Science* **312**, 1967 (2006).
11. Materials and methods are available as supporting material on *Science* Online.
12. T. Romero, M. A. Castellanos, F. B. de Waal, *Proc. Natl. Acad. Sci. U.S.A.* **107**, 12110 (2010).
13. C. Mohr, A. C. Rowe, O. Blanke, *Br. J. Psychol.* **101**, 277 (2010).
14. G. B. Glavin, W. P. Paré, T. Sandbak, H. K. Bakke, R. Murison, *Neurosci. Biobehav. Rev.* **18**, 223 (1994).
15. W. P. Paré, G. B. Glavin, *Neurosci. Biobehav. Rev.* **10**, 339 (1986).
16. J. F. Lucke, C. D. Baton, *J. Exp. Soc. Psychol.* **16**, 214 (1980).
17. G. E. J. Rice, *Psychol. Rec.* **14**, 165 (1964).
18. J. J. Lavery, P. J. Foley, *Science* **140**, 172 (1963).
19. M. L. Reger, D. A. Hovda, C. C. Giza, *Dev. Psychobiol.* **51**, 672 (2009).
20. F. B. de Waal, *Annu. Rev. Psychol.* **59**, 279 (2008).
21. F. Warneken, M. Tomasello, *Br. J. Psychol.* **100**, 455 (2009).
22. S. Preston, in *Encyclopedia of Animal Behavior*, Mark Bekoff, Ed. (Greenwood Press, Westport, CT, 2004), vol. 2, D-P.
23. G. D. Wills, A. L. Wesley, F. R. Moore, D. A. Sisemore, *Neurosci. Biobehav. Rev.* **7**, 315 (1983).
24. J. Decety, *Ann. N. Y. Acad. Sci.* **1231**, 35 (2011).
25. E. Hatfield, R. L. Rapson, Y. C. Le, in *The Social Neuroscience of Empathy*, J. Decety, W. J. Ickes, Eds. (MIT Press, Cambridge, MA, 2009), pp. 19–30.
26. S. D. Preston, F. B. M. de Waal, *Behav. Brain Sci.* **25**, 1, discussion 20 (2002).
27. J. B. Silk, in *The Oxford Handbook of Evolutionary Psychology*, R. I. M. Dunbar, L. Barrett, Eds. (Oxford Univ. Press, Oxford, 2007), pp. 115–126.

Acknowledgments: This research was supported by grants from the National Institute on Drug Abuse (DA022978 and DA022429) to P.M. and from NSF (BCS-0718480) to J.D. The assistance of I. Boni, A. Brimmer, U. Fonio, K. Hellman, A. Logli, J. Peralta, K. Ragsdale, D. Rodgers, M. Sales, J. Wang, A. Weiss, and K. Yuan is gratefully acknowledged.

Supporting Online Material

www.sciencemag.org/cgi/content/full/334/6061/1427/DC1
Materials and Methods
Fig. S1
Table S1
Reference (*28*)

7 July 2011; accepted 18 October 2011
10.1126/science.1210789

ERRATUM

Post date 27 January 2012

Reports: "Empathy and pro-social behavior in rats" by I. Ben-Ami Bartal *et al.* (9 December 2011, p. 1427). On p. 1428, the last full paragraph of column 1 was incorrect. The paragraph should be replaced by this corrected text: "All female rats (6/6) and most male rats (17/24) in the trapped condition became door-openers. Female rats in the trapped condition opened the restrainer door at a shorter latency than males on days 7 to 12 ($P < 0.01$, MMA, Fig. 3A), consistent with suggestions that females are more empathic than males (*7*, *12*, *13*). Furthermore, female rats were also more active than males in the trapped condition ($P < 0.001$, ANOVA) but not in the empty condition (Fig. 3B).

Populin et al.,

Rhesus Monkeys (Macaca mulatta) Do Recognize Themselves in the Mirror:

Implication for the Evolution of Self-Recognition,

PLOS ONE, vol. 5 (Sept. 29, 2010)

OPEN ACCESS Freely available online

PLoS one

Rhesus Monkeys (*Macaca mulatta*) Do Recognize Themselves in the Mirror: Implications for the Evolution of Self-Recognition

Abigail Z. Rajala[1], Katharine R. Reininger[2], Kimberly M. Lancaster[2], Luis C. Populin[1,2,3,4]*

1 Neuroscience Training Program, University of Wisconsin-Madison, Madison, Wisconsin, United States of America, **2** Department of Anatomy, University of Wisconsin-Madison, Madison, Wisconsin, United States of America, **3** Eye Research Institute, University of Wisconsin-Madison, Madison, Wisconsin, United States of America, **4** Department of Psychology, University of Wisconsin-Madison, Madison, Wisconsin, United States of America

Abstract

Self-recognition in front of a mirror is used as an indicator of self-awareness. Along with humans, some chimpanzees and orangutans have been shown to be self-aware using the mark test. Monkeys are conspicuously absent from this list because they fail the mark test and show persistent signs of social responses to mirrors despite prolonged exposure, which has been interpreted as evidence of a cognitive divide between hominoids and other species. In stark contrast with those reports, the rhesus monkeys in this study, who had been prepared for electrophysiological recordings with a head implant, showed consistent self-directed behaviors in front of the mirror and showed social responses that subsided quickly during the first experimental session. The self-directed behaviors, which were performed in front of the mirror and did not take place in its absence, included extensive observation of the implant and genital areas that cannot be observed directly without a mirror. We hypothesize that the head implant, a most salient mark, prompted the monkeys to overcome gaze aversion inhibition or lack of interest in order to look and examine themselves in front of the mirror. The results of this study demonstrate that rhesus monkeys do recognize themselves in the mirror and, therefore, have some form of self-awareness. Accordingly, instead of a cognitive divide, they support the notion of an evolutionary continuity of mental functions.

Citation: Rajala AZ, Reininger KR, Lancaster KM, Populin LC (2010) Rhesus Monkeys (*Macaca mulatta*) Do Recognize Themselves in the Mirror: Implications for the Evolution of Self-Recognition. PLoS ONE 5(9): e12865. doi:10.1371/journal.pone.0012865

Editor: Jan Lauwereyns, Kyushu University, Japan

Received June 9, 2010; **Accepted** August 26, 2010; **Published** September 29, 2010

Funding: This work was supported by grants from the National Science Foundation (NSF) (IOB-0517458) and National Institutes of Health (NIH) (DC003693). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

* E-mail: Lpopulin@wisc.edu

Introduction

Mirror self-recognition, measured with the mark test [1], is thought to be an indicator of self-awareness [2,3], the capacity to comprehend that one exists as an individual separate from thoughts, other individuals, and the environment. Some chimpanzees [1] and orangutans [4], like humans [5], pass the mark test and, therefore, are self-aware. Macaques, on the other hand, are thought to lack self-awareness because, with few exceptions [6], they have consistently failed the mark test and have shown persistent social responses towards mirrors [1], even after prolonged exposure [7] and training [8].

The mark test [1], the standard test for self-recognition, is performed after first exposing an animal to the mirror, during which time the behavior may change from social interactions directed towards the reflection to self-directed behaviors [1], indicating that it may have learned to recognize its reflection as its own. The actual test consists of the application of marks on the animal's face while anesthetized, then exposure to a mirror after recovery. If the animal touches the marks, acknowledging their presence on its face, it is concluded that it has passed the test and thereby verifies the observations that suggested that it recognizes itself in the mirror [1] and, therefore, is self-aware [3].

Determining that an individual of a given species, an ape or a monkey for instance, can recognize itself constitutes a monumental problem because one cannot know objectively what is the creature's cognitive process; for a human, one cannot know what he or she is thinking. The mark test is thought to provide an objective solution to this problem. By touching the mark on its face, not the mark on the mirror, the animal is thought to show not only that it has detected the presence of the mark on its face but, fundamentally, to have judged the mark as foreign to the image of itself, demonstrating, therefore, that if has a concept of self.

The results of the mark test have been used to delineate a fundamental divide in cognitive function between hominoids and all other species [9,10], but recent evidence has called this assertion into question. Some elephants [11], dolphins [12], and magpies [13] have passed the mark test thereby demonstrating that the ability to learn to recognize one's self in a mirror has evolved independently along different branches of the evolutionary tree [13].

It is important to note that despite its objectivity and the fact that it has become a benchmark, the mark test is not free from controversy [14]. For instance, it may fail to properly measure the cognitive abilities of species that do not self-groom or rely heavily on senses other than vision [15]. Furthermore, it may share the limitations of comparative studies of cognitive function that fail to distinguish between differences in ability and differences in performance [16–18]. It may be possible, therefore, that the monkey has fallen on the wrong side of the cognitive divide.

Observations of two rhesus monkeys that had been prepared for behavioral/electrophysiological studies with a head implant led us to question the assertion that monkeys do not recognize themselves in the mirror and, therefore, lack self-awareness. These monkeys held mirrors and looked into them while grooming. The results of two experiments with mirrors of different sizes, reflectivity, and location confirmed our initial observations that indicate that these animals do in fact recognize themselves in the mirror.

Results

Initial observations

Figure 1A,B shows a sample of the observations that led us to question that rhesus monkeys cannot recognize themselves in the mirror. Upon being returned to his cage after experiments this monkey moved in front of the mirror (Fig. 1A), or held it at the appropriate angle with one hand while grooming the area around the implant with the other (Fig. 1B). The images in Movie S1 (supplemental materials) illustrate that the monkey engaged in these behaviors for several minutes at a time. As reported in chimpanzees during the mark test [1], the monkey smelled, licked, and looked at his fingers while grooming in front of the mirror, indicating that he understood that the area being groomed was clearly his. Similar behaviors were observed in a second monkey. Although they occasionally groomed the area around the implant in the absence of the mirror, their gaze was not fixed in any particular location. When grooming was guided by mirror viewing, the monkeys always turned to face it and looked into it. Furthermore, there were no attempts to touch or groom the image in the mirror, which would have suggested that the monkey saw the reflection as another animal. Most importantly, no social responses were observed during the periods in which the monkeys looked at themselves and groomed in front of the mirror.

Because these behaviors had not been reported in the literature, these two monkeys were given the mark test and, consistent with previous reports [1,7], they failed. In no instance did they show behaviors directed at the marks dyed on their faces. Thus, we had two conflicting pieces of evidence. On the one hand, both monkeys failed the mark test, which as discussed above is the standard test for self-recognition [1]. On the other hand, both monkeys exhibited behaviors that were unequivocally self-directed and guided by looking into the mirror (see Movie S1).

Since both monkeys had failed the mark test, it was imperative to design experiments around other objective measures of behavior that would allow us to determine if these monkeys exhibited self-recognition. Anderson [19] outlined the following criteria to objectively determine if an animal displays mirror self-recognition: (1) the spontaneous development of mirror-guided self-directed behaviors, such as examining parts of the body that are unseen without the aid of a mirror, and (2) the disappearance

Figure 1. Examples of monkey self-directed behaviors in front of the mirror. (A,B) images from video recordings taken over the course of approximately eight months following initial observations. In each photograph the hand used for grooming is highlighted with a red arrow. In (A) the monkey leaned to his left while sitting on the perch to be able to look at himself in the mirror. In (B) The same monkey held the mirror at the appropriate angle for viewing himself with the right hand while grooming the area around the implant with the left. (C,D) Self-directed behaviors with the large mirror from two other monkeys. View of the implants have been masked for discretion (A–C).
doi:10.1371/journal.pone.0012865.g001

of social responses directed toward mirrors. Two experiments that included these measures were carried out to resolve the contradiction.

Experiment 1

Although our initial observations appeared to demonstrate that monkeys use the mirror to look at and groom themselves spontaneously at any moment, the possibility existed that in response to somatosensory stimulation from being cleaned, they groomed the implant area regardless of the availability of the mirror. Accordingly, the small mirrors were removed for one week, then placed back on the cage of each of five subjects, including the two animals initially observed using mirrors to groom, for five one-hour sessions videotaped on separate days; videotaping took place before cleaning the implant area to avoid providing somatosensory cues. In addition, as a control, the animals were also videotaped in five one-hour sessions using the same mirror with the reflective surfaces covered with black plastic. We hypothesized that no differences in behavior should be observed if the reflectivity of the mirror was irrelevant and the animal was simply holding or sitting in front of an object and staring at it. Conversely, if important, the mirror should reveal self-directed actions and reduction and eventual disappearance of social responses [19].

On average, monkeys looked at the small mirror significantly longer than the black control ($p<0.05$), approximately once every 2.5 minutes (Fig. 2A), but the duration of the looks, despite a trend for being longer than the looks into the actual mirror, was not significantly different (Fig. 2B). We hypothesize that the animals persisted in looking at the black control because it was attached to the same frame used to hold the regular mirror and stopped looking when they realized that the object was not a mirror, as revealed by the significantly larger number of looks directed at the mirror versus the control (Fig. 2A).

Except for a few instances from one of the five subjects tested, no social behaviors were observed with either the mirror or the control (Table 1). In addition, as described below, some of the monkeys used the small mirror to examine parts of the body they could not see directly. We computed the rate in which they spontaneously touched or groomed the area around the implant and other unseen areas of the body (genitals) with and without the mirror. An equal rate of touching would have indicated that the mirror was irrelevant. The data in Figure 2C indicate otherwise. The rate of touching when the mirror was present was nearly tenfold greater than without the mirror. The data have been normalized because of the small number of spontaneous touches in the control. These observations are consistent with Anderson's [19] assertions regarding behavioral events that suggest self-recognition.

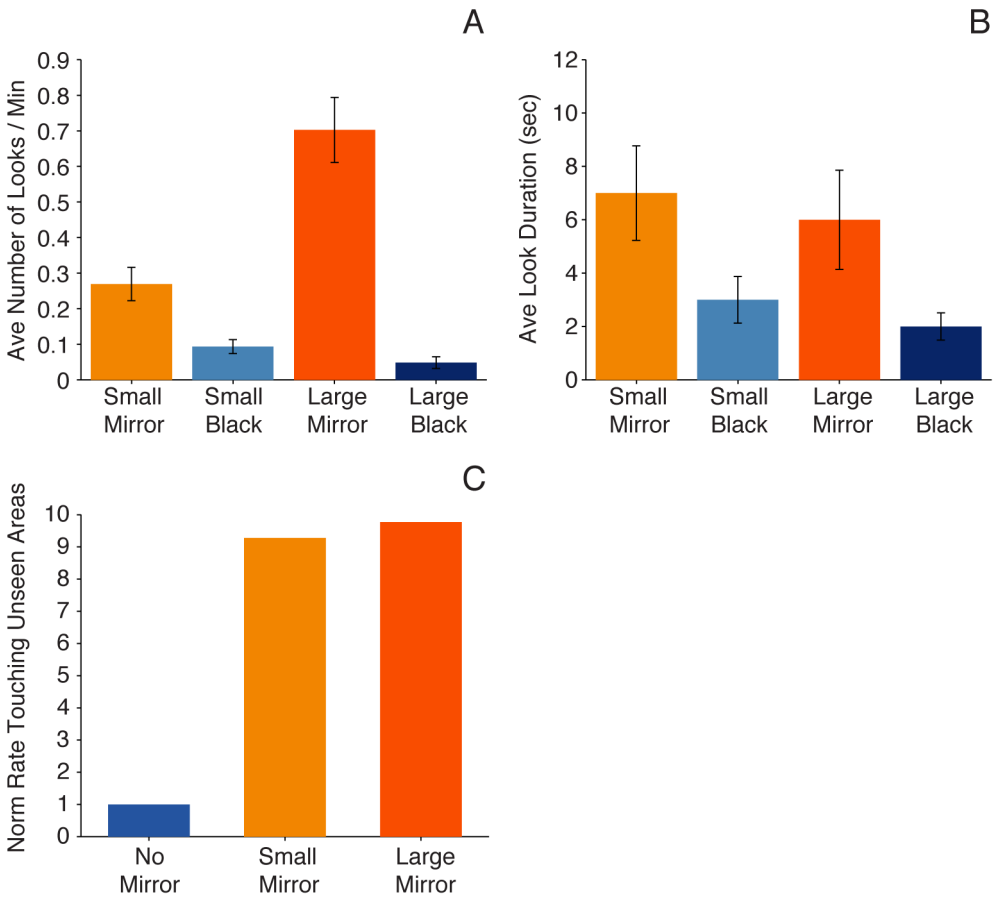


Figure 2. Quantification of mirror-directed behaviors. (A) Average number of looks in the mirror per minute recorded in the large and small mirror sessions and their corresponding controls covered with black, non-reflective plastic. (B) Average look duration for the mirrors and black controls. (C) Rate of touching unseen areas, the area around the implant on the head and genitals as described in B of Table 2, in the small and large mirror sessions normalized to the control. Control represents the no-mirror condition. Data from all five monkeys studied are included in this figure. All behaviors involved the monkeys moving or moving the mirror with their hands or feet to obtain the appropriate angle to look at themselves.
doi:10.1371/journal.pone.0012865.g002

Table 1. Average Behavior per minute or five subjects.

Behavioral Category	A	B	C	D	E	F	G
Large Mirror							
Mean	0.490	0.064	0.201	0.004	0.106	0.030	0.021
SE	0.115	0.033	0.049	0.002	0.066	0.007	0.011
Large Black							
Mean	0.029	0.000	0.039	0.000	0.005	0.000	0.020
SE	0.018	-	0.014	-	0.003	-	0.009
Small Mirror							
Mean	0.160	0.061	0.058	0.003	0.028	0.002	0.000
SE	0.042	0.018	0.017	0.001	0.016	0.002	0.000
Small Black							
Mean	0.046	0.007	0.043	0.000	0.003	0.000	0.015
SE	0.013	0.005	0.012	-	0.003	-	0.007

doi:10.1371/journal.pone.0012865.t001

Experiment 2

Despite the positive results of the previous experiment, the possibility existed that the small size of the mirror coupled with it being hung outside the cage may have mitigated the monkeys perceiving the images in the mirror as threatening conspecifics and thus made the observed behaviors possible. Accordingly, a large mirror in which the monkeys could see their entire body was introduced. This mirror had one reflective side, was hung in the upper half of a double space cage, and could be swiveled. It was reasoned that this arrangement would provide the monkeys room to observe themselves, inspect the backside of the mirror, or avoid it if threatened by the reflection.

The introduction of the large mirror was met with curiosity. Figure 1C,D shows two monkeys as they held the large mirror with their hands and feet while looking at themselves (see also Movie S2). The first interactions were varied and included looking behind the mirror, presumably seeking the monkey they observed in the reflection. The contingency with the mirror was quickly established, however, and social behaviors subsided during the first session.

Monkeys looked at themselves more than twice as often in the large than in the small mirror (Fig. 2A), possibly due to the novelty associated with it. This measure comprises all instances of actively looking in the mirror without social behaviors, listed under the *Self-examination* heading in Table 2. Specifically those in which the monkey turned toward the mirror, positioned it at the appropriate angle to look into it, or shifted its position to match the moving mirror in order to maintain the appropriate angle of view. In control sessions the average number of looks was smaller ($p<0.05$) than with the mirror; there were no differences in the number of looks between the controls of the two experiments (Fig. 2A). Interestingly, in control sessions two monkeys tore the cover exposing part of the mirror and looked into it intently.

The number of looks into the mirror was significantly larger than the control during the first thirty minutes ($p<0.05$), declining slightly in the second half of the session (Fig. 3A). Throughout the first session the number of looks declined for the mirror and the control; the number of looks at the control was practically zero after 30 min. Few or no interactions were documented with the control in sessions 2–5. The monkeys appeared to simply ignore the black, non-reflective object.

One of the most important findings concerns the difference in the rate of occurrence of self-directed and social behaviors directed towards the large mirror (Fig. 3B). Social behaviors occurred at a lower rate ($p<0.05$) than self-directed behaviors. Fundamentally, unlike in previous reports in monkeys [1], their rate decayed significantly from the first to the second session ($p<0.05$), remaining at negligible levels in the subsequent three. This is similar to observations in chimpanzees [1,20] and consistent with one of Anderson's [19] assertions that diminishing and ultimately extinguishing social behaviors are indicative of self-recognition during mirror tests. Lastly, the monkeys looked into the large mirror approximately once a minute, a rate that decreased slightly across all five sessions but the decline did not reach significance. The duration of the looks directed at the large mirror and its control were similar to the duration of the looks directed at the small mirror and control in Experiment 1 but, despite a trend for longer looks into the mirrors, the differences did not reach significance (Fig. 2B).

Table 2. Behavioral categories and descriptions.

Behavior	Description
Self-examination	
A) Looking at himself in mirror	Specific orienting or positioning of his body in front of mirror and intent self-examination
B) Looking at himself in mirror while touching otherwise unseen areas	Specific orienting in front of mirror and grooming implant area or examining genitals
C) Looking at himself in mirror while holding it	Grabbing the mirror and specifically orienting it to self-examine
D) Looking at himself in mirror while touching otherwise unseen areas and holding it	Holding mirror in position while intently looking at reflection and grooming implant area or manipulating genitals
Exploratory	
E) Using mirror to look at environment (as a tool)	Angling the mirror to indirectly examine areas of the environment or neighboring conspecifics
G) Looking behind the mirror	Examining space behind or around mirror
Social	
F) Behaviors observed when an animal comes into contact with an unknown conspecific	Signs of aggression or submission such as charging the mirror, open-mouth threats, or lip smacking

doi:10.1371/journal.pone.0012865.t002

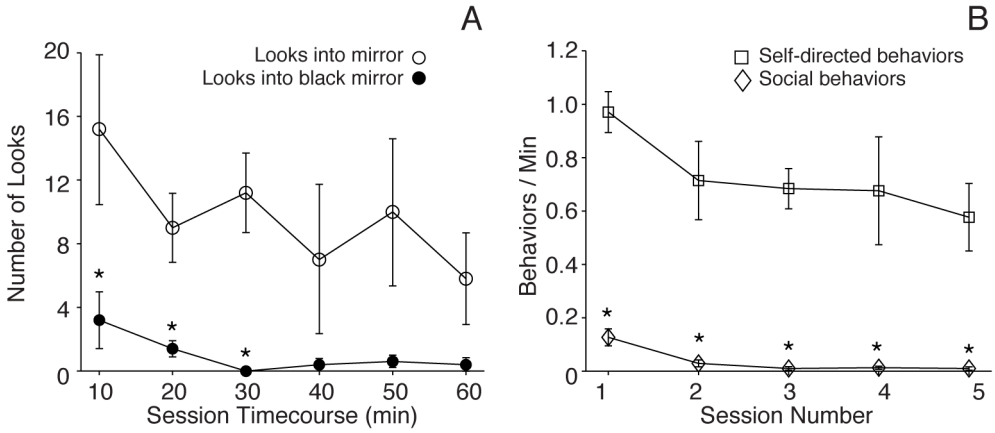


Figure 3. Quantification of mirror-directed behaviors during the first session and across five sessions. (A) Number of looks in the large mirror and large black-covered mirror during the first session. The one-hour session was broken up into 10-minute bins. (B) Number of looks into the large mirror and number of social behaviors directed at the large mirror per minute. The number of social behaviors in the last four sessions declined significantly ($p<0.05$) relative to the first. The standard bars represent standard errors and the asterisks indicate significance (t-test, $p<0.05$).
doi:10.1371/journal.pone.0012865.g003

In addition, the monkeys used the mirrors extensively to look at their genitals (Fig. 4). This behavior was first observed after the head implant of one monkey was removed to avert a potential infection, after which he continued to use the small mirror but instead of observing and grooming the top of his head, he began inspecting and touching his genitals (see Movie S3); the implant was reattached later successfully. All five monkeys used the mirror to look at areas of their bodies they could not see directly. Sometimes they used one hand to hold the mirror in place (Fig. 4B) and moved or manipulated their genitals (Fig. 4B,C), while other times they performed acrobatics in what appear to be an effort to obtain a better view (Fig. 4D and Movies S4, S5). These observations are consistent with another of Anderson's [19] assertions concerning mirror-guided behaviors that are indicative of self-recognition and could be categorized under Povinelli et al.'s [20] classification of self-exploratory behavior used as a positive

Figure 4. Example of monkeys examining their genital area in front of the mirrors. The red arrows point to the manipulation of the genitals (B,C). (D) Acrobatics such as this were commonly observed during inspection of genital area.
doi:10.1371/journal.pone.0012865.g004

indicator of mirror self-recognition in chimpanzees. As shown in Figure 2C, the rate of looking at unseen areas, the genitals in particular, was ten times smaller in the absence of the mirror.

Notably monkeys that had not been implanted were not observed using the mirror suggesting that the implant constituted a relevant stimulus, a ''super mark,'' that prompted them to look. We confirmed this by observing the behavior of two monkeys the day after the implant was attached. After some hesitation both monkeys began to look at themselves in the mirror and to examine the area around the implant. Unequivocally more revealing was that they attempted to pull the head post off their heads while looking in the mirror, a behavior that subsided after a few attempts and was not observed again. Most importantly, these behaviors were mirror-guided and self-directed but never directed toward the reflection in the mirror.

Discussion

Here we have shown that rhesus monkeys, though failing the mark test, demonstrate behaviors indicative of mirror self-recognition. They use the mirror to groom their head implants and inspect unseen areas of their bodies such as their genitals. Though we cannot objectively claim that these animals are self-aware, all the pieces are there to suggest that, in some form, they are.

If the ability to demonstrate self-recognition were innate, as suggested by Gallup [7], and could be explained solely on evolutionary grounds, one would expect that most, if not all members of a given species would or would not pass the test [21]. As it turns out, only a fraction of chimpanzees shows signs of mirror self-recognition [20,21]. Furthermore, one would not expect a phylogenetic gap in the expression of this ability, a conclusion derived from the fact that gorillas fail to show signs of mirror self recognition and fail the mark test [20], while orangutans, though lower evolutionarily, do [22]; but see [23,24] for positive evidence from two different gorillas. Note: even in children the proportion that exhibit self-recognition at a particular point in development varies as a function of intelligence level, cultural background, and type of self-recognition test administered [25–27].

A more likely explanation, however, is that behaviors indicative of mirror self-recognition are learned by establishing a contingency between self-produced movement and the reflection. The capability to learn and establish such a contingency and the form in which it is expressed is likely to vary across species. The question arises, therefore, as to the conditions that facilitate the establishment of the contingency.

Overall, the data are consistent with the saliency hypothesis [16], which postulates that an alteration in an individual's body must be highly salient to draw attention to the mirror image. Accordingly, the changes imposed on the appearance of monkeys in the standard mark test, as with more extensive markings in cotton-top tamarins [28], are not sufficient to draw the animal to touch the marks while looking in the mirror. The head implant, on the other hand, constitutes a relevant change that motivates the subject to use the mirror to inspect the area around it.

The sudden onset of self-directed behaviors in front of the mirror suggests that the monkeys either developed this ability *de novo* as a result of the surgery or were aware that they could see themselves in the mirror but were unable, perhaps due to gaze aversion, or uninterested in looking at themselves until a sufficiently relevant change took place - implantation of the head cap, therefore, simply triggered the display of this ability. Based on the length of the exposure to mirrors of these monkeys before they received the implants (all grew up with mirrors and were exposed to them constantly throughout their lives as part of their enrichment program), we conclude that the data are consistent with the latter.

We hypothesize that for the monkeys in this study the implant constituted a ''super mark'' that, coupled to their prior experience with the small mirror, the mobility of both mirrors, and the monkey's direct access to them, facilitated the manifestation of these behaviors. Future study should reveal what are the most effective experimental conditions, including mirror configurations and the time required to develop the contingency.

The mark test [1], therefore, is an inadequate measure of self-recognition for rhesus monkeys. A similar argument can be made for the results of studies of other species that rely heavily on audition or olfaction, as the mark test relies solely on vision, because they may reveal some form of self-recognition if tested differently [15,29]. More fundamentally, the mark test may not be enough to reveal that members of a given species are self-aware [14].

These observations, taken together, demonstrate that rhesus monkeys do recognize themselves in the mirror and, therefore, have the fundamental elements to have the capacity to be self-aware. Accordingly, we conclude that behavioral differences between hominoids and lower primates are not the result of cognitive deficits in the latter, but rather of a different position on the underlying evolutionary continuity of mental functions [6,15,30].

Materials and Methods

Five male rhesus monkeys (*Macaca mulatta*) 5–13 years of age that had been exposed to mirrors as part of their enrichment throughout most of their lives were studied. A sixth monkey, who had also received a head implant, showed no interactions with the mirror and thus was not included in the study. The mirror was two-sided, set in a plastic frame measuring 3×4.75 inches, hung outside the cage, and could be swiveled. All five subjects had been prepared for behavioral/electrophysiological experiments with a head implant, the area around which was cleaned before experiments with a dry cotton swab. The implant consisted of a block of acrylic (Ortho Resin, Justi Products, Oxnard, CA) ranging in size ~(40 mm–100 mm×40–80 mm). The acrylic was blue in color and held (1) a lightweight titanium head post used for holding a water spout in front of the animal's lips during head-unrestrained oculomotor experiments and to restrain the head to clean and care for the area surrounding the implant [31], (2) connectors for the eye coils [32] used to record eye movements with the scleral search coil technique [33], and (3) a cylinder to insert microelectrodes into the brain for physiological recordings. Two implants, one with and one without a recording cylinder are shown in Figure S1. The implants were attached to the skull of the subjects with human grade titanium screws.

The animals were housed individually and provided double the space, 12.4 cu ft, typically provided for rhesus monkeys. Data were acquired in the room where the animals were housed. Video recordings followed the initial observations using a webcam without humans present. The animals continued to participate in their assigned experiments. Five one-hour sessions were videotaped for the two mirror sizes, small and large (12×24 inches set in a metal frame), and corresponding controls in which the mirrors were covered with black non-reflective plastic. The non-reflective controls were used to determine if similar behaviors took place when the monkeys could not see themselves.

The data were scored offline for self-directed and social behaviors in front of the mirror according to the categories outlined in Table 2.

All behaviors scored were active and purposeful, that is the monkey either positioned the mirror with his hand to look at himself or moved to attain the appropriate angle for viewing himself. These are similar to the criteria used to characterize the behavior of chimpanzees [20]. Of particular importance were self-exploration, defined as manipulation of areas not visible without use of the mirror (e.g., the anal-genital area) used to classify animals as showing positive evidence of self-recognition, and social behaviors, aggressive or appeasing gestures suggesting that the monkey sees a conspecific [20]. Three observers, aware of the hypothesis being tested, viewed and scored the first group of data collected according to the behavioral categories listed in Table 2. The formula used by Povinelli et al. [20] was used to calculate reliability where the percentage of agreement between observers = total instances of agreement/total opportunities for agreement with L.C. Populin used as the standard for comparison. A congruency between the scoring of three observers exceeded 95% in the first group of video data obtained thus only one observer scored the remaining data. The small proportion of inconsistencies among the three observers primarily comprised the length of brief behaviors such as glances into the mirror; they were resolved by consensus after frame-by-frame review of the pertinent sections of the video record. All efforts were made to ameliorate suffering of the animals. Specifically, all procedures were approved by the University of Wisconsin Animal Care Committee and were in accordance with the National Institutes of Health *Guide for the Care and User of Laboratory Animals*.

Supporting Information

Figure S1 Head implants. (A) Basic head implant used for behavioral experiments. The acrylic holds a titanium head post and two connectors for eye coils. (B) Head implant used for physiological experiments. A recording cylinder, 19 mm in diameter, has been added to the basic implant to allow the insertion of microelectrodes.
Found at: doi:10.1371/journal.pone.0012865.s001 (1.35 MB TIF)

Movie S1 Self-directed behavior in front of the mirror. This movie shows a monkey waking up from a nap, then reaching for the small mirror outside his cage, positioning it to view himself, and grooming the area around the implant while looking at himself. A green mark used for the mark test, which he failed, is still visible on his left cheek. The view of the head implant has been blocked for discretion.
Found at: doi:10.1371/journal.pone.0012865.s002 (0.33 MB MOV)

Movie S2 Typical use of the large mirror by monkeys. This movie shows a monkey using the large mirror inside his cage to view his neighbor and to examine himself. Note the position of his right leg, which is elevated thereby exposing his genital area. For nearly one minute the monkey observes himself without signs of social behaviors directed at the mirror.
Found at: doi:10.1371/journal.pone.0012865.s003 (1.10 MB MOV)

Movie S3 This movie shows a monkey inspecting the lower part of his body and genitals using the small mirror. He looks over his shoulder to view his backside and genitals. Note that toward the end of the movie he reaches with his hand between his legs and pushes his genitals forward into view, confirming, therefore, that he is examining them in the mirror. This movie was recorded after the implant had been removed from this monkey.
Found at: doi:10.1371/journal.pone.0012865.s004 (1.12 MB MOV)

Movie S4 Use of the big mirror to inspect genitals; two clips are shown in succession. First the monkey positions the mirror, orients and lifts his left, then grabs his genitals while looking attentively. Second, the monkey directly looks between his legs, then turns toward the mirror to view the same part of his body.
Found at: doi:10.1371/journal.pone.0012865.s005 (0.89 MB MOV)

Movie S5 Monkey performing acrobatics in front of the mirror to view his backside and genitals. First the monkey looks between his legs while pushing his genitals with his hand. Second, he hangs upside down from the top of his cage while attempting to view his genital area from this angle.
Found at: doi:10.1371/journal.pone.0012865.s006 (3.12 MB MOV)

Acknowledgments

We are grateful to Chris Coe, John Harting, Steve Shelton, and Charles Snowdon for comments on an earlier version of this manuscript. We also thank C Daly, P Esser, M Gallardo, K Sayles, and K Tegt for outstanding animal care.

Author Contributions

Conceived and designed the experiments: AZR KRR KML LCP. Performed the experiments: AZR KRR KML LCP. Analyzed the data: AZR KRR KML LCP. Contributed reagents/materials/analysis tools: AZR KRR KML LCP. Wrote the paper: AZR KRR KML LCP.

References

1. Gallup GG, Jr. (1970) Chimpanzees: self-recognition. Science 167: 86–87.
2. Gallup GG, Jr. (1977) Self-recognition in primates: a comparative approach to the bidirectional properties of consciousness. Am Psychol 32: 329–338.
3. Gallup GG, Jr. (1982) Self-awareness and the emergence of mind in primates. Am J Primatol 2: 237–248.
4. Lethmate J, Dücker G (1973) Untersuchungen zum Selbsterkennen im Spiegel bei Orangutans und einigen anderen Affenarten. Z. Tierpsychol 33: 248–269.
5. Amsterdam B (1972) Mirror self-image reactions before age two. Dev Psychobiol 5: 297–305.
6. Boccia ML (1994) Mirror behavior in macaques. In: Parker ST, Mitchell RW, Boccia ML, eds. Self-awareness in animals and humans. New York: Cambridge University Press. pp 350–360.
7. Gallup GG, Jr. (1977) Absence of self-recognition in a monkey (Macaca fascicularis) following prolonged exposure to a mirror. Dev. Psychobiol 10: 281–284.
8. Anderson JR (1983) Responses to mirror image stimulation and assessment of self-recognition in mirror- and peer-reared stumptail macaques. Q J Exp Psychol 35B: 201–212.
9. Gallup GG, Jr. (1987) Self-awareness. Comp Primate Biol 2B: 3–16.
10. Povinelli DJ (1993) Reconstructing the evolution of mind. Am Psychol 48: 493–509.
11. Plotnik JM, de Waal FBM, Reiss D (2006) Self-recognition in an Asian elephant. PNAS 103: 17053–17057.
12. Reiss D, Marino L (2001) Mirror-self-recognition in the bottlenose dolphin: a case of cognitive convergence. PNAS 98: 5937–5942.
13. Prior H, Schwarz A, Güntürkün O (2008) Mirror-induced behavior in the Magpie (Pica pica): evidence of self-recognition. PLoS Bio 6: 1642–1650.
14. Mitchell RW (1993) Mental models of mirror-self-recognition: two theories. New Ideas Psychol 11: 295–325.
15. Eglash AR, Snowdon CT (1983) Mirror-image responses in pygmy marmoset (Cebuella pygmaea). Am J Primatol 5: 211–219.
16. Hauser MD, Kralik J, Botto-Mahan C, Garrett M, Oser J (1995) Self-recognition in primates: phylogeny and the salience of species-typical features. PNAS 92: 10811–10814.
17. MacPhail EM (1987) The comparative psychology of intelligence. Behav Brain Sci 10: 645–695.
18. MacPhail EM (1987) Intelligence: a comparative prospective. In: Blackemore C, Freenfield S, eds. Mindwaves: thoughts on intelligence, identity and consciousness. Oxford: Blackwell. pp 177–194.
19. Anderson JR (1994) The monkey in the mirror: a strange conspecific. In: Parker ST, Mitchell RW, Boccia ML, eds. Self-awareness in animals and humans. New York: Cambridge University Press. pp 315–329.

20. Povinelli DJ, Rulf AB, Landau KR, Bierschwale DT (1993) Self-recognition in chimpanzees (Pan troglodytes): distribution, ontogeny, and patterns of emergence. J Comp Psychol 107: 347–372.
21. Swartz KB, Evans S (1991) Not all chimpanzees (Pan troglodytes) show self-recognition. Primates 32: 483–496.
22. Suarez SD, Gallup GG, Jr. (1981) Self-recognition in chimpanzees and orangutans, but not gorillas. J Hum Evol 10: 175–188.
23. Patterson FGP, Cohn RH (1994) Self-recognition and self-awareness in lowland gorillas. In: Parker ST, Mitchell RW, Boccia ML, eds. Self-awareness in animals and humans. New York: Cambridge University Press. pp 373–290.
24. Swartz KB, Evans S (1994) Social and cognitive factors in chimpanzee and gorilla mirror behavior and self-recognition. In: Parker ST, Mitchell RW, Boccia ML, eds. Self-awareness in animals and humans. New York: Cambridge University Press. pp 189–206.
25. Bard KA, Todd BK, Bernier C, Love C, Leavens DA (2006) Self-awareness in human and chimpanzee infants: what is measured and what is meant by the mark and mirror tests? Infancy 9: 191–219.
26. Fryrear JL, Kodera TL, Kennedy MJ (1981) Self-recognition ability in mentally retarded adolescents. J Psychol 104: 123–131.
27. Keller H, Yovsi R, Borke J, Kartner J, Jensen H, et al. (2004) Developmental consequences of early parenting experiences: self-recognition and self-regulation in three cultural community. .
28. Hauser MD, Miller CT, Liu K, Gupta R (2001) Cotton-top tamarins (Saguinus oedipus) fail to show mirror-guided self-exploration. Am J Primatol 53: 131–137.
29. Gallup GG, Jr. (1979) Self-awareness in primates. Am Scientist 67: 417–421.
30. Griffin DR (1976) The question of animal awareness. Rockefeller University Press.
31. Populin LC (2006) Monkey sound localization: head-restrained versus head-unrestrained orienting. J Neurosci 26: 9820–9832.
32. Judge SJ, Richmond BJ, Chu FC (1980) Implantation of magnetic search coils for measurement of eye position: an improved method. Vision Res 20: 535–537.
33. Robinson DA (1963) A method of measuring eye movement using a scleral search coil in a magnetic field. IEEE Trans Biomed Eng 10: 137–145.

Chimera, THEFREEDICTIONARY,

available online at http://www.thefreedictionary.com/ Chimera (last accessed Nov. 25, 2013)

thefreedictionary.com

Chi·me·ra also **Chi·mae·ra** (kī-mîr'ə, kĭ-)

n.

1. *Greek Mythology* A fire-breathing she-monster usually represented as a composite of a lion, goat, and serpent.
2. An imaginary monster made up of grotesquely disparate parts.

chi·me·ra also **chi·mae·ra** (kī-mîr'ə, kĭ-)

n.

1.
a. An organism, organ, or part consisting of two or more tissues of different genetic composition, produced as a result of organ transplant, grafting, or genetic engineering.
b. A substance, such as an antibody, created from the proteins or genes of two different species.
2. An individual who has received a transplant of genetically and immunologically different tissue.
3. A fanciful mental illusion or fabrication.

[Middle English chimere, *Chimera*, from Old French, from Latin chimaera, from Greek khimaira, *chimera, she-goat*; see ghei- in Indo-European roots.]

chimera, **chimaera** [kaɪˈmɪərə kɪ-]

n

1. (Myth & Legend / Classical Myth & Legend) *(often capital) Greek myth* a fire-breathing monster with the head of a lion, body of a goat, and tail of a serpent
2. (Fine Arts & Visual Arts / Art Terms) a fabulous beast made up of parts taken from various animals
3. a wild and unrealistic dream or notion
4. (Life Sciences & Allied Applications / Genetics) *Biology* an organism, esp a cultivated plant, consisting of at least two genetically different kinds of tissue as a result of mutation, grafting, etc.

[from Latin *chimaera,* from Greek *khimaira* she-goat, from *khimaros* he-goat]

chi•me•ra or chi•mae•ra (kɪˈmɪər ə, kaɪ-)

n., pl. **-ras.**

1. (*often cap.*) a monster of classical myth, commonly represented with a lion's head, a goat's body, and a serpent's tail.
2. any horrible or grotesque imaginary creature.
3. a fancy or dream.
4. an organism composed of two or more genetically distinct tissues.

[1350–1400; Middle English < Latin *chimaera* < Greek *chímaira* she-goat; akin to Old Norse *gymbr,* E *gimmer* ewe-lamb one year (i.e., one winter) old, Latin *hiems* winter (see hiemal)]

Thesaurus

Legend: Synonyms | Related Words | Antonyms

Noun 1. Chimera - (Greek mythology) fire-breathing female monster with a lion's head and a goat's body and a serpent's tail; daughter of Typhon

- Chimaera
- Greek mythology - the mythology of the ancient Greeks
- mythical creature, mythical monster - a monster renowned in folklore and myth

2. chimera - a grotesque product of the imagination

- chimaera
- imagery, imaging, mental imagery, imagination - the ability to form mental images of things or events; "he could still hear her in his imagination"

Based on WordNet 3.0, Farlex clipart collection. © 2003-2012 Princeton University, Farlex Inc.

chimera

noun illusion, dream, fantasy, delusion, spectre, snare, hallucination, figment, ignis fatuus, will-o'-the-wisp *He spent his life pursuing the chimera of perfect love.*

Collins Thesaurus of the English Language – Complete and Unabridged 2nd Edition. 2002 © HarperCollins Publishers 1995, 2002

Translations

Select a language: ----------

Greely,

Defining Chimeras . . . and Chimeric Concerns,

AM. J. BIOETHICS, vol. 3, no. 3 (Summer 2003)

their lives miserable and give them the status of freaks? Again, parallel problems exist for hybrids across animal species.

I am grateful to Robert and Baylis for making me rethink my own cavalier dismissal of crossing species barriers as a spurious moral issue. While their thesis acknowledges that this concern might be biologically and conceptually ill-founded, if taken seriously it should make us deepen our thinking regarding the adaptability of our moral categories to our ability to manipulate life. ■

References

Gaskell, G., et al. 1997. Europe ambivalent on biotechnology. *Nature* 387(6636):845–47.

Pursel, V. G., C. A. Pinkert, K. F. Miller et al. 1989. Genetic engineering of livestock. *Science* 244(4910):1281–88.

Robert, J. S., and F. Baylis. 2003. Crossing species boundaries. *The American Journal of Bioethics* 3(3):1–13.

Rollin, B. E. 1992. *Animal rights and human morality.* Buffalo: Prometheus.

———. 1995. *The Frankenstein syndrome: Ethical and social issues in the genetic engineering of animals.* New York: Cambridge University Press.

Defining Chimeras . . . and Chimeric Concerns

Henry T. Greely, Stanford University

Chimera, n. . . . 1. a. A fabled fire-breathing monster of Greek mythology, with a lion's head, a goat's body, and a serpent's tail (or according to others with the heads of a lion, a goat, and a serpent), killed by Bellerophon.

Oxford English Dictionary (1989)

The original chimera turns out to be surprisingly undefined. Did Bellerophon, riding Pegasus, slay a monster with the heads of three different species or a one-headed beast with parts from three species? This lack of clear definition exists in contemporary discussions of the ethics of nonmythological chimeras, including in the useful article by Jason Scott Robert and Françoise Baylis, "Crossing Species Boundaries" (2003). In their third paragraph Robert and Baylis list a broad set of possible types of chimeras before, in their fourth paragraph, focusing on human-to-animal embryonic chimeras. I believe that we can achieve a better understanding of the ethical issues raised by chimeras—and, indeed, whether the category "chimera" is useful in ethical discussion of contemporary biology—by defining *chimera* more exhaustively and then examining the concerns associated with different types of chimeras. In this commentary, therefore, I first offer a taxonomy of chimeras and then speculate on how that taxonomy might illuminate the ethical issues the category raises. I conclude that ethical issues are not raised by whether something is a chimera but on the basis of three other questions about the chimeric organism: its "humanity," its "naturalness," and its proposed uses.

The *Oxford English Dictionary* (OED) did not give a biological definition of *chimera* in its 1971 edition, although the famous second edition of *Webster's Unabridged Dictionary* had already provided a botanical definition by 1934:

> "4. *Bot.* A mixture of tissues of different genetic constitution in the same part of a plant."

In its 1989 second edition the OED added, as the fourth figurative definition, the following:

> "d. *Biol.* . . . An organism (commonly a plant) in which tissues of genetically different constitution co-exist as a result of grafting, mutation, or some other process."

The OED traced the term to a German scientist, H. Winkler, in 1907.

Robert and Baylis go beyond all of these definitions, not only in the specifics of some of their proposed chimeras, unanticipated by dictionary writers, but by including hybrids. I believe their broader approach is appropriate. The core idea in the biological use of *chimera* is captured by the following broad definition: "a single biological entity that is composed of a mixing of materials from two or more different organisms." This broad definition can then be played out across four important dimensions:

1. the biological constituents that are mixed;
2. the relationship between the two organisms being mixed;
3. how the mixing is done ("naturally" or "unnaturally," by which, for this purpose, I mean through technical human intervention); and
4. when the mixing takes place.

The biological constituents mixed vary substantially. In current uses they can be cells, tissues, or larger body parts. They can be gametes (eggs and sperm), as in hybrids. Or they can be genes—individually, collectively, or, via nuclear transfer, as whole genomes.

The organisms can be related in various ways. They might be from the same "type," "breed," or "race" of the same species. They might be from different subparts of the same species. They might be from different but closely related species. Or they might be from distantly related species.

Some forms of "mixing" occur naturally, as the mixing of egg and sperm produces, in sexually reproducing species, a single biological entity that is composed of a mixing of materials from two different organisms. Others can be done only with human intervention using relatively nontechnical (and often long-practiced) methods, such as the common practice of grafting limbs from one species of fruit tree onto the trunk of a different species. Still other forms of mixing, such as the creation of embryonic chimeras across species lines, require technical (and relatively new) human intervention.

The significance of the timing of the mixing comes largely from the potential effects of the mixing. Mixing at the time of the formation of a zygote or in an early embryo has broad potential implications for the resulting organism; by contrast, a kidney transplant in an adult seems likely to have a narrower and more defined effect on the recipient. Gamete mixtures or nuclear transfers take place, necessarily, at the beginning of an organism's existence; the timing issue is more important in mixing genese, cells, tissues, or body parts.

Tables 1, 2, and 3 are an attempt to capture the ramifications of this approach to defining chimeras, providing examples for some (but not all) of the possible variations. (The rows filled in as "none?" simply reflect the limits of my knowledge or imagination.)

Table 1. Mixing Cells, Tissues, or Body Parts

	Naturally	*Unnaturally*
Same species, same breed		
early	mosaic twins	embryonic mosaics
late	mother with fetal cells	blood transfusions, transplants
Same species, different breed		
early	interracial mosaic twins	embryonic mosaics
late	mother with fetal cells	interracial transplants
Different but related species		
early	none?	embryonic mosaics
late	none?	pig heart valves
Distant species		
early	symbiotes	embryonic mosaics
late	symbiotes?	transplants

Table 2. Mixing Gametes

	Naturally	*Unnaturally*
Same species, same breed		
	sexually-produced offspring	IVF children
Same species, different breed		
	interracial offspring	interracial IVF children
	"mixed-breed" pets	male dog big, female dog small, surrogate mother
Different but related species		
	mules, hinnies, ligers, tions	IVF hybrids
Distant species		
	none?	none?

Table 3. Mixing Genes or Genomes

	Naturally	*Unnaturally*
Same species, same breed		
early	somatic-cell mutations	germ-line gene therapy
late	somatic-cell mutations	somatic cell gene therapy
Same species, different breed		
early	none?	interracial germ-line gene therapy
late	none?	interracial somatic-cell gene therapy
Different but related species		
	none?	gene transfers, Chinese hamster ovary cells used to produce human proteins
Distant species		
	retroviruses	bacterial or yeast production of human proteins

Attempts at definition have their own definitional problems. I have little problem considering lichen as chimeras. These early symbiotic mixtures of algae and fungi that appear, to the casual human observer, to be a single organism and where the presence of each species is necessary for the other's survival. Less clear to me is whether, for example, the fact that normal human beings have lots of intestinal bacteria should lead us to consider most people as human-bacterial chimeras. Humans probably contain as many bacterial cells in their intestines as they have in their

whole bodies, and those bacteria seem to play useful but not essential roles for their human "hosts."

Similarly, one might want to think about chimeras from outside naturally occurring biological species—the transfer into an organism of genes that are newly created, for example—or from nonbiological sources, such as prostheses. Is a man with a wooden leg a chimera made from a human and a tree? One could even imagine some kind of nonmaterial "mental" chimerism. Moving the memories or personality of a person into a different person, into an organism from a different species, or into a computer might, if feasible, be considered a kind of chimerism.

I do not intend the taxonomy I have proposed for different kinds of chimeras to be definitive. The more important question is whether it is useful. I think it is.

What kinds of chimeras are likely to raise serious concerns, among either academics or among the contemporary North American public? Nothing done "naturally" seems likely to raise ethical concerns, although interracial procreation did raise ethical concerns—and justify felony charges—in some parts of North America within human memory. Among the "unnatural" combinations, mixing human and nonhuman life forms seems most likely to cause concern. (Robert and Baylis restrict their discussion to nonhuman animals, but it is not entirely clear why plants would not raise similar issues; bacteria, because of their relative invisibility, might pose a slightly different case.)

Moving nonhuman parts into human beings seems troubling. But, after a few early reports of patient qualms, the use of pig heart valves for medical procedures now raises little concern. Apart from pragmatic fear of the passage of disease and some animal rights concerns that are quite distinct from issues of chimerism (the animal rights critique of raising pigs for organs is not, I believe, substantially different from the animal rights critique of raising pigs for bacon), other plausible single organ xenotransplants into human beings seem unlikely to be heavily controversial. On the other hand, if it were feasible to transplant a chimpanzee brain into a human, or if a human were given a large number of organs from nonhuman sources, people might worry whether the resulting organism was really human.

In the other direction, moving human "parts" into nonhuman beings has proven acceptable when the result was a cell making human proteins for human medical use. The creation of the SCID-hu mouse with a human immune system, as a tool for researching human immune system function, has not raised any outcry. Creating a mouse with a brain made from human neurons, as proposed by at least one researcher, has attracted some press attention and does raise some concern. Putting human brain tissue into nonhuman primates can be even more problematic.

Creating chimeras that do not involve human beings seem to raise weaker concerns. The mule—a cross between a male donkey and a female horse—is not generally shunned. [I am told, however, that Jewish law, while allowing observant Jews to buy and use mules, does not allow them to make mules (Zoloth, personal communication).] Concerns about genetically-modified food might involve chimeric concerns, expressed perhaps as concern about moving genes from fish into fruit, but concerns about genetically-modified organisms might also apply to genetic modifications that stay within species lines, such as modifications that increase the expression of growth hormone.

In summary, chimeras that are produced "naturally" seem to raise few concerns. Many "unnatural" chimeras are also uncontroversial. Chimeras made by moving nonhuman parts into human beings would raise concerns when they are significant enough to cast doubt on the humanity of the recipient. Chimeras made by moving human parts into nonhuman beings would raise concerns when they are significant enough to raise the question of the possible humanity of the recipient. In both cases the "importance" of the parts—brains and gametes are more important than heart valves or skin—and the number of parts moved—transplanting five visceral organs would be more troubling than transplanting one—seem significant. So do the uses of such part-human, part-nonhuman chimeras. Making a chimera of a human and a nonhuman is much less controversial when done for medical purposes than if such a creature were made for entertainment or "art." The acceptability of totally nonhuman chimeras might also depend on their uses—chimeras as human food might raise special concerns for some because they are eaten. Although chimeric cotton or chimeric trees might raise environmental or other concerns, those concerns might not hinge on the chimeric nature of the organism. And, particularly in the nonhuman cases, the concern might arise more from the "unnatural" status of the organism than from its source in two different organisms.

The *Oxford English Dictionary* does provide other definitions of *chimera.* The 1989 second edition includes this:

> "3. *fig.* . . . b. An unreal creature of the imagination, a mere wild fancy; an unfounded conception (The ordinary modern use)."

As an ethical concern, chimerism per se might itself be "an unfounded conception." The fact that something is or isn't a chimera does not in itself raise ethical concerns. A new type of organism might raise concerns because of the possibility that it could create confusion about human versus nonhuman identity, because of the "unnaturalness" of its creation, or because of the perceived frivolity of

its use—whether or not it meets anyone's definition of *chimera*. ■

Acknowledgment

The author is grateful to the participants in the April 2003 workshop at Dalhousie University, "Interspecific Chimaeras: Scientific Necessity, Moral Abomination, Neither, or Both?" and to its organizers—Françoise Baylis, Jason Scott Robert, and Fern Brunger.

References

The Oxford English Dictionary, 2nd. ed. 1989. Oxford, U.K.: Clarendon Press.

Robert, J. S., and F. Baylis. 2003. Crossing species boundaries. *The American Journal of Bioethics* 3(3):1–13.

Webster's New International Dictionary of the English Language (2nd ed., unabridged 1934).

Crossing Species Boundaries and Making Human-Nonhuman Hybrids: Moral and Legal Ramifications

A. M. Chakrabarty, University of Illinois College of Medicine

People often react with horror, disgust, or simply indifference when asked about the advisability of creating part-human, part-nonhuman chimeras. Few people will express a positive feeling about this. Ingrained in people's minds is the idea that birds, animals, or fish are creatures created by God to be separate and distinct from human beings and should remain as such. Although hybrids among closely related animals are known (e.g., mules) and certainly plant hybrids are well-known, somehow crossing the so-called evolutionary barrier through scientific interventions does not resonate well with most people; it is considered an overreach for scientists to play God. This then becomes a highly emotional issue when one talks about creating artificial human-nonhuman animal hybrids without clearly defining the nature of the hybrids or the purpose of creating them.

In their thought-provoking article "Crossing Species Boundaries," Jason Scott Robert and Françoise Baylis (2003) critically examine why this is so. Why do people think that species identity is fixed by nature? Why should natural separation be maintained and the boundaries not be breached? They argue convincingly that the biological species concept is flawed and that there are enough variations for a strict definition of species to be meaningless. If species cannot be defined, then the fear of crossing the evolutionary boundary is irrational. They point out from the genome sequences that there is a variable but identifiable relatedness between the nucleotide sequences of *Homo sapiens* and other animals, worms, flies, and so on, including plants. They argue that there is no unique DNA sequence in the human genome, as far as is known, that points to the uniqueness of *Homo sapiens* as compared to the rest of the animal world. Indeed, the DNA sequence identity between chimpanzees and human beings is very high, between 98.4 and 98.8%, close to the 99.9% identity among human beings. Yet, people will be loath to accept a chimpanzee as akin to a human being, their gentle nature and above-average intelligence notwithstanding!

So, what's so unique about human beings? Robert and Baylis consider the often promoted argument that language skill is unique to human beings. They point out, however, that not all human beings speak or write a language and that, although we don't necessarily understand what they say, dolphins do many things when instructed, thus showing a high level of communication skill. I remember that as a child I used to visit the home of my sister, who had a parrot that I always thought was kind of stupid. Whenever I would enter my sister's home, the parrot would start "Here comes the jerk! Here comes the jerk." I think he was coached by my nephew; nevertheless, I believed strongly that all birds in general, and parrots in particular, had no intelligence or language skills and were particularly deficient in recognizing super-intelligent human beings! But they did speak a human language!

Having found no rational reason why there should be any ethical debate about the prospect of crossing species boundaries between human and nonhuman animals, Robert and Baylis conclude that part of the reason for people's repugnance to accept such hybrids is because they are thought to be unnatural, perverse, offensive, or frightening. People also believe that they have moral reasons not to accept human-animal hybrids, as they would certainly perform roles different from the known societal roles animals normally hold, such as being sources of food, performers of hard labor, transports, objects of hunting, and so on. Whatever the reasons, Robert and Baylis find them

Boklage,

Embryogenesis of chimeras, twins and anterior midline asymmetries,

HUMAN REPRODUCTION, vol. 21, no. 3 (2006)

Human Reproduction Vol.21, No.3 pp. 579–591, 2006 doi:10.1093/humrep/dei370
Advance Access publication October 27, 2005.

Embryogenesis of chimeras, twins and anterior midline asymmetries

Charles E.Boklage[1]

Laboratory of Behavioral and Developmental Genetics, Department of Pediatrics, 3N72 Brody School of Medicine, East Carolina University, Greenville NC 27834 USA

[1]To whom correspondence should be addressed. E-mail: boklagec@mail.ecu.edu

Human spontaneous chimerism, with one body built from cells of both twins of a dizygotic (DZ) pair, is supposed to be extremely rare, arising from the exchange of blood cells through placental anastomoses. Mosaicism is supposed to be far more common, arising from single zygotes by embryonic mutation. Because typical diagnosis of mosaicism can neither identify nor exclude chimerism, 'mosaicism' may often be chimerism undiscovered. Evidence shows chimerism arises primarily from DZ embryo fusion and is not rare, although it has negligible probability under the hypothesis of independent double ovulation and independent embryogenesis. If, instead, DZ twin embryos begin development as a single cell mass, chimerism is likely. This would be consistent with observations that DZ twins develop as differently from singletons as monozygotic twins do with regard to embryogenic establishment of asymmetries of midline neural-crest-driven structures of brain, face and heart. Chimerism is a significant component of human embryonic development that deserves closer attention as a mechanism of developmental variation. The 'common knowledge' understanding of twinning mechanisms is at best inadequate. The importance of the difference lies in what we can learn from chimerism about human embryogenesis and the cellular origins of structures and functions basic to the business of becoming human.

Key words: twins/chimeras/mosaicism/anterior midline asymmetries/human embryogenesis

Introduction

Spontaneous human chimerism has lately drawn increasing notice. As a plot device in television crime drama, the victim knows exactly who hurt her, but DNA from his cheek swab indicates that he is only a brother to the source of the DNA from the rape kit … until his chimerism is discovered. A world-class athlete accused of boosting his endurance by transfusion of extra red blood cells tried to excuse the extra antigens in his samples as a spontaneous sole-survivor twin chimerism (Henderson, 2005). His defence was dismissed, perhaps for the wrong reasons. A woman needing an organ donor was told that two of her three sons were appropriately related to each other, but not to her … until she was found to have a germ-line chimerism producing two different families of germ cells (Yu *et al.*, 2002). When boy–girl twins (opposite sex, OS = proof of dizygosity) are delivered in a single chorion (monochorionicity, MC = proof of monozygosity) (Miura and Niikawa, 2005), astonished questions arise—the only credible answer to which seems to be that the cells from which those dizygotic (DZ) twins developed were together in a single mass of cells around which a single trophoblast/chorion differentiated during the first few days of embryogenesis. They grew from there into separate bodies, with one or both of them carrying souvenir cells of the other's genotype. Their reciprocal chimerism is discovered only because of investigations of that MC-OS-DZ discrepancy.

Chimeras are not visibly different from the rest of us unless a developmental anomaly in one of the cell lines, or sex discordance between the cell lines, sometimes causes a visibly abnormal phenotype. Without such cause for notice (as would usually be the case), they are impossible to differentiate from single-genotype people by ordinary observation and seriously difficult to identify even with the best of the newest biomedical technologies. Cases are discovered in the population with low frequency and high technical difficulty, creating the pervasive false impression that they are rare. Critical consideration of their cellular origins should improve understanding of human developmental biology, especially with respect to the cellular origins and developmental consequences of twinning, and the intimately related establishment of normal asymmetries of structure and function. Much of what is offered as biological background is not supported by physical evidence and is probably wrong. The object of this work is to assemble the available evidence into a coherent and useful idea of what we should learn from the special embryogenic events that lead to the development of these special people.

Subjects of the analysis

We are concerned here only with spontaneous chimerism in individuals whose mixed cell lines arose without medical artifice—not from transfusion or other tissue transplantation. Because experimental chimeric mammals and birds have been powerful tools for studies of developmental biology, some of their characteristics will be mentioned to help with understanding what we might expect to see in, and learn from, spontaneous human chimeras.

Churchill's Medical Dictionary (1989) defines a chimera as: 'an organism composed of two or more genetically distinct cell types.' In her review of the biology of the human chimeras known in 1983, Tippett (1983) says: 'a chimera has cells from two or more zygotes.' The definition in Churchill's Medical Dictionary (1989) mentions somatic mutation as a possible source of chimerism, but goes on to say: 'it occurs in humans most commonly when the blood of dizygous twins mixes *in utero*.' The definition in the Online Medical Dictionary (2004) does not mention mixing of bloods, but offers fusion of embryos first among the possible origins suggested.

Chimeras = Mosaics? Mosaics = Chimeras? Both? Neither?

In the Online Medical Dictionary (2004), 'chimera' is the last word in their definition of 'mosaic'. In Anderson *et al.* (1951), we find: 'a mosaic is formed of cells of a single zygote lineage.' Churchill's Medical Dictionary (1989) defines 'mosaic' as: 'in genetics, an individual whose cells consist of at least two genotypically distinct populations that arose after fertilization through somatic mutation or somatic nondisjunction.'

In the actual everyday practice of clinical genetics, a diagnosis of 'mosaicism' results from cytogenetic analysis carried out for prenatal diagnosis or for explaining a congenital phenotype which a clinical geneticist believes might represent an aneuploidy. Bodies composed partly of normal cells and partly of chromosomally abnormal cells are not very rare in such situations, appearing with a frequency in direct proportion with the clinical intuition of the geneticist choosing patients to be tested in that way. No such investigation is made with regard to 'multifactorial' or single gene anomalies. In neither case can I find any consideration that phenotypic variation might ever be due to mixed genotypes and proportional to the fractions of abnormal versus normal cells.

Therefore, 'mosaicism' is—not by theoretical definition, but as a matter of everyday clinical genetic understanding and practice—a cytogenetic phenomenon.

When a newborn, or an adult never properly diagnosed before being found in an institutional population, shows signs of chromosomal anomaly, blood samples are taken in the expectation of finding an abnormal genotype to explain the phenotype. Sometimes many, even most, of the cells are normal and the diagnosis is 'mosaicism'. If the technicians cannot find at least two or three identically abnormal chromosome sets in cells from 50 white cell clones, then the patient will often lose a pinch of skin from under each arm to provide fibroblasts for culture and further testing. Some mosaicisms not detectable in blood do show up in skin, often with different normal versus abnormal proportions in samples from the two arms. When no evidence of the expected anomaly can be found in the blood or skin of such a patient, the belief usually lingers that there are abnormal cells in there somewhere—either in tissues not sampled, or previously active in embryogenesis but having died off to a presently undetectable level. We do frequently find cell line fractions in samples from 'mosaic' individuals varying over time (Hansen *et al.*, 1984) and we have, after all, examined only a few cells from only one or two tissues.

When the technicians find the all-aneuploid or part-normal-part-aneuploid mixture of cells that they sought, the search is over. Samples are usually not tested for differences other than those found in the karyotype. The studies that typically yield a diagnosis of mosaicism do not expect chimerism, can seldom recognize it, and cannot exclude it. The laboratory may be motivated to additional efforts by certain sex chromosome differences between the cell lines, or the obvious involvement of more than one chromosome, such that a single segregation anomaly becomes an implausible answer (Wiley *et al.*, 2002).

The cell line differences typically observed in mosaicism are supposed to have arisen from post-zygotic (mitotic) error. Some change is supposed to have occurred in one of the cell divisions in embryogenesis, descendants of which mutated cell persist as additional cell line/s among the normal cells. The most common such finding is partial trisomy; to explain which we suppose that *anaphase lag* has occurred in an embryonic mitosis, producing trisomic and monosomic daughter cell lines by causing both chromatids of one member of one chromosome pair to be incorporated into the same daughter cell nucleus (cf. Cupisti *et al.*, 2003; Katz-Jaffe *et al.*, 2004), and leaving the other daughter cell missing one copy of that chromosome. However, we almost never find any cells with the autosomal monosomy corresponding to a discovered partial trisomy.

The mitotic error model for mosaicism generally accepted among clinical geneticists, the story usually told to medical students and to the parents of such patients, has become the standard answer by repetition alone. It is neither the only possible way to explain the routinely incomplete observations nor the most likely when all available evidence is considered together.

'Mosaics' identified clinically in this way are not rare among people with aneuploidy syndromes, particularly among those with relatively mild phenotypes. When we do undertake cytogenetic prenatal diagnosis by chorionic villus sampling, ~2% of such samples yield two cell lines, generally recognized as differing only because of an autosomal trisomy in some fraction of the cells (Viot, 2002). Most such cases are called examples of 'confined placental mosaicism', because we find the fetus itself normal at amniocentesis later in the pregnancy and normal at delivery. Unless the discovered 'mosaicism' involves a sex chromosome difference or at least two different chromosomes, no further examination is considered necessary (Falik-Borenstein *et al.*, 1994). There have been passing mentions of the possibility of a vanished twin as the source of the abnormal cells (Tharapel *et al.*, 1989; Kennerknecht *et al.*, 1991), but I find no published record of that prospect having been considered in any depth.

'Germline mosaicism' has become a routine explanation for certain apparent departures from Mendelian inheritance. When

a highly penetrant dominant disease allele disappears and reappears in a pedigree ('skips a generation'), when an autosomal dominant or X-linked recessive disorder appears as if by new mutation generating an abnormal allele not found in samples from either parent and then repeats in siblings (which a new mutation is highly unlikely to do), 'germline mosaicism' sometimes seems less improbable than the number or kinds of new mutations necessary to explain the observations (Cutler *et al.*, 2004; Ferreiro *et al.*, 2004; Gloyn *et al.*, 2004). Germline mosaicism may be declared, to explain such discrepancies between siblings, and the mixed genotype parent is identified as such only later by further investigation (Mayr *et al.*, 1981; Yu *et al.*, 2002). The chimeric woman reported by Yu *et al.* (2002) would not have been discovered but for the level of genotyping involved in seeking a transplant donor, and the shock value of questionable maternity.

Finding chimeras

We do not expect to find chimeras because most of us are ignorant of their existence and the informed few just know they are too rare and bizarre to require consideration. We don't look for them because we don't expect to find them and we don't find them until we trip over evidence we cannot ignore. The human spontaneous chimeras identified as such to date comprise only the small fraction of all chimeras in the human population which we have been unable to ignore.

Most known chimeras have become known in one of two ways. There is blind chance, among people with unremarkable phenotypes, who are discovered in some genotyping situation to carry three or four, instead of one or two, alleles at multiple loci (Tippett, 1983; Bromilow and Duguid, 1991; Mifsud *et al.*, 1999; Drexler *et al.*, 2005). Routine blood-banking tests are nearly blind to small admixtures; unless there happen to be informative allele configurations in the subject's family for several of the routinely tested loci, and the minority genotype constitutes a substantial fraction of all cells examined, chimerism will generally not be discovered that way. One recent case was found when a surgical patient experienced acute intravascular hemolysis after transfusion of what more sensitive testing proved to be a unit of chimeric blood (Pruss *et al.*, 2003).

And there is sex. Most of the other chimeras we know about have been found because of a sex difference between the cell lines in a chimeric individual, manifested by anomalies of sexual anatomy or maturation or function, causing a search for an explanation for the odd sexual phenotype, leading to discovery of mixed cell lines (Verp *et al.*, 1992; Strain *et al.*, 1998).

Monochorionic boy-girl twins may be the most dramatic kind of mixed-sex anomaly (Souter *et al.*, 2003)—both sexes are no more 'normal' inside one chorion than inside one body. Whether or not we now know how they do that, we have every reason to believe they had to be together in a single mixed-sex embryonic cell mass when trophoblast differentiation occurred in the first few days of embryogenesis with both of them inside. Non-sexual developmental anomalies, if sufficiently visible, may also trigger appropriate investigation (Nyberg *et al.*, 1992). Predominance of sexual maldevelopment among discovered developmental anomalies is to be expected due to the relatively benign nature of most sex development anomalies and the high level of interest it attracts. 'Boy or girl?' is still very often the first question society asks about each of its new members. My students are always astonished to learn how often the answer to that standard question is not perfectly clear and the harm that may come from forcing the issue.

Lessons from experimental chimeras

Many thousands of experimental chimeras have been generated for studies of embryogenesis and development (Gardner and Davies, 2000; Nagy and Rossant, 2001; Gardner, 2002; Tam and Rossant, 2003; Le Douarin, 2004). Transgenic animals, such important research tools in modern biotechnology, begin as chimeras, grown from embryos into which cells of a modified genotype have been inserted. In some of those, some of the extra cells will enter germ-line developmental pathways and produce gametes with the modified genotype. If the introduced mutation is compatible with viable development, this may allow for the breeding of whole-body transgenic organisms. Often, we learn at least as much from differences in development and functionality between the different cell types in the bodies of chimeric individuals. Those research chimeras would be useless for many of their intended purposes if chimerism tended to be homogeneous. It is characteristic of animal chimeras to be patchy, with one (piece of) tissue composed primarily of one cell type and the next of the other. Koopmans *et al.* (2005) show chimerism was never present in every organ examined from any single individual. It follows that failure to detect chimerism in blood or any other one particular sampled tissue is negligible evidence against the presence of chimerism in any other part of the same body. This is especially true when the tests in question are confined to cytogenetic analyses or routine blood antigen genotyping, or even a high-resolution genome scan performed on DNA from a single tissue, especially if signals from extra alleles are ignored as noise (if <30% of peak signal) or declared to have come from contaminated samples (if >30%) (cf. Ewen *et al.*, 2000).

Spontaneous chimeras are DZ twins (or mothers)

Some cases of human spontaneous chimerism may arise from embryonic or fetal cells colonizing a mother's body (Lo *et al.*, 1996; Reed *et al.*, 2004; Stevens *et al.*, 2004; Khosrotehrani and Bianchi, 2005; Koopmans *et al.*, 2005; Lambert *et al.*, 2005). This occurs, in some cases, with no pregnancy having survived to recognition. In all such cases, extra alleles must match the father of the conceptus from which the extra cells arose.

With the exception of this fetal-in-maternal chimerism, human spontaneous chimeras are products of DZ twinning events. DZ twinning is the only naturally-occurring human circumstance in which embryos with different genotypes are available to colonize one another. This is not the same as twin birth. Neither the delivery of the co-twin, nor any oddity of the placenta, nor any other evidence or suggestion of twinship is required. Chimerism arises from twin embryogenesis; it is not a function of gestation or delivery as twins.

If the genotypes of the cell lines in a human chimera are incompatible with belonging to siblings, then the chimerism is not spontaneous. Genotype data from parent/s or sibling/s may be required for a definitive answer to that question, which requirement might constitute a difficulty in the investigation of any case with no available first-degree relatives. However, even when the extra genotype clearly could be that of a sibling, if there are antibodies against the extra antigens, then the extra cell line producing those antigens was probably not present in the embryo before the establishment of immune self-tolerance. Cell lines in a spontaneous chimera will in general be cross-tolerant sibling lines.

Results

Chimeras are not rare

At upwards of one in 12, chimerism cannot be considered rare among liveborn DZ twins, and its occurrence in >20% of DZ triplet sets has to be called common (van Dijk *et al.*, 1996). The immunohistochemical method those workers used has a long history of reliable specificity and exquisite sensitivity (to detect one cell in 10 000 or more), but its use there was limited in scope. That work was performed under the assumption that chimerism in twins occurs exclusively by way of mixing of blood alone via placental anastomoses. Only blood was examined. All possibility of chimerism in other tissues was ignored. Their toolkit included fluorescent antibodies for a few marker antigens and their sample included only twins and triplets born alive as such. They could not have detected chimerism in any set the members of which were concordant for all of their marker antigens, nor in any individual whose second cell line had not survived to the time of testing, nor in any individual whose chimerism occurred only in tissues other than blood. Knowing parental genotypes would have given a better understanding of the relevant probabilities. The frequencies they report, astonishing as they are against the background of general understanding then and still, represent only a fraction of the chimerism among multiple conceptions.

'Kinds' of chimeras?

According to the literature, one might suppose that there are two or more 'kinds' of spontaneous human chimerism, differentiated by the imagined mechanisms of their origins. 'Dispermic', 'whole body', 'generalized' and 'tetragametic' are labels that have been used for cases acknowledged to have arisen from fusion of DZ twin embryos. Chimerism is said to be of this type when it is found in tissues other than blood or when adequate genotyping shows the twin cell lines to be discordant for paternal alleles (Osinska and Woloszyn, 1971; Dauber *et al.*, 1999; Wiley *et al.*, 2002). Otherwise, it is usually imagined to be of the supposedly more common 'twin' chimera type.

'Twin' chimeras are supposed to be chimeric in blood only, and to have become such by way of exchanging blood cells through anastomoses between their placental circulations (Angela *et al.*, 1976; Hosoi *et al.*, 1977; Pausch *et al.*, 1979; Bird *et al.*, 1980; Gilgenkrantz *et al.*, 1981). It has, however, become clear that chimeras among delivered DZ twins are far more common (van Dijk *et al.*, 1996) than blood vessel anastomoses between dichorionic placentas (Robertson and Neer, 1983; Bjoro and Bjoro, 1985; Lage *et al.*, 1989; Benirschke, 1990, 1992, 1995; Machin *et al.*, 1995; Benirschke and Masliah, 2001; Foschini *et al.*, 2003). There are nowhere near enough anastomoses between dichorionic placentas to account for the observed frequency of chimeras. This can reasonably be considered to refute that traditional supposition. Reports of finding chimerism only in blood arise overwhelmingly from situations in which no tissue other than blood was examined.

Twin-to-mother-to-twin transfer?

An alternative explanation which we cannot presently exclude out-of-hand would be the transfer of blood between twins by way of the maternal circulation. We have known for a while, and made good use of the knowledge, that fetal cells are commonly found in the maternal circulation. We use fetal cells in maternal blood samples as substrate for non-invasive prenatal diagnoses. Detection in a mother's body of 'microchimerism' (small colonies of cells from her child or children), even decades after the corresponding pregnancy, and the prospect that those foreign cells might cause graft-versus-host 'autoimmune' disorder/s in the mother, has recently drawn attention (Stevens *et al.*, 2004; Khosrotehrani and Bianchi, 2005; Lambert *et al.*, 2005).

However, women with no history of pregnancy or transfusion are also commonly found to be chimeric in autopsy specimens of internal organs (tissue-specific cells, not just blood cells passing through, and in no case was the chimerism found in every organ examined from any given woman). The explanation offered as most likely was that the extra cells came from pregnancies that failed before clinical or maternal recognition (Koopmans *et al.*, 2005; cf. Boklage, 1990). That work was performed, for better understanding of transplant surgery results, by probing for cells that included Y-chromosome DNA sequences. For present purposes, clearly that approach ignores approximately half of all fetal-to-maternal-transfer chimerisms—in which the conceptuses providing colonizing cells were female. Furthermore, that approach allows for no proper further investigation of the prospect that some of the chimerism found in women with no history of pregnancy or transfusion may have arisen from their own embryogeneses rather than from unrecognized pregnancies. Extra alleles could and should be traced to determine whether the 'foreign' cells match mates or parents or siblings. (If they are products of conception, they must match the father of the conceptus. If arising from her own embryogenesis, they should match her parents or siblings. Only the latter should occur in virgin females.)

I have found the theoretical possibility mentioned, but have found no demonstration in human subjects that nucleated cells move from maternal to fetal blood with any frequency remotely comparable with that of fetal-to-maternal transfer. It should be easier, because maternal antigens in a fetus should encounter no immune resistance and would be expected to acquire permanently all benefits of self-tolerance when established by the child's immune system. I have been unable to find documentation of any significant frequency of permanent

maternal-to-fetal exchange of cells capable of ongoing development and the establishment of permanent colonies. Reed *et al.* (2004) have shown that maternal cells sometimes colonize a fetus, but they found it only in association with an HLA-DQ A1*0501 allele in the mother. Lo *et al.* (1996) found maternal DNA in almost half of their cord blood samples, but only at PCR sensitivity 1000-fold greater than that which sufficed to demonstrate all of the fetal-to-maternal transfers in their sample. This is not satisfying evidence that the average fetus routinely incorporates from the maternal circulation functional nucleated cells with the developmental potential to establish permanent chimerism.

DZ twins are not just womb mates

It is clear that most twin conceptions do not result in twin births. Survival of both members of a pair of twins from fertilization to term is rare (~1 in 50 in apparently optimal circumstances). There is a sole survivor from ~25% of twinning events and none from the rest. Sole survivors of twin conceptions are several times more common among live births than twins. By conservative estimate, sole survivors of multiple conceptions are at least as frequent as one live birth in eight (Boklage, 1990, 1995), roughly 10 times the frequency of twin pairs among all deliveries. Given that most spontaneous human chimeras discovered to date have been under the lifelong impression that they had always been singletons, there is no reason to suppose chimerism would be less frequent among sole survivors of DZ conceptions than it is among liveborn DZ twins (van Dijk *et al.*, 1996). We must infer that most chimeras are born single.

The traditional assertion that the excess prenatal mortality among twins is due to monozygotic (MZ) twins is gratuitous and wrong. Direct examination with good zygosity diagnosis shows that same sex DZ twins are at least as vulnerable to fetal and neonatal mortality as the MZs are (Boklage, 1985, 1987*a*).

The many ways in which twins of both zygosities differ in their development from singletons (Boklage, 2005*a*) do not result from gestation or delivery as twins, but from circumstances of embryogenesis peculiar to twinning—specifically from those parts of embryogenesis in which brain, craniofacial and behavioral asymmetries are established (Boklage, 1987*b*,*c*, 2005*a*; Gardner, 2001; Sudik *et al.*, 2001; Golubovsky, 2002, 2003*a*,*b*). DZ twins are developmentally at least as different from singletons as the MZs are, and in very much the same ways. The differences concentrate in embryogenic asymmetry variations of anterior midline structures.

Oddities of asymmetry development in twins have been falsely assumed to be routine and exclusive to the MZs from generations of folklore to the effect that MZ twins arise from some mechanical 'splitting' event whereby the embryo is torn in two and incipient structural asymmetries are disrupted and must find ways to realign if development is to continue ('… what should have been the left side of Harry had to become the right side of George …'). As witness, the enduring currency of the notion that same-sex twins discordant for handedness must be 'late-splitting' 'mirror-image' MZ twins (cf.Boklage, 1981; Derom *et al.*, 1996).

DZs, on the other hand, are supposed to come from separate and independent double (ovulation + fertilization + embryogenesis). According to that supposition, DZ twins have no reason to develop at all differently from singletons, especially in the establishment of structural and functional asymmetries in early embryogenesis and especially not to differ from singletons in the same ways that MZs do. But they do. They do just that, in every relevant way that they have been measured. DZ twins are not developmentally equivalent to singletons. The differences between DZ twins and singletons are very similar to the differences between MZ twins and singletons, and are not compatible with the expectations of independent double ovulation and independent embryogenesis as their origin (Harlap *et al.*, 1985; Boklage, 2005*a*).

Monochorionic male-female twins? That can't be right!

The male–female chimeric monochorionic DZ twins (MCOSDZs) reported by Souter *et al. (*2003) are considered in the editorial of the same journal issue (Redline, 2003) as disproving dogma because they contradict the doctrine that monochorionicity is proof positive of monozygosity. Those presentations, however, leave a strong impression that they are seeing those MCOSDZs as a freakish exception that might almost rather prove the rule, caused perhaps by one or more of the ways that artificial reproductive technologies bring extra developmental vulnerabilities. But … cells did it, cells never do anything they don't 'know how' to do, and cells don't know anything about the rules we have imagined for them. Dismissing or ignoring them is not okay. 'How?' seems likely to be important. 'Dogma' and 'doctrine' are not words too strong for this use. At the Fifth International Congress on Twin Studies in Amsterdam in 1986, a young physician from Glasgow tried to tell us about three monochorionic pairs among 12 in his sample, in whom he had found (with testing more extensive and more sensitive than the usual zygosity genotyping) discordant blood grouping markers suggesting dizygosity (Mortimer, 1987). The pillars of the Society came crashing down about his head. The tenor of the response from the floor was: '... of course, one must know, of course, that only monozygotic twins can be monochorionic. Results such as yours suggesting otherwise must have come from a very unreliable laboratory …'

The foundations of the MC = MZ dogma as discussed in Redline (2003) are from Husby *et al.* (1991) and Vlietinck *et al. (*1988). Those studies were performed to test the applicability of Weinberg estimates of zygosity fraction against genotyped samples of twins. No twins who were identified in the studied birth records as monochorionic (these investigators did not attend the deliveries) were found to differ clearly at any of the loci tested, and they found no boy–girl twins recorded as monochorionic, which would have required further investigation if it were not summarily dismissed as obvious error.

Given that monochorionic twins apparently without exception do have placental anastomoses through which they exchange blood, concordance for the handful of blood antigen markers used to test zygosity in these samples cannot be considered overwhelming evidence. Souter *et al.* (2003) reported that the initial genotyping of the MCOSDZ pair they reported

was consistent with monozygosity. Using a nearly identical panel of markers in an experimental control not mentioned in Husby *et al.* (1991) or Vlietinck *et al.* (1988), Nylander and colleagues found genotypes concordant for all tested markers, consistent with criteria for confident diagnosis of monozygosity, in approximately one quarter of the boy–girl pairs in their samples. They 'corrected' their results from the same-sex pairs accordingly (Nylander, 1974; Nylander and Corney, 1977) and called the corrected results consistent with Weinberg method expectations without addressing the implication of reduced polymorphism among the parents of twins.

A number of other MCDZ pairs have been reported (Nylander and Osunkoya, 1970; Iselius *et al.*, 1979; Bieber *et al.*, 1981; Vietor *et al.*, 2000; Quintero *et al.*, 2003; Williams *et al.*, 2004; Yoon *et al.*, 2005), plus the recent cluster of six such pairs reported by Miura and Niikawa (2005). The MC pair reported in Bieber *et al.* (1981) was investigated because one member was acardiac; extensive genetic differences proved dizygosity. The MC twins reported in Yoon *et al.* (2005) were investigated because of visible discordance for what proved to be Beckwith–Wiedemann syndrome. They were found to be DZ, discordant also for Klinefelter syndrome and several unlinked marker loci. All of the others in these references are boy–girl pairs, without which unignorable oddity monochorionicity would have been unremarkable and the possibility that they were dizygotic would almost certainly not have been investigated.

The Beckwith–Wiedemann syndrome, by the way, is reported to be excessively frequent in monozygotic twin pairs, with an excess of female pairs, and almost always discordant (Weksberg *et al.*, 2002; Bestor, 2003). The excess of twin pairs associated with Beckwith–Wiedemann has been identified as monozygotic, in spite of substantially discordant phenotypes … generally because of sex-concordant monochorionicity. It is not clear that the level of genotyping capable of discovering chimeric dizygosity was performed in any of the reported cases.

There is ample reason to suppose, and to test the prospect carefully, that monochorionic DZ twins are also rather more frequent than finding them is.

Two into one, and back

In thousands of experiments in which all or part of one experimental mammalian embryo has been put inside another one, of same or different genotype (or sex or strain or species), the result is not twinning but single chimeric offspring or embryo failure (cf. Gardner and Davies, 2000; Gardner, 2002). The development of separate twin bodies from a single embryonic cell mass (regardless of the number of genotypes among those cells) requires the cellular behaviour of a monozygotic twinning event ... subsets of the cells in the mass must establish two distinct systems of body symmetries, two sets of head–tail, back–belly and left–right axes.

This is all there is to 'splitting'. In the first few cell divisions, molecular decisions are made about where the head and the tail are supposed to go, who gets to be back and which has to be belly, and which cell will get the transcription factor subsystem that will determine that its progeny will later migrate into the gonadal ridges to induce the differentiation of the gonads and become the gametes. Unless something is badly wrong, the entire three-dimensional armature is microscopically visible as soon as the location of the prochordal plate and/or the primitive streak becomes apparent to mark anterior versus posterior and leave left–right no choice because dorsal–ventral has already been clear for a few days. All the axes are quite clear by the sixth or seventh day because it takes a while for the cells to show up in their proper places after the organizing decisions are made. This is only a day or so after the zona comes off, so all those decisions must normally be made while still inside the zona. The zona pellucida is elastic. It's tight in there. No room in there for anything that could be visualized as a 'split'. No ripping. No tearing. No child's hair to tie the one embryo almost in two á la Spemann. The cells just set themselves up in two patterns. As we traditionally interpret the meaning of chorionicity: if such twins are to be dichorionic (apparently, but hardly proven to be, the more common outcome), the separate systems of body axes must be established within the first 1–3 days post-fertilization. A few hours less quickly, and they assume the extra gestational hazards of monochorionicity.

We have no evidence of any constraint on the final allotment, between the twins, of cells of the different genotypes. The results in van Dijk *et al.* (1996), limited to what can be understood from blood alone, show some very small numbers of cells of the co-twin's genotype and some quite substantial fractions, some reciprocal exchanges and some apparently one-way.

On the fusion of male + female embryos

The normal excess of males in human births in spite of most reports showing excess male losses throughout pregnancy apparently can be explained by observations that a paternally imprinted X-chromosome (normally present only in female embryos) substantially slows female embryogenesis (Boklage, 2005*b*). Much faster early development in male versus female embryos would seem likely to predict a predominance of male phenotypes for mixed-sex chimeric individuals, and might be expected to suppress (below the theoretical binomial half) the frequency of live-born chimeric twin pairs appearing as normal boy and normal girl.

Same-sex pairs are found in excess among delivered DZs (James, 1992)—in spite of prenatal losses concentrated in same-sex pairs (Rydhstroem and Heraib, 2001)—among which SSDZs are at least as vulnerable as MZs (Boklage, 1985, 1987a). This follows the pattern behind the 'secondary sex ratio' (Boklage, 2005*b*) and suggests the parallel possibility that the excess of SSDZ pairs at birth, in spite of excess losses among SS pairs throughout pregnancy beyond embryogenesis, may be established by excessive failure of OSDZ pairs in embryogenesis, before pregnancy recognition. Overgrowth of male cells in mixed-sex embryos could cause OS chimeric embryos to appear later in pregnancy as male twins. Most sex-chimeric mice become fertile males (Tarkowski, 1998).

The members of normal, ordinary, dichorionic live born male–female pairs clearly have not developed independently.

They do not have the normal statistically obvious sex differences in craniofacial development found in singletons and members of same-sex twin pairs (Boklage, 1984), and both members of male-female pairs show fetal and neonatal mortality that is significantly lower than their counterparts in same-sex pairs (Boklage, 1985, 1987a).

The excess of males in human births appears due to a paternally imprinted X-chromosome retarding female embryogenesis relative to that of males (Boklage, 2005*b*). The male excess at birth is lower for fathers of African descent than for white European fathers, and higher for Asian fathers. This could mean that the more permissive the paternal X-imprint, the more females, the more twins, and the more male-female twins reach term birth. Also differing over these populations in the same order: average female age at menarche, at first birth and at last birth. Also in the same order, the earlier the trophoblast differentiates, and the greater is the fraction of dichorionic pairs among same-sex pairs and the lower the fraction male among monochorionic pairs and still more so among monoamnionic pairs. The more permissive the paternal X-imprint, the faster apparently moves every aspect of reproduction in females.

Miura and Niikawa (2005) have supposed that artificial reproduction technologies (ART) might be promoting chimerism because all the MCOSDZ pairs they discovered were products of ART procedures in Japan. Given that they would not have found the chimerism in any of those cases had not monochorionic boy–girl twins attracted their closer attention, given that natural Japanese twins are known for their low frequency of OS pairs, given the survival issues surrounding all twins, and given the male > female embryonic growth rate discrepancy, I propose that ART need not increase the probability of chimerism in general as suggested by Miura and Niikawa (2005), but instead need only make rates of male and female embryogenesis more equal by an epigenetic effect, such that the female cells in mixed-sex chimeras would be less likely to be outgrown or pushed aside into an ineffective minority—the better to see 'normal' boy + 'normal' girl twins at birth. There is good and growing evidence that ART protocols in current use are associated with disorders of imprinting (Paoloni-Giacobino and Chaillet, 2004; Gardner and Lane, 2005; Maher, 2005; Shiota and Yamada, 2005). Normal sex-dependent differences in speed of human embryogenesis are reported absent in IVF embryos (Dumoulin *et al.*, 2005).

Discussion

If natural DZ twins must in general arise from independent double ovulations and independent embryogeneses, then spontaneous chimerism should probably be even more rare than it has been imagined to be.

The evidence, however, shows that chimerism is not at all rare and that it must arise primarily from fusion of DZ embryos—an outcome very difficult to explain beginning from independent double ovulation and embryogenesis. What we know about the chimeras we have found and the ways we have found them demands the inference that those human chimeras who have been identified as such constitute a small minority, and that the undiscovered majority are normal people whose chimerism will most probably never be discovered. Human spontaneous chimeras are common; only those identified as such are rare. Chimeric individuals whose bodies are composed of two normal cell lines, or in whose bodies cells of an abnormal line constitute an ineffective minority or exist only in tissues unlikely to be sampled, must constitute the majority of all chimeras and draw no special attention.

Dichorionic twin placentas grow together ('fuse') about half the time, but anastomoses between them are very rare in either zygosity. Spontaneous chimerism is not rare; therefore, placental anastomosis cannot be the way most chimerism happens. If we should wish to maintain the tradition that chimerism results overwhelmingly from mixing of blood alone against the evidence that chimerism is far more common than placental anastomoses between DZ twins, then there is a need for exciting new evidence showing that exchange of pluripotent cells between DZ twins can and does occur quite commonly by way of the maternal circulation. Until such evidence can be gathered, I must infer that spontaneous human chimeras arise primarily from fusion of DZ twin embryos and seldom if ever from fusion of their placental circulations. Many cases of chimerism can be explained only by fusion of DZ embryos, but I can find no case proven to have arisen from exchange of blood alone between DZ co-twins via either placental anastomoses or passage from one twin to the other through the maternal circulation. Chimerism of blood alone is reported overwhelmingly from circumstances in which only blood was examined.

It was suggested that I should consider 'stress effects related to having multiple embryos in a single womb' as a possible 'cause of characteristics specific to both MZ and DZ twins'—rather than sharing a history of deriving two body symmetries from a single embryonic cell mass. The differences at issue here—in a/symmetry-dependent development of neural tube, cardiac tubes, craniofacial structures and brain function—all depend upon cellular/molecular axis-definition processes which must occur in the first few days, while the conceptus is microscopic and probably before even hormonal communication with the mother. Any stresses at issue here seem certain to be internal to the embryogenic process, and it seems important that the outcomes do not differ by zygosity.

The question arises whether DZ twins from independent double ovulation might become monochorionic without spending time together in a single cell mass, perhaps by being close enough at blastogenesis that their respective chorion-precursor trophoblasts might fuse around them. Because blastogenesis and trophoblast differentiation normally happen inside the zona pellucida, premature removal or fusion of the two zonae would be topologically essential to allow cells of the respective trophoblasts even to touch. To arrange such events for experimental purposes, as mentioned above, requires removal of the zonae. In general, the two inner cell masses coalesce as well. Roughly half of all pairs of dichorionic placentas, regardless of zygosity, appear as fused later in pregnancy. Recognition of their dichorionicity in spite of such fusion is not trivial, but routine.

Opposite-sex twins are roughly half of all DZ twins and roughly a third of all live born white European twins. The fraction

of all twins who are OS is larger among live born twins of African ancestry than among white European twins, and smaller among live born twins of Asian ancestry. Except for certain very rare anomalies, opposite-sex twins are dizygotic. (The assumption that OS twins should be exactly half of all live born DZ twins is an approximation, crude at best for any group other than healthy white European twins. The other standard assumption required for any faith in the utility of the Weinberg method of estimating zygosity fractions, namely that OS twins are developmentally equivalent to SS-DZ twins, and thus developmentally representative of all DZ twins, is nonsense.)

Monochorionic twins as a group have more problems than dichorionic twins as a group, but they constitute about half of live born MZ twins of African ancestry (Nylander, 1974; Nylander and Corney, 1977), about two-thirds of live born white European monozygotic twins (Vlietinck *et al.*, 1988; Husby *et al.*, 1991) and >80% (Yoshida and Soma, 1984) of Japanese MZs. (The old Weinberg-based assertion that only DZ twinning varies over subpopulations, while MZ twinning is constant, has persisted in spite of these variations in the biology of MZ twinning.) Monochorionicity has been considered certain proof of monozygosity. Rarely, monochorionic twins are of opposite sex because one has normal 46,XY cells and his twin is a 45,X Turner syndrome female missing the second sex chromosome in all of her cells (extrapolation assumed from a non-mosaic blood karyotype). We call those 'heterokaryotic', monozygotic twins. They are supposed to have arisen from a single zygote, but they have different karyotypes due to anomalous X,Y chromosome segregation in embryogenesis (that would be textbook mosaicism followed by twinning—one might wonder whether there are in fact no autosomal counterparts). 45X,46XY–heterokaryotic MZ twins cause us no theoretical anxiety as long as we can believe that the 45,X female has no 46,XX cells.

Beware of the dogma

Twins who are both opposite-sex (46,XX and 46,XY) and monochorionic raise very different issues. It is not supposed to be possible. It does, however, occur. Therefore, it can. It can occur only by way of embryo fusion. That is what makes it so 'wrong'. The MC = MZ doctrine is only a corollary of an older and deeper dogma at issue in these considerations—the 'common knowledge' that DZ twins just do arise from double ovulation (Boklage, 2005*a*). Only because of that article of faith is the idea of monochorionic, dizygotic twins any sort of surprise in the first place. The same idea is all that stands in the way of understanding chimerism as primarily the result of DZ twin embryo fusion, having little or nothing to do with exchanging only blood through placental anastomoses. Monochorionic DZ pairs particularly and obviously, and spontaneous chimerism in general, imply and require that some fraction of DZ twins have spent at least part of their embryonic lives in a single cell mass. This is extremely unlikely in the shadow of the DZ double ovulation dogma, but not so much if we can drag it out from under there into better light (Boklage, 1987*a*,*b*, 2005*a*). Spontaneous chimeras via DZ embryo fusion, and especially MCDZs, satisfy predictions of an alternate model for the cellular origin of DZ twins—which arises from a list of observations that the hypothesis of independent double ovulation cannot satisfy.

Mechanism(s)

Plausible cellular alternatives to independent double ovulations as source of DZ twinning would have them arising from daughter cells of single secondary oocytes divided symmetrically before sperm entry ('tertiary oöcyte twins' (Boklage, 1987*b*,*c*), often called 'polar body twins'), or those same two half-genomes in an as-yet-undivided secondary oöcyte (Golubovsky, 2002,2003*a*,*b*; St Clair and Golubovsky, 2002). Some find it easier to think of this as a 'rescue' pathway for over-ripe or otherwise compromised oocytes (cf. Bomsel-Helmreich and Papiernik-Berkhauer, 1976; Harlap *et al.*, 1985; Boklage, 1987*b*,*c*). In all of the possible mechanisms, there must be two paternal pronuclei (generally from two sperm cells, but diploid sperm are apparently not yet conclusively ruled out), achieving syngamy with two maternal pronuclei arising from the second meiotic division of the secondary oocyte nucleus, one of which 'should have been' discarded in the second polar body. The maternal pronuclei may be in one cell with an unfinished second meiotic division, or two (tertiary oocytes) after a symmetrical second meiotic division. All variations have the final common expectation of two syngamies producing two zygotes inside a single zona pellucida—indistinguishable from any other two-cell embryo except that those first two cells are of different genotypes. The existence of MCDZ twins requires that it be possible; the apparent origins and distribution of chimerism require that it be frequent.

Assuming that only mothers could influence any probability of twinning by double ovulation, we must suppose that the well-documented paternal effects on probability of DZ twinning (Carmelli *et al.*, 1981; Sathananthan *et al.*, 2001; Golubovsky, 2002; St Clair and Golubovsky, 2002; cf. Tesarik, 2005) are exerted through monovular DZ twinning. The frequency of triploidy shows an ample supply of doubled contributions from both maternal and paternal sources (McFadden and Langlois, 2000; Zaragoza *et al.*, 2000; McFadden *et al.*, 2002; Golubovsky, 2003*a*,*b*). Other major pieces of this puzzle include: (i) suspension of the second meiotic division pending sperm penetration; (ii) the dependency of syngamy and early embryogenic cell division on the centrosomal material and centriole/s provided by the sperm (van Blerkom *et al.*, 1995; Palermo *et al.*, 1997; Sathananthan, 1997; Sutovsky and Schatten, 2000); (iii) the need for the oocyte to conduct a major rearrangement of the sperm chromatin to transform it into a functional paternal pronucleus (Gioia *et al.*, 2005); and (iv) other changes in the oocyte after ovulation (reviewed in Boklage, 1987*b*,*c*). This system of interactive processes required to complete fertilization provides a plausible focus for questions of paternal influence and monovular DZ twinning.

For embryogeneses beginning from a configuration of two zygotes in a single zona, a single chimeric offspring would seem at least as likely as the formation of separate twin bodies. If separation is achieved (requiring the same cellular behaviours as monozygotic twinning), so that concurrent embryogeneses

may proceed in parallel beyond that intersection, the likelihood that the two embryos would carry souvenir cells of each other's genotype seems high.

The existence of monochorionic dizygotic twins provides an unavoidable lesson: twin zygotes, same sex or different, do at least sometimes form a single mixed embryo from which they may emerge as viable twins, often carrying samples of cells from each other mixed in as they build their separate bodies. In such chimeric embryos, spontaneous internal definition of two body symmetries occurs, perhaps most commonly before (dichorionic) but at least sometimes after (monochorionic) cellular commitment to the differentiation of the trophoblast. We have no evidence as to the relative frequencies of those two possibilities. To date, the number of discovered monochorionic DZs is small, but not particularly small compared with the probability of finding them without looking. Any prospect a fused embryo will have for development to live birth as two separate individuals requires the very same cellular event as monozygotic twinning, namely, to create two systems of body symmetry axes inside a single mass of cells, so that they can begin and continue to grow out as two bodies. The twin bodies that may be built upon those cellular/molecular armatures are dizygotic but not independent—they have been at least temporarily within the same one embryonic cell mass. According to the evidence accumulated here, this occurs with much greater frequency than previously imagined, with many more cases undiscovered for want of asking the necessary questions. Unless exchange of pluripotent stem cells between twin fetuses through the maternal circulation can be shown to be routine, such an outcome seems highly improbable for twin embryos from independent oocytes. Chimerism, would, however, be quite ordinary for twin embryos that begin development within a single zona pellucida.

Immunology

Another prediction may be in order. There is lore to the effect that co-twins diagnosed as monozygotic should be perfect tissue transplant donors for each other, and that DZ co-twins should be no better than any other siblings, with only ~25% chance of matching for the primary transplant-compatibility genes. While grafts or transplants between twins who are supposed to be 'identical' do not always take without some immunosuppression (Golembe *et al.*, 1979; Hinterberger *et al.*, 1997), I have found no published evidence that transplant efforts between HLA-non-identical DZ twins have been made on many occasions and constantly failed. Perhaps it has been faithfully assumed that DZ co-twins would be as limited as singleton siblings as source of necessary tissue transplants … assumptions of that sort are certainly common around twins. If DZ twins are reciprocally chimeric as often as they have been reported to be (van Dijk *et al.*, 1996), let alone as much more often than that as I am arguing here, and if that chimerism has been in place since embryogenesis—before and during their immune systems' establishment of self-tolerance, then it seems likely that somewhat more than the HLA-identical ~25% of DZ co-twins might in fact be reciprocally suitable transplant partners (Nylander, 1974; Summers and Shelton, 1985). Such DZ transplant tolerance will occasionally be unidirectional—when only one of the co-twins carries cells of the other's genotype, the single-genotype twin may be expected to reject tissue from the chimeric co-twin. This latter prospect, in turn, might explain some of the transplant difficulties between twins thought to be 'identical' because of sex-concordance and monochorionicity.

Embryogenesis of anterior midline functional asymmetries

The human brain appears to surpass substantially any other kind in the extent and importance of left-right asymmetry in its functionalities. Left-handers plus the ambidextrous comprise a minority variously estimated at ~10% of the population. They differ from the 'strictly' right-handed folks in many ways. According to most genetic models still given any consideration, these 'nonrighthanders' (NRH) constitute a random half of a minority whose members lack the cellular or molecular determinants required to establish the normal/majority human brain function asymmetry. Twins and their parents and siblings (very importantly, of both zygosities equally, and independently of chorionicity among the MZs) have a clear excess frequency of NRH (Boklage, 1981, 1987*b*,*c*; Derom *et al.*, 1996). The malformations that are excessive among twins (neural tube defects, orofacial clefts and congenital heart defects most prominently—all midline/fusion anomalies) are excessive also among first-degree relatives of twins, and all have strong associations with NRH among singleton victims and their first-degree relatives as well. Clearly, neither twin gestation nor twin birth, nothing about twinship beyond associated heritable variations in embryogenesis, causes any of these developmental asymmetry anomalies, because their single born parents and siblings and offspring and unrelated singletons show the same associations. In most of these relationships, there is no zygosity difference. Where there is a zygosity difference, the relationships tend to be stronger among DZ than among MZ twins (e.g. Klaning *et al.*, 2002). This is strongly contrary to the old notions that anomalies such as these belong strictly to the MZs because of their exclusive involvement with some odd sort of embryogenesis. There is no escape from the inference that DZ embryogenesis is more or less exactly as odd as that of the MZs, and no reason to suppose it could get that way beginning with independent double ovulation.

Variations such as these in brain function asymmetry are associated with virtually every oddity of human mental or behavioural development and function. The exact cellular and molecular processes of defining two systems of brain and body symmetry axes from within a single embryonic cell mass, and the results thereof, might reasonably be imagined to differ from the usual embryogenic protocol of defining only one developmental armature per embryonic cell mass. Whatever that system of differences may be, this phase of embryogenesis must be where the symmetry development differences originate between singletons and twins. The developmental differences in embryogenic asymmetry determination between DZ twins and singletons as groups are not occasional or accidental: groupings calculated from patterns of craniofacial development are coherent and highly statistically significant (Boklage,

1984) with negligible overlap. These results represent symmetry determination specific to the head and the neural crest, involved with midline fusion, craniofacial, brain and behavioural development (Boklage, 1984, 1987bc, 2005; Klaning *et al.*, 2002; Klar, 2005; Mitchell and Crow, 2005). The results give no reason to suppose that only some DZs, any less than all, are developmentally different from singletons. That system of asymmetry differentiations might or might not differ from any other such processes (Levin, 2004, 2005) that appear later and might not involve neural crest mesenchyme. DZ twins and MZ twins are equally different from singletons in patterns of craniofacial asymmetry development. Twin heads are built differently from singleton heads. In the major components of that system of differences, MZ and DZ twins differ from singletons equally. Any part of the MZ developmental oddities arising from their having developed from two different body symmetry systems in a single cell mass happens to DZ embryos in the same or very similar ways.

I am convinced that the normal process of establishing the body's axes of a/symmetry is initiated primarily by a cascade of epigenetic mechanisms anchored in the fundamental asymmetry of the DNA. A sizable body of excellent work (cf. Levin's reviews, 2004, 2005) has demonstrated cascades of transcription control mechanisms that contribute to defining the embryonic origins of structural and functional asymmetries. All such systems reported to date begin with a signal that is already reliably asymmetric. They cannot therefore be considered to have answered the fundamental question, but only to have pushed the question back a little. How are we to suppose that the gene encoding the first transcription factor signal in the cascade knows how to kick things off by always first producing its product from cells on one side of the embryo and always the same side? (Please note that we can only say which side if we know which side is which, and I must insist that the cells had to 'know' that before they could set themselves up so that we could see it.) I have suggested DNA as the source because it brings the necessary reliable asymmetry forward from the beginning, and cells of every living thing appropriately questioned have demonstrated ways to know the difference between old strand and new, leading strand and trailing strand (Pierucci and Zuchowski, 1973; Dalgaard and Klar, 2001*a*,*b*; Klar, 2004*a*) for their use in allocating the modifications that constitute their epigenetic programming (Santos and Dean, 2004).

From such a perspective, it seems that an embryo with cells of two genotypes (and epigenotypes) would be more likely than a single-genotype embryo to establish two systems of embryogenic body axes. This would be entirely consistent with, and might help to explain, the fact that all reproductive procedures that involve artificially induced ovulation (which necessarily and always departs from natural oocyte maturation) increase frequencies of both polyzygotic and apparently monozygotic twinning events (Derom *et al.*, 1987; Hankins and Saade, 2005).

The presence of two distinct and potentially incompatible genomes and epigenomes in one embryo, each working from its own logic to establish its own version of structures around and across the midline, might interfere with normal determination of functional asymmetries. Most cases of functional asymmetries of body and brain differing from those in the normally lateralized majority could find their explanation in twin embryogenesis or chimerism and associated anomalies of epigenetic control. That grouping will include, and may help to explain, nearly every individual any of whose functional asymmetries of brain or body differ from those of the majority, including but probably not limited to natural NRHs, most cases of midline fusion malformations, most cases of functional psychosis, alcoholism, or spontaneous seizure disorders, and most cases of genitalia-discordant sexual orientation (Klar, 2004*b*).

The oddities by which we may rarely discover spontaneous chimerism are not required for its occurrence, and there is no reason to imagine that spontaneous chimerism is a quantum–mechanical event that owes its existence to being observed. In fact, a substantial fraction of us are built of cells that grew from zygotes that might have become two people, with different genomes and different epigenomes, different (and potentially conflicting) systems of genes and gene expression patterns responsible for directing the construction and function of bodies and brains. And, with only those exceptions in which one or both of the cell lines causes a visible problem, chimerism in general makes no difference we now know how to interpret as such, and no one need ever know.

The fraction of the population who are chimeric might be as high as 10% or more. Conservatively estimated, at least one live birth in eight is a product of a twin conception, the majority of which bring with them to delivery neither a co-twin nor any other overt evidence of their twin history (Boklage, 1990,1995).

The capacity for reflection provided by the structural and functional duality of the human mind-brain is arguably its greatest distinction from, and its greatest evolutionary advantage over, the brain of any other organism. Reflection is the mental substrate of self-awareness, and of the creative power of experiment and comparison. It provides the survival-value luxuries of the products of those processes and the safety of offline rehearsal. The mechanisms underlying the development of the necessary dual functionalities are closely involved at cellular and molecular levels with the mechanisms and consequences of twinning, which must be understood to include chimerism.

Summary

(i) Human spontaneous chimerism is common—plausibly of the order of 10% of the population.

(ii) Most spontaneous chimeras are DZ twins who have exchanged cells as embryos. Some are mothers colonized by cells from offspring *in utero*—some of whom never had a recognized pregnancy.

(iii) Most chimeras, like most twins, are born single.

(iv) Chimerism rarely if ever arises from placental anastomoses.

(v) Twin embryogenesis is associated with anomalies of midline fusion asymmetries, affecting twins of both zygosities equally and in the same ways.

(vi) Midline asymmetries of nervous system, face and heart are established in the same first few cell divisions of embryogenesis in which twinning occurs.

(vii) Every anomaly attributed to odd embryogenesis in MZ twins happens with equal or greater frequency in DZ twins.
(viii) DZ embryogenesis is at least as odd as that of MZ twins.
(ix) There is no evidence that any pair of natural DZ twins ever came from double ovulation.
(x) DZ embryogenesis happens the same way as MZ embryogenesis—defining and growing out two body symmetries from a single mass of cells.
(xi) Some DZ twins are monochorionic and some monochorionic twins are DZ; the same could be true of monoamnionic twins.
(xii) Chimeras, like other DZ twins, arise from monovular embryos
(xiii) Many non-HLA-identical DZ twins will be mutually excellent tissue transplant donors; sometimes, it will only work one-way.
(xiv) Many 'mosaic' cell lines will be found to be chimeric if properly tested.
(xv) Autopsy specimens are a reasonable place to look for chimerism in tissues other than blood.

Acknowledgements

Thanks to Amar Klar, Mikhail Golubovsky and Kurt Benirschke for challenging arguments. Support for this work has come from: NIH grant HD 22507 to the author at East Carolina University, genotyping services under contract N01-HG-65403 to the Center for Inherited Disease Research at Johns Hopkins University, The Children's Miracle Network, Deans Laupus and Kragel, and the Pediatrics Department of the Brody School of Medicine at East Carolina University.

References

Anderson D, Billingham RE, Lampkin GH and Medawar PB (1951) The use of skingrafting to distinguish between monozygotic and dizygotic twins in cattle. Heredity 5,379–397.

Angela E, Robinson E and North D (1976) A case of twin chimerism. J Med Genet 13,528–530.

Benirschke K (1990) The placenta in twin gestation. Clin Obstet Gynecol 33,18–31.

Benirschke K (1992) The contribution of placental anastomoses to prenatal twin damage. Hum Pathol 23,1319–1320.

Benirschke K (1995) The biology of the twinning process: how placentation influences outcome. Semin Perinatol 19,342–350.

Benirschke K and Masliah E (2001) The placenta in multiple pregnancy: outstanding issues. Reprod Fertil Dev 13,615–622.

Bestor TH (2003) Imprinting errors and developmental asymmetry. Philos Trans R Soc Lond B Biol Sci 358,1411–1415.

Bieber FR, Nance WE, Morton CC, Brown JA, Redwine FO, et al. (1981) Genetic studies of an acardiac monster: evidence of polar body twinning in man. Science 213,775–777.

Bird GW, Gibson M, Wingham J, Mackintosh P, Watkins W and Greenwell P. (1980) Another example of haemopoietic chimaerism in dizygotic twins. Br J Haematol 46,439–445.

Bjoro K Jr and Bjoro K (1985) Disturbed intrauterine growth in twins: etiological aspects. Acta Genet Med Gemellol (Roma) 34,73–79.

Boklage CE (1981) On the distribution of nonrighthandedness among twins and their families. Acta Genet Med Gemellol 30,167–187.

Boklage CE (1984) Differences in protocols of craniofacial development related to twinship and zygosity. J Craniofac Genet 4,151–169.

Boklage CE (1985) Interactions between opposite-sex dizygotic twins and the assumptions of Weinberg method epidemiology. Am J Hum Genet 37,591–605.

Boklage CE (1987a) Race, zygosity and mortality among twins: Interactions of myth and method. Acta Genet Med Gemellol 36,275–288.

Boklage CE (1987b) Twinning, nonrighthandedness and fusion malformations: Evidence for heritable causal elements held in common. Am J Med Genet 28,67–84.

Boklage CE (1987c) The organization of the oocyte and embryogenesis in twinning and fusion malformations. Acta Genet Med Gemellol 36,421–431.

Boklage CE (1990) Survival probability of human conceptions from fertilization to term. Int J Fertil 35,75–94.

Boklage CE (1995) The frequency and survival probability of natural twin conceptions. In Keith LG, Papiernik E, Keith DM and Luke B (eds) Multiple Pregnancy: Epidemiology, Gestation and Perinatal Outcome. Parthenon, New York, USA, pp.41–50.

Boklage CE (2005a) Biology of human twinning: a needed change of perspective. In Blickstein I and Keith LG (eds) Multiple Pregnancy: Epidemiology, Gestation and Perinatal Outcome. Taylor and Francis/Parthenon, New York and London, pp.255–264.

Boklage CE (2005b) The epigenetic environment: secondary sex ratio depends on differential survival in embryogenesis. Hum Reprod 20,583–587.

Bomsel-Helmreich O and Papiernik-Berkhauer, E (1976) Delayed ovulation and (monozygotic) twinning. Acta Genet Med Gemellol 25,73–76.

Bromilow IM and Duguid JK (1991) Blood group chimaerism: a possible further example. Med Lab Sci 48,212–216.

Carmelli D, Hasstedt S and Anderson S (1981) Demography and genetics of human twinning in the Utah Mormon genealogy. Prog Clin Biol Res 69A,81–94.

Churchill's Illustrated Medical Dictionary (1989) Konigsberg R (managing ed.) Churchill Livingstone, New York, Edinburgh, London, Melbourne.

Cupisti S, Conn CM, Fragouli E, Whalley K, Mills JA, et al. (2003) Sequential FISH analysis of oocytes and polar bodies reveals aneuploidy mechanisms. Prenat Diagn 23,663–668.

Cutler JA, Mitchell MJ, Smith MP and Savidge GF (2004) Germline mosaicism resulting in the transmission of severe haemophilia B from a grandfather with a mild deficiency. Am J Med Genet A. 129,13–15.

Dalgaard JZ and Klar AJ (2001a) Does *S. pombe* exploit the intrinsic asymmetry of DNA synthesis to imprint daughter cells for mating-type switching? Trends Genet 17,153–157.

Dalgaard JZ and Klar AJ (2001b) A DNA replication-arrest site RTS1 regulates imprinting by determining the direction of replication at mat1 in *S. pombe*. Genes Dev 15,2060–2068.

Dauber EM, Glock B, Mayr D and Mayr WR (1999) A case of dispermic chimerism: importance of DNA polymorphisms. J Biol Regul Homeost Agents 13,5–6.

Derom C, Derom R, Vlietinck R, Van den Berghe H and Thiery M. (1987) Increased monozygotic twinning rate after ovulation induction. Lancet 329,1236–1238.

Derom C, Thiery E, Vlietinck R, Loos R and Derom R (1996) Handedness in twins according to zygosity and chorion type: a preliminary report. Behav Genet 26,407–408.

Drexler C, Glock B, Vadon M, Staudacher E, Dauber EM, et al. (2005) Tetragametic chimerism detected in a healthy woman with mixed-field agglutination reactions in ABO blood grouping. Transfusion 45,698–703.

Dumoulin JC, Derhaag JG, Bras M, Van Montfoort AP, Kester AD, et al. (2005) Growth rate of human preimplantation embryos is sex dependent after ICSI but not after IVF. Hum Reprod 20,484–491.

Ewen KR, Bahlo M, Treloar SA, Levinson DF, Mowry B, et al. (2000) Identification and analysis of error types in high-throughput genotyping. Am J Hum Genet 67,727–736.

Falik-Borenstein TC, Korenberg JR and Schreck RR (1994) Confined placental chimerism: prenatal and postnatal cytogenetic and molecular analysis, and pregnancy outcome. Am J Med Genet 50,51–55.

Ferreiro V, Szijan I and Giliberto F (2004) Detection of germline mosaicism in two Duchenne muscular dystrophy families using polymorphic dinucleotide (CA)n repeat loci within the dystrophin gene. Mol Diagn 8,115–121.

Foschini MP, Gabrielli L, Dorji T, Kos M, Lazzarotto T, et al. (2003) Vascular anastomoses in dichorionic diamniotic-fused placentas. Int J Gynecol Pathol 22,359–361.

Gardner RL (2001) The initial phase of embryonic patterning in mammals. Int Rev Cytol 203,233–290.

Gardner RL (2002) Human experimental chimaerism: chimaeras as a source of stem cells. Reprod Biomed Online 5,103.

Gardner RL and Davies TJ (2000) Mouse chimeras and the analysis of development. Methods Mol Biol 135,397–424.

Gardner DK and Lane M (2005) Ex vivo early embryo development and effects on gene expression and imprinting. Reprod Fertil Dev 17,361–370.

Gilgenkrantz S, Marchal C, Wendremaire Ph and Seger M (1981) Cytogenetic and antigenic studies in a pair of twins: a normal boy and a trisomic 21 girl with chimera. Prog Clin Biol Res 69A,141–153.

Gioia L, Barboni B, Turriani M, Capacchietti G, Pistilli MG, Berardinelli P and Mattiolo M (2005) The capability of reprogramming the male chromatin after fertilization is dependent on the quality of oocyte maturation. Reproduction 130,29–39.

Gloyn AL, Cummings EA, Edghill EL, Harries LW, Scott R, et al. (2004) Permanent neonatal diabetes due to paternal germline mosaicism for an activating mutation of the KCNJ11 gene encoding the Kir6.2 subunit of the beta-cell potassium adenosine triphosphate channel. J Clin Endocrinol Metab 89,3932–3935.

Golembe BL, Ramsay NK, Krivit W, Nesbit ME, Coccia PF, Roell SL and Kersey JH (1979) Rejection after bone marrow transplantation for aplastic anemia using an identical twin, followed by permanent success utilizing immunosuppression. J Pediatr 95,569–571.

Golubovsky MD (2002) Paternal familial twinning: hypothesis and genetic/medical implications. Twin Res 5,75–86.

Golubovsky MD (2003a) Postzygotic diploidization of triploids as a source of unusual cases of mosaicism, chimerism and twinning. Hum Reprod 18,236–242.

Golubovsky MD (2003b) Higher mole and lower dizygotic twin rate in Japan: are these oddities associated with the same reproductive errors? Hum Reprod 18,1753–1755.

Hankins GV and Saade GR (2005) Factors influencing twins and zygosity. Paediatr Perinat Epidemiol 19 Suppl 1,8–9.

Hansen HE, Niebuhr E and Lomas C (1984) Chimeric twins. T.S. and M.R. re-examined. Hum Hered 34,127–130.

Harlap S, Shahar S and Baras, M (1985) Overripe ova and twinning. Amer J Hum Genet 37,1206–1215.

Henderson J (2005) Hamilton won't go down without a fight. DenverPost.com. Article first posted 22 May 2005. http://www.denverpost.com/search/ci_2749984

Hinterberger W, Rowlings PA, Hinterberger-Fischer M, Gibson J, Jacobsen N, Klein JP, Kolb HJ, Stevens DA, Horowitz MM and Gale RP (1997) Results of transplanting bone marrow from genetically identical twins into patients with aplastic anemia. Ann Intern Med 126,116–122.

Hosoi T, Yahara S, Kunitomo K, Saji H and Ohtsuki Y (1977) Blood chimeric twins: an example of blood cell chimerism. Vox Sang 32,339–341.

Husby H, Holm NV, Gernow A, Thomsen SG, Kock K and Gurtler H (1991) Zygosity, placental membranes and Weinberg's rule in a Danish consecutive twin series. Acta Genet Med Gemellol 40,147–152.

Iselius L, Lambert B, Lindsten J, Tippett P, Gavin J, et al. (1979) Unusual XX/XY chimerism. Ann Hum Genet 43,89–96.

James WH (1992) The current status of Weinberg's differential rule. Acta Genet Med Gemellol 41,33–42.

Katz-Jaffe MG, Trounson AO and Cram DS (2004) Mitotic errors in chromosome 21 of human preimplantation embryos are associated with non-viability. Mol Hum Reprod 10,143–147.

Kennerknecht I, Barbi G, Djalali M, Just W, Vogel W and Terinde R (1991) Uncommon chromosomal mosaicism in chorionic villi. Prenat Diagn 11,569–575.

Khosrotehrani K and Bianchi DW (2005) Multi-lineage potential of fetal cells in maternal tissue: a legacy in reverse J Cell Sci 118,1559–1563.

Klaning U, Bocker Pedersen C, Bo Mortensen P, Ohm Kyvik K and Skytthe A (2002) A possible association between the genetic predisposition for dizygotic twinning and schizophrenia. Schizophr Res 58,:31

Klar AJ (2004a) An epigenetic hypothesis for human brain laterality, handedness and psychosis development. Cold Spring Harb Symp Quant Biol 69,499–506.

Klar AJ (2004b) Excess of counterclockwise scalp hair-whorl rotation in homosexual men. J Genet 2004 83,251–255.

Klar AJ (2005) 1927 study supports a current genetic model for inheritance of human scalp hair-whorl orientation and hand-use preference traits. Genetics 170,2027–2030.

Koopmans M, Kremer Hovinga ICL, Baelde HJ, Fernandes RJ, de Heer E, et al. (2005) Chimerism in Kidneys, Livers and Hearts of Normal Women: Implications for Transplantation Studies. Amer J Transplantation 5,11495–11502.

Lage JM, Vanmarter LJ and Mikhail E (1989) Vascular anastomoses in fused, dichorionic twin placentas resulting in twin transfusion syndrome. Placenta 10,55–59.

Lambert NC, Pang JM, Yan Z, Erickson TD, Stevens AM, et al. (2005) Male microchimerism in women with systemic sclerosis and healthy women who have never given birth to a son. Ann Rheum Dis 64,845–848.

Le Douarin NM (2004) The avian embryo as a model to study the development of the neural crest: a long and still ongoing story. Mech Devel 121,1089–1102.

Levin M (2004) The embryonic origins of left-right asymmetry. Crit Rev Oral Biol Med 15,197–206.

Levin M (2005) Left-right asymmetry in embryonic development: a comprehensive review. Mech Dev 122,3–25.

Lo YMD, Lo ESF, Watson N, Noakes L, Sargent IL, et al. (1996) Two-way cell traffic between mother and fetus: biologic and clinical implications. Blood 88,4390–4395.

Machin G, Bamforth F, Innes M and McNichol K (1995) Some perinatal characteristics of monozygotic twins who are dichorionic. Am J Med Genet 55,71–76.

Maher ER (2005) Imprinting and assisted reproductive technology. Hum Mol Genet 14 (Spec No. 1),R133–138.

Mayr WR (1981) Human (germline) chimerism. Rev Fr Transfus Immunohematol 24,19–26.

McFadden DE and Langlois S (2000) Parental and meiotic origin of triploidy in the embryonic and fetal periods. Clin Genet 58,192–200.

McFadden DE, Jiang R, Langlois S and Robinson WP (2002) Dispermy—origin of diandric triploidy: brief communication. Hum Reprod 17,3037–3038.

Mifsud NA, Haddad AP, Hart CF, Holdsworth R, Condon JA, et al. (1999) Serologic and molecular investigations of a chimera. Immunohematol 15,100–104.

Mitchell RL and Crow TJ (2005) Right hemisphere language functions and schizophrenia: the forgotten hemisphere? Brain 128,963–978.

Miura K and Niikawa N (2005) Do monochorionic dizygotic twins increase after pregnancy by assisted reproductive technology? J Hum Genet 50,1–6.

Mortimer G (1987) Zygosity and placental structure in monochorionic twins. Acta Genet Med Gemellol 36,417–420.

Nagy A and Rossant J (2001) Chimaeras and mosaics for dissecting complex mutant phenotypes. Int J Dev Biol 45,577–582.

Nyberg RH, Haapala AK and Simola KO (1992) A case of human chimerism detected by unbalanced chromosomal translocation. Clin Genet 42,257–259.

Nylander PP (1974) The placenta and zygosity of twins. Acta Genet Med Gemellol 46,9–22.

Nylander PP and Osunkoya BO (1970) Unusual monochorionic placentation with heterosexual twins. Obstet Gynecol 36,621–625.

Nylander PP and Corney G (1977) Placentation and zygosity of twins in northern Nigeria. Ann Hum Genet 40,323–329.

Online Medical Dictionary (1997–2004) http://cancerweb.ncl.ac.uk/omd/index.html The CancerWEB Project. Published by the Department of Medical Oncology, University of Newcastle, UK.

Osinska M and Woloszyn T (1971) A rare case of embryonic chimerism in human twins. Arch Immunol Ther Exp (Warsz) 19,657–665.

Palermo GD, Colombero LT and Rosenwaks Z (1997) The human sperm centrosome is responsible for normal syngamy and early embryonic development. Rev Reprod 2,19–27.

Paoloni-Giacobino A and Chaillet JR (2004) Genomic imprinting and assisted reproduction. Reprod Health 1,6–12.

Pausch V, Bleier I, Dub E, Kirnbauer M, Weirather M, et al. (1979) A further case of chimeric twins: genetic markers of the blood. Vox Sang 36,85–92.

Pierucci O and Zuchowski C (1973) Non-random segregation of DNA strands in Escherichia coli B/r. J Mol Biol 80,477–503.

Pruss A, Heymann GA, Hell A, Kalus UJ, Krausch D, et al. (2003) Acute intravascular hemolysis after transfusion of a chimeric RBC unit. Transfusion 43,1449–1451.

Quintero RA, Mueller OT, Martinez JM, Arroyo J, Gilbert-Barness E, et al. (2003) Twin-twin transfusion syndrome in a dizygotic monochorionic-diamniotic twin pregnancy. J Matern Fetal Neonatal Med 14,279–281.

Redline RW (2003) Nonidentical twins with a single placenta; disproving dogma in perinatal pathology. N Engl J Med 349,111–114.

Reed AM, McNallan K, Wettstein P, Vehe R and Ober C (2004) Does HLA-dependent chimerism underlie the pathogenesis of juvenile dermatomyositis? J Immunol 172,5041–5046.

Robertson EG and Neer KJ (1983) Placental injection studies in twin gestation. Am J Obstet Gynecol 147,170–174.

Rydhstroem H and Heraib F (2001) Gestational duration, and fetal and infant mortality for twins vs singletons. Twin Res 4,227–231.

St Clair JB and Golubovsky MD (2002) Paternally derived twinning: a two century examination of records of one Scottish name. Twin Res 5,294–307.

Santos F and Dean W (2004) Epigenetic reprogramming during early development in mammals. Reproduction 127,643–651.

Sathananthan AH (1997) Mitosis in the human embryo: the vital role of the sperm centrosome (centriole). Histol Histopathol 12,827–856.

Sathananthan AH, Ratnasooriya WD, de Silva PK, Menezes J (2001) Characterization of human gamete centrosomes for assist reproduction. Ital J Anat Embryol 106 (2 Suppl2):61–73.

Shiota K and Yamada S (2005) Assisted reproductive technologies and birth defects. Congenit Anom (Kyoto) 45,39–43.

Souter VL, Kapur RP, Nyholt DR, Skogerboe K, Myerson D, et al. (2003) A report of dizygous monochorionic twins. N Engl J Med 349,154–158.

Stevens AM, McDonnell WM, Mullarkey ME, Pang JM, Leisenring W and Nelson JL (2004) Liver biopsies from human females contain male hepatocytes in the absence of transplantation. Lab Invest 84,1603–1609.

Strain L, Dean JC, Hamilton MP and Bonthron DT (1998) A true hermaphrodite chimera resulting from embryo amalgamation after in vitro fertilization. N Engl J Med 338,166–169.

Sudik R, Jakubiczka S, Nawroth F, Gilberg E and Wieacker PF (2001) Chimerism in a fertile woman with 46,XY karyotype and female phenotype. Hum Reprod 16,56–58.

Summers PM and Shelton JN (1985) Long-term acceptance of full thickness body skin grafts between Bos Taurus–Bos indicus chimeric twins. Aust J Exp Biol Med Sci 63,329–332.

Sutovsky P and Schatten G (2000) Paternal contributions to the mammalian zygote: fertilization after sperm-egg fusion. Int Rev Cytol 195,1–65.

Tam PP and Rossant J (2003) Mouse embryonic chimeras: tools for studying mammalian development. Development 130,6155–6163.

Tarkowski AK (1998) Mouse chimeras revisited: recollection and reflections. Int J Dev Biol 42,903–908.

Tesarik J (2005) Paternal effects on cell division in the human preimplantation embryo. Reprod Biomed Online 10,370–375.

Tharapel AT, Elias S, Shulman LP, Seely L, Emerson DS and Simpson JL (1989) Resorbed co-twin as an explanation for discrepant chorionic villus results: non-mosaic 47,XX,+16 in villi (direct and culture) with normal (46,XX) amniotic fluid and neonatal blood. Prenat Diagn 9,467–472.

Tippett P (1983) Blood group chimeras. Vox Sang 44,333–359.

van Blerkom J, Davis P, Merriam J and Sinclair J (1995) Nuclear and cytoplasmic dynamics of sperm penetration, pronuclear formation and microtubule organization during fertilization and early preimplantation development in the human. Hum Reprod Update 1,429–461.

van Dijk BA, Boomsma DI and de Man AJ (1996) Blood group chimerism in human multiple births is not rare. Am J Med Genet 61,264–268.

Verp MS, Harrison HH, Ober C, Oliveri D, Amarose AP, et al. (1992) Chimerism as the etiology of a 46,XX/46,XY fertile true hermaphrodite. Fertil Steril 57,346–349.

Vietor HE, Hamel BC, van Bree SP, van der Meer EM, Smeets DF, et al. (2000) Immunological tolerance in an HLA non-identical chimeric twin. Hum Immunol 61,190–192.

Viot G (2002) Confined placental mosaicism: definition, consequences and outcome. J Gynecol Obstet Biol Reprod (Paris) 31(1 Suppl),S70–S74.

Vlietinck R, Derom R, van den Berghe H and Thiery M (1988) The validity of Weinberg's rule in the East Flanders Prospective Twin Survey (EFPTS) Acta Genet Med Gemellol 37,137–141.

Weksberg R, Shuman C, Caluseriu O, Smith AC, Fei YL, Nishikawa J, Stockley TL, Best L, Chitayat D, Olney A, Ives E, Schneider A, Bestor TH, Li M, Sadowski P and Squire J (2002) Discordant KCNQ1OT1 imprinting in sets of monozygotic twins discordant for Beckwith-Wiedemann syndrome. Hum Mol Genet 11,1317–1325.

Wiley JE, Madigan M, Christie JD and Smith AW (2002) Dispermic chimerism with two abnormal cell lines, 47,XY, +21 and 47,XX, +12. Am J Med Genet 107,64–66.

Williams CA, Wallace MR, Drury KC, Kipersztok S, Edwards RK, et al. (2004) Blood lymphocyte chimerism associated with IVF and monochorionic dizygous twinning: case report. Hum Reprod 19,2816–2821.

Yoon G, Beischel LS, Johnson JP and Jones MC (2005) Dizygotic twin pregnancy conceived with assisted reproductive technology associated with chromosomal anomaly, imprinting disorder, and monochorionic placentation. J Pediatrics 146,565–567.

Yoshida K and Soma H (1984) A study of twin placentation in Tokyo. Acta Genet Med Gemellol 33,115–120.

Yu N, Kruskall MS, Yunis JJ, Knoll JH, Uhl L, et al. (2002) Disputed maternity leading to identification of tetragametic chimerism. N Engl J Med 346,1545–1552.

Zaragoza MV, Surti U, Redline RW, Millie E, Chakravarti A and Hassold TJ (2000) Parental origin and phenotype of triploidy in spontaneous abortions: predominance of diandry and association with the partial hydatidiform mole. Am J Hum Genet 66,1807–1820.

Submitted on August 22, 2005; resubmitted on September 26, 2005; accepted on September 28, 2005

Polzin et al.,

Production of Sheep-Goat Chimeras by Inner Cell Mass Transplantation,

J. OF ANIMAL. SCI., vol. 65, pp. 325-330 (1987)

Production of Sheep-Goat Chimeras by Inner Cell Mass Transplantation

V. J. Polzin, D. L. Anderson, G. B. Anderson, R. H. BonDurant, J. E. Butler, R. L. Pashens, M. C. T. Penedo and J. D. Rowe

J ANIM SCI 1987, 65:325-330.

PRODUCTION OF SHEEP-GOAT CHIMERAS BY INNER CELL MASS TRANSPLANTATION[1]

V. J. Polzin[2], D. L. Anderson[3], G. B. Anderson[2,4], R. H. BonDurant[5], J. E. Butler[2,6], R. L. Pashen[5], M. C. T. Penedo[3] and J. D. Rowe[7]

University of California, Davis 95616

ABSTRACT

Embryos were surgically flushed from goats and sheep on d 6 and 7, respectively, following the first day of estrus (d 0). After enzymatic removal of the zonae pellucidae, inner cell masses were isolated from caprine blastocysts by immunosurgery. The intact inner cell masses were injected into ovine blastocysts with the aid of a micromanipulator. Twenty-two manipulated blastocysts were surgically transferred into 12 ovine recipients. Nine ewes gave birth to a total of 13 young (59% embryo survival). Ten were classified by serum electrophoretic assays or karyotypes as lambs, one as a kid, and two as interspecific chimeras.

(Key Words: Sheep, Goat, Chimera, Blastocyst, Biotechnology.)

Introduction

Intraspecific chimeras have been successfully produced in several species, but chimeras between two different species have been produced only in mice (Mus musculus-Mus caroli; Rossant and Frels, 1980), cattle (Bos taurus-Bos indicus; Summer et al., 1983), and between sheep and goats (Fehilly et al., 1984). Interspecific chimeras are useful biological models for the study of cell deployment during fetal development because each cell contains species-specific markers that distinguish it from cells of the other species. Mammalian chimeras are generally considered to have allogeneic tolerance of the component lines, and interspecific chimeras also provide a model for the study of maternal-fetal incompatibilities (Rossant et al., 1982; Croy et al., 1985).

Successful development to term of interspecific and chimeric pregnancies between sheep and goats has been accomplished by manipulation of chimeric embryos such that species of trophoblast and recipient is the same (Fehilly et al., 1984; Meinecke-Tillman and Meinecke, 1984). We describe here the production of sheep-goat chimeras by immunosurgical isolation of caprine inner cell masses (ICM), their injection into ovine blastocysts and transfer of the resulting chimeric embryos to ovine recipients.

Materials and Methods

Does of various breeds (Alpine, Toggenburg, Saanen and Nubian) and crossbred ewes of Finnish Landrace, Rambouillet and Suffolk breeding were used as embryo donors. Intravaginal pessaries containing 40 mg fluorogestone acetate[8] were inserted for 12 d to synchronize estrus. Donors were superovulated with twice-daily injections of porcine follicle stimulating hormone[9] in decreasing doses of 5 and 5, 4 and 4, and 3 and 3 mg, respectively, starting 1 d before sponge removal. Injection of goats began 12 h after the start of the superovulatory treatment of ewes in an attempt to induce ovulation 12 to 24 h after that of the ewes. Caprine donors also were given a single injection of cloprostenol[10] at sponge removal.

[1] The authors thank Susan Donahue for her technical assistance, Jacques Staats and Lee Millon for their for their preparation and analysis of chromosome karyotypes, and Keith Maddock for operation of the cytofluorograph.

[2] Dept. of Anim. Sci.

[3] Serol. Lab.

[4] To whom reprint requests should be addressed.

[5] Dept. of Reprod.

[6] Present address: LHRRB, Harvard Med. School, Boston, MA 02115.

[7] Vet. Med. Teaching Hospital.

[8] Chrono-gest pessaries, Intervet, France.

[9] Burns-Biotec, Omaha, NE.

[10] Estrumate, Haver-Lockart, Shawnee, KS.

Received August 20, 1986.
Accepted February 5, 1987.

Donors were observed twice daily for estrus following sponge removal and receptive females mated to fertile males of the same species.

Embryos were collected at laparotomy as described by Anderson et al. (1981) from does and ewes on d 6 and 7, respectively, after the first day of estrus (d 0). Embryos were cultured at 37 C in drops of Whitten's medium (Whitten and Biggers, 1968) supplemented with .5% bovine serum albumin (BSA) under silicone medical fluid[11] in a humidified atmosphere of 5% carbon dioxide in air. This culture system was used except where otherwise described.

For isolation of the caprine inner cell masses, blastocysts were treated with .5% pronase in Whitten's medium without BSA for 20 to 30 s to remove the zonae pellucidae. Following zona removal, embryos were gently rinsed and cultured in Whitten's medium for 1 to 2 h to allow them to recover from the effects of pronase. Blastocysts were then cultured in a 1:8 dilution of non-heat-treated rabbit anti-sheep antiserum for 20 to 30 min at which time the trophoblastic cells began to lyse. Antisera were produced by the method described by Solter and Knowles (1975). The embryos were then rinsed three times to remove excess antiserum, and finally drawn in and out of a small-bore glass pipette (approximately 75 μm id) mounted on a Leitz micromanipulator[12]. Lysed trophoblastic cells were removed, leaving an intact ICM.

Isolated caprine ICM were injected into ovine blastocysts by a technique modified from that reported by Butler et al. (1985). The manipulators were arranged with a beveled injection pipette on one side and an embryo-holding pipette on the other. The caprine ICM was drawn into the injection pipette and held directly opposite the ovine blastocyst on the holding pipette. The ovine blastocyst was maintained on the holding pipette by gentle suction, such that its ICM was as far from the tip of the injection pipette as possible. With a swift precise forward motion the tip of the injection pipette was introduced into the blastocoele and the ICM was ejected. Embryos were cultured for 10 to 12 h and then examined microscopically to determine which ones had incorporated the injected ICM with the recipient ICM. Embryos that had failed to incorporate the caprine ICM, due either to demise of the injected cells or failure of the cells to be successfully injected into the blastocoelic cavity, were classified as non-chimeric.

All injected embryos (both chimeric and non-chimeric) were surgically transferred into crossbred ovine recipients in which estrus had been induced 24 h after that of the donor ewes by a single injection of cloprostenol. For 2 wk following transfer, recipients were observed daily for estrus with the aid of vasectomized rams. Ultrasonography was used at 45 d of gestation to confirm pregnancy.

Offspring were examined for chimerism by visual inspection and by gel electrophoresis of red cell and serum proteins. Hemoglobin was resolved by isoelectric focusing on polyacrylamide gel (Braend and Johanses, 1983). Glucose phosphate isomerase (GPI) was resolved on starch gel (Manwell and Baker, 1977) and transferrin on polyacrylamide gel (Juneja et al., 1981). In addition, chromosomal analysis of lymphocytes obtained from leukocyte culture was carried out as described by Lin et al. (1976). The lymphocytes were stained with Giemsa stain in Sorenson's buffer. As a difference in size exists between the red blood cells of sheep and goats (Fehilly et al., 1985), distribution of red blood cell size was also determined using a cytofluorograph[13] and compared with red blood cells from normal sheep and goats.

Skin biopsies were taken under aseptic conditions from an overt sheep-goat chimera and cultured to confluent monolayers using the modified procedures of Martin (1973). The biopsy specimens were washed five times, to remove hair and red blood cells, with sterile Hank's balanced salt solution supplemented with penicillin (100 units/ml), streptomycin (100 μg/ml), and amphiteracin B (2.5 μg/ml). The explants were cultured in Eagle's basal medium with 15% fetal calf serum and 50 μg/ml gentamycin sulfate. Karyological preparations of the fibroblast cells were made as described by Hsu (1973). Cells were stained as described previously.

Results

Results are summarized in table 1. Twenty-two ovine blastocysts were injected with a foreign ICM and on microscopic examination

[11] Dow Corning 360, 20 Cs viscosity, Dow Corning, Midland, MI.
[12] E. Leitz, Wetzler, West Germany.
[13] Model 50-H, Ortho Diagnostics, Westwood, MA.

TABLE 1. OFFSPRING PRODUCED FROM OVINE BLASTOCYSTS INJECTED WITH IMMUNOSURGICALLY-PREPARED CAPRINE ICM

No. blastocyst pairs		No. recipients			No. young		
Total	Chimeric blastocysts	Total	Pregnant (45 d)	Pregnant (term)	Lambs	Kids	Inter-specific chimeras
22	13 (59%)	14	10	9 (64%)	10[a]	1	2

[a]One lamb from a set of twins was stillborn.

nine were judged to be non-chimeric. These included five in which injection failed and resulted in the ICM being deposited in the perivitelline space. The four remaining non-chimeric embryos contained injected cells within the blastocoelic cavity but the cells appeared not to have survived during the culture period. In each of the four embryos, the caprine ICM cells had disaggregated into as many as three or four pieces while being drawn into the injection pipette. It was not always possible to know with certainty, however, that blastocysts classified as non-chimeric had not incorporated a few caprine cells.

In two cases recipient ovine blastocysts were punctured such that the injection pipette passed completely through the ovine blastocoelic cavity and ICM prior to deposition of the caprine ICM into the blastocoele. Although this appeared to cause considerable damage to the ovine ICM, the embryos re-expanded in culture and appeared morphologically normal by microscopic examination. Moreover, the donor ICM appeared to have been incorporated with the remaining ovine ICM and the embryos were classified as being chimeric.

All 22 embryos were transferred into 14 ewes. Eight ewes received two embryos each, while six ewes received one each. Three ewes returned to estrus within 2 wk of transfer and of these, two had each received two embryos. Another ewe that had been given one embryo had not been observed in estrus, but was found not to be pregnant at d 45 by ultrasonography. One ewe, given two embryos and diagnosed pregnant at 45 d, failed to deliver fetuses at term. Nine ewes gave birth to a total of 13 young, representing a pregnancy rate of 64% and an embryo survival rate of 59%.

Of the 13 animals born (including one lamb born dead), 10 had the general appearance of lambs. Of the remaining three, one female was judged to be an overt sheep-goat chimera because her coat contained patches of both wool and hair (figure 1). The shape of her head, the size and position of her ears and the symmetrical color patterns on her face and legs resembled her caprine parentage (Nubian × Alpine). Her blood, however, had only sheep-type transferrin, hemoglobin and GPI. Chromosomal analysis of blood lymphocytes also indicated only sheep cells and the size of her red cells was within the normal range for sheep. Skin biopsies were taken at sites containing only wool, only hair, and both wool and hair.

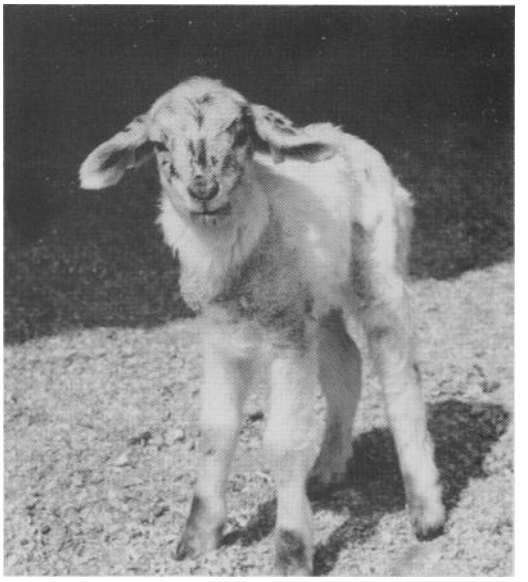


Figure 1. Overt female sheep-goat chimera produced from the injection of a caprine ICM (Alpine × Nubian) into an ovine blastocyst (Rambouillet × Rambouillet).

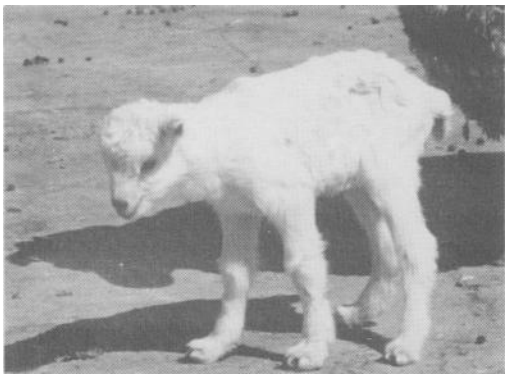


Figure 2. Male sheep-goat chimera produced from the injection of a caprine ICM (Alpine × Saanen) into an ovine blastocyst (Finnish Landrace × Finnish Landrace).

Chromosomal analysis of fibroblast cells grown in culture demonstrated both sheep (2n=54) and goat (2n=60) cells were present in all biopsies of the skin of this animal. Her sibling, produced from the same parental combination, appeared to be a normal Rambouillet male lamb.

One of the remaining young that resembled a white male kid (Alpine × Saanen) died 26 h after birth (figure 2). Necropsy results revealed no developmental abnormalities and no specific cause of death could be determined. Serum samples taken prior to death demonstrated interspecific chimerism in the transferrin proteins of this animal (figure 3), and this animal was judged to be a sheep-goat chimera. No blood cells were available for GPI typing, chromosomal analysis or red cell sizing.

A second kid born to a recipient ewe appeared to be a normal Toggenburg male (figure 4); only the caprine parents (Toggenburg × Toggenburg) were represented in the blood traits studied. Hemoglobin, transferrin, karyotype and red cell size were all typical of the caprine species. This kid developed from a chimeric blastocyst in which the ovine ICM was thought to have been damaged during the injection procedure.

Blood samples taken from the remaining lambs, both at birth and at 1 mo of age, indicated no blood chimerism in transferrin, hemoglobin or GPI. Except for the stillborn lamb, which was not studied, these animals also exhibited ovine chromosomes in their blood

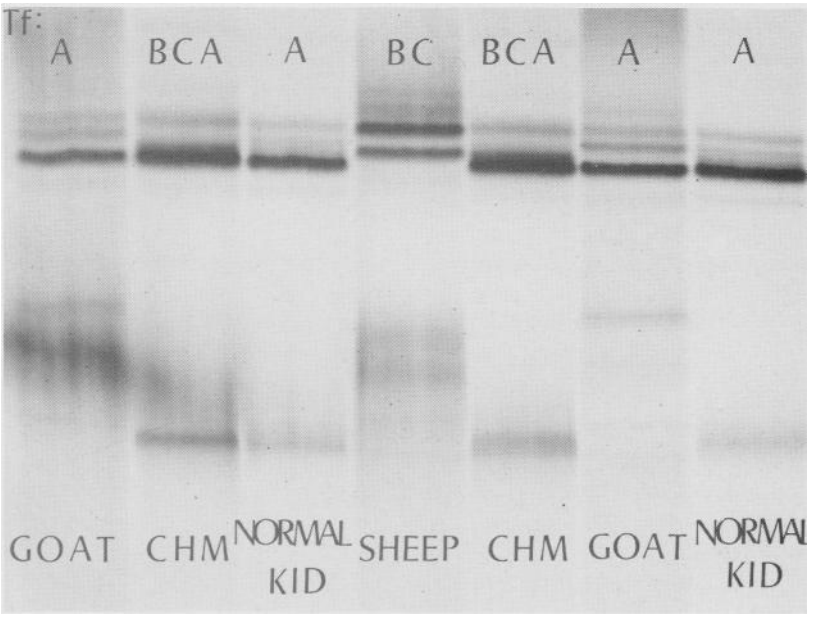


Figure 3. Polyacrylamide gel of transferrin (Tf) types of the male interspecific chimera (CHM), the ovine parents, the caprine parents, and a normal 1-d-old kid. The two ovine and two caprine parents had the same transferrin variants, respectively.

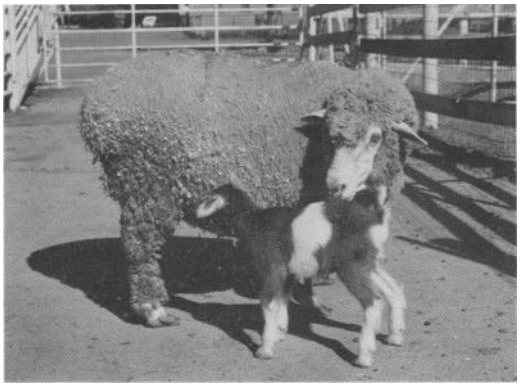


Figure 4. Toggenburg kid born to a Rambouillet ewe.

lymphocytes and red cells within the normal size range for sheep.

Discussion

Immunosurgery has been used previously to isolate viable ICM from mouse (Solter and Knowles, 1975), rabbit (Babinet and Bordenave, 1980) and sheep (Butler et al., 1985) embryos. It was thought that immunosurgery on goat embryos could severely damage the ICM because it is only partly covered by the trophoblastic cell layer in early development (Amoroso et al., 1942; McLaren, 1972). Despite this concern, the method allowed the ICM to be isolated from a goat embryo as a compact group of cells. Damage to the ICM that occurred was attributed in part to problems encountered in drawing the ICM into the injection pipette.

There were several ways in which the foreign and "host" ICM of any injected blastocyst could potentially develop: if incorporation took place, a chimera would develop; if the injected ICM was excluded, a lamb would be born; and if the injected ICM replaced the "host" ICM, a kid would result. From the offspring born, it was demonstrated that all of these different possibilities occurred. A similar outcome was observed by Fehilly et al. (1984) and Butler et al. (1985) in their production of sheep-goat and sheep-sheep chimeras, respectively. It is therefore possible to use a relatively simple technique such as blastocyst injection to replace one ICM with another, although currently little control can be exercised over which cells will develop. Inner cell mass transfer may be useful in overcoming the interspecies embryo transfer barrier. By maintaining the trophoblastic integrity of the recipient species, it is apparently possible to "mask" antigens of a foreign fetus from the mother's immune system, thereby increasing its chances of survival. Domestic animals could be used as recipients for chimeric blastocysts containing ICM of evolutionarily-related endangered species if contributions of the "host" ICM to fetal development could be blocked.

The data presented show that procedures for immunosurgical isolation of ICM and blastocyst injection developed for the production of intraspecific ovine chimeras (Butler et al., 1985) can be extended to the production of interspecific sheep-goat chimeras. The results also indicate that these procedures can be used to permit interspecific embryo transfer by allowing development of a fetus of one species within trophoblast of the recipient species. In both cases, the technique of blastocyst injection allows transfer of the manipulated embryos directly to the recipient that will carry the pregnancy to term, eliminating the need for an intermediate recipient. This becomes especially important in cases where only limited numbers of embryos are available since the fewer steps involved in manipulation of the embryos allows less opportunity for embryos to be lost.

Literature Cited

Amoroso, E. C., W.F.B. Griffiths and W. J. Hamilton. 1942. The early development of the goat (Capra hircus). J. Anat. 76:377.

Anderson, G. B., G. E. Bradford and P. T. Cupps. 1981. Length of gestation in ewes carrying lambs of two different breeds. Theriogenology 16:119.

Babinet, C. and G. R. Bordenave. 1980. Chimeric rabbits from immunosurgically-prepared inner-cell-mass transplantation. J. Embryol. Exp. Morphol. 60:429.

Braend, M. and K. E. Johnanses. 1983. Haemoglobin types in Norwegian horses. Anim. Blood Groups Biochem. Genet. 14:305.

Butler, J. E., G. B. Anderson, R. H. BonDurant and R. L. Pashen. 1985. Production of ovine chimeras. Theriogenology 23:183 (Abstr.).

Croy, B. A., J. Rossant and D. A. Clark. 1985. Effects of alterations in the immunocomponent status of Mus musculus females on the survival of transformed Mus caroli embryos. J. Reprod. Fertil. 74:479.

Fehilly, C. B., S. M. Willadsen, A. R. Dain and E. M. Tucker. 1985. Cytogenetic and blood group studies of sheep/goat chimaeras. J. Reprod. Fertil. 74:215.

Fehilly, C. B., S. M. Willadsen and E. M. Tucker. 1984.

Interspecific chimaerism between sheep and goats. Nature 307:634.
Hsu, T. C. 1973. Preparation and analysis of karyotypes and idiograms. In: P. F. Kruse, Jr. and M. K. Patterson (Ed.) Tissue Culture Methods and Applications. pp 764–767. Academic Press, New York.
Juneja, R. K., K. Christensen, E. Anderson and B. Gahne. 1981. Frequencies of transferrin types in various breeds of domestic dogs. Anim. Blood Groups Biochem. Genet. 12:79.
Lin, C. C., D. R. Newton, W. K. Smink and R. B. Church. 1976. A rapid and simple method for the isolation and culture and leukocytes for chromosome analysis in domestic animals. Can. J. Anim. Sci. 56:27.
Manwell, C. and C. M. Baker. 1977. Ammotragus lervia: Barbary sheep or Barbary goat? Comp. Biochem. Physiol. 58B:267.
Martin, G. M. 1973. Human skin fibroblasts. In: P. S. Kruse, Jr. and M. K. Patterson (Ed.) Tissue Culture Methods and Applications. pp 39–43. Academic Press, New York.
McLaren, A. 1972. The Embryo. In: C. R. Austin and R. V. Short (Ed.) Reproduction in Mammals, Book 2, Embryonic and Fetal Development. pp 1–42. University Press, Cambridge.
Meinecke-Tillman, S. and B. Meinecke. 1984. Experimental chimeras–Removal of reproductive barrier between sheep and goat. Nature 307:637.
Rossant, J. and W. I. Frels. 1980. Interspecific chimeras in mammals: Successful production of live chimeras between Mus musculus and Mus caroli. Science 208:419.
Rossant, J., V. M. Mauro and B. A. Croy. 1982. Importance of trophoblast genotype for survival of interspecific murine chimeras. J. Embryol. Exp. Morph. 69:141.
Solter, D. and B. B. Knowles. 1975. Immunosurgery of mouse blastocyst. Proc. Natl. Acad. Sci. (USA) 72:5099.
Summer, P. M., J. B. Shelton and K. Bell. 1983. Synthesis of primary Bos taurus-Bos indicus chimeric calves. Anim. Reprod. Sci. 6:91.
Whitten, W. K. and J. D. Biggers. 1968. Complete development in vitro of the preimplantation stages of the mouse in a simple chemically defined medium. J. Reprod. Fertil. 17:899.

Science: It's a Geep,

TIME MAGAZINE (Feb. 27, 1984)

Back to Article Click to Print

TIME

Monday, Feb. 27, 1984

Science: It's a Geep

Crossbreeding goats and sheep

It looks like a zookeeper's prank: a goat dressed in a sweater of angora. But the odd-looking creature that appeared on the cover of the journal Nature last week is no joke. The animal is a crossbreed of two entirely different species, a goat and a sheep. Inevitably, it has been dubbed a geep.

Now 18 months old and thriving, the geep was produced by the latest tricks of embryo manipulation. Scientists at the Institute of Animal Physiology in Cambridge, England, mingled new embryos from both sheep and goats when each consisted of no more than four to eight cells. Ultimately, these were placed in the wombs of surrogate sheep or goat mothers and allowed to grow to term. Such hybrids are called chimeras (after the mythic monster with a lion's head, goat's body and serpent's tail).

Because each embryo came originally from the fertilized eggs of both a goat and a sheep, the animals had four parents.

The Cambridge experimenters produced a total of six animals with characteristics of both sheep and goats. Only one of them, however, had blood proteins from both species. That animal behaves like a goat and has even tried mating with female goats, but like another hybrid, the mule, its sperm are defective. At Justus-Liebig University in Giessen, West Germany, other embryo manipulators also reported producing goat-sheep.

Though such experimenting is sure to trigger debate, scientists point to practical benefits: it should make it easier to rear embryos of endangered species in the wombs of other species or even create hybrids as valuable as the indomitable mule.

Click to Print

Find this article at:
http://content.time.com/time/magazine/article/0,9171,921546,00.html

Greely et al.,

Thinking About the Human Neuron Mouse,

AM. J. BIOTECH, vol. 7, no. 5 (May 2007)

Published in final edited form as:
Am J Bioeth. 2007 May ; 7(5): 27–40.

Thinking About the Human Neuron Mouse

Henry T. Greely,
Stanford University

Mildred K. Cho,
Stanford University

Linda F. Hogle, and
University of Wisconsin, Madison

Debra M. Satz
Stanford University

> Tonight I ask you to pass legislation to prohibit the most egregious abuses of medical research . . . [which include] creating human-animal hybrids.
>
> — George W. Bush, 2006 State of the Union Address (2006)

Dr. Irving Weissman, a professor in the departments of pathology and developmental biology at Stanford University (Stanford, CA), approached one of the authors of this article in February 2000 with ethical questions about interesting experiments he was considering. The most interesting experiment would begin with an inbred strain of mouse that begins to form brains during very early fetal development, but, several days before birth, died as a result of the death of most or all of the developing neurons in their brains (the glial cells that make up approximately 90% of the brain are unharmed). Weissman proposed to transplant human brain stem cells into the fetal mice, just before their own neurons died. His hope was to produce a living mouse with a functioning brain made up of mouse glial cells and human-derived neurons. This mouse could then be used to study human neurons *in vivo* in a laboratory animal, similar to the way the severe combined immunodeficiency (SCID)-hu mouse, which Weissman had helped developed in the late 1980s, allowed the study of the human immune system inside laboratory mice.

That conversation led to the creation of a five-person working group that, after meeting for more than one year, in early 2002 reported to Dr. Weissman that it believed his proposed experiments could be performed ethically, subject to some guidelines. The report has never been published and the experiments, for reasons not associated with the report, were never performed. Yet both the experiments and, to a lesser extent, the report have been subjects of discussion and debate (DeWitt 2002; Krieger 2005; Wade 2005; Weiss 2004), and the issue of human/non-human chimeras has only grown more controversial, leading even to proposed criminal legislation that has the “unambiguous” support of the President of the United States (S. 1373; Brownback 2005).

This article is a revised version of our report, updated to reflect nearly five years of debate about the ethical issues surrounding the creation and use of human/non-human chimeras. That debate has taken place in scholarly journals, important policy reports, and the halls of Congress. We believe our analysis has interest as one of the earliest efforts to come to grips with the implications of this scientific research and as an example of a “benchside consult,” an effort

Address correspondence to Henry T. Greely, Stanford Law School, Crown Quadrangle, 559 Nathan Abbott Way, Stanford, CA 94305-8610. E-mail: hgreely@stanford.edu.

to provide ethics-based advice on research in progress. More importantly, we also believe that it remains, with slight modifications, a useful approach to such experiments. Our report focuses on transplanting human neural progenitor cells into non-human brains and so falls well within whatever boundaries define "neuroethics," but it also has broader implications for the creation of other kinds of human/non-human chimeras, including some with non-biological components.

This article begins by describing the debate over human/non-human chimeras. It then focuses on our case study, Weissman's proposed experiments aimed at creating what we have called the "human neuron mouse." It provides some background on the experiments and discusses their potential benefits and their risks and costs before providing our recommendations to Dr. Weissman (and, now, others contemplating similar experiments). The article ends with some broader conclusions about the ethics of research with human/non-human chimeras.

Some readers will, no doubt, be disappointed that neither this article, nor the original report, attempts to answer the question whether conferring human-like mental characteristics on non-human animals is, or is not, ethically appropriate. We concluded that this fascinating question just was not plausibly raised by Weissman's proposed experiments. To emphasize that question in the context of these, or similar, experiments would give too much credence to a sensational misreading of this research; as we note in our last section, the question does need further work.

THE DEBATE OVER HUMAN/NON-HUMAN CHIMERAS

Although the definitions and meanings of chimeras are numerous and complex (Greely 2003), for the purposes of this article chimeras are creatures with cells, tissues or organs from individuals of two different species (interspecific chimeras). In spite of President Bush's language, hybrids are not chimeras but are, instead, the result of sexual reproduction involving individuals of different species, as a mule is a hybrid resulting from the mating of a male donkey with a female horse. Human/non-human chimeras can be created in two different directions, by putting human cells or tissues into non-human animals or by putting non-human cells or tissues into humans. This article discusses only the first; the second topic is more often referred to as *xenotransplantation* and is the subject of wide-ranging debates, mainly about its safety. (Interestingly, at least some experiments have transplanted non-human neural cells into human brains with long-term survival [Deacon 1997].) This section of the article reviews the scientific, ethical, and policy discussions that have taken place concerning the first method.

The Continuing Creation of Human/ Non-Human Chimeras

The science and politics of human stem cells have combined to keep human/non-human chimeras a scientifically relevant issue. Weissman hoped to make human neuron mice largely so the mice could serve as model organisms for studying human cells. But as interest, scientific and popular, grows in human stem cell research, human/non-human chimeras are likely to take on broader uses. Before anyone makes new clinical use of human stem cells—or any clinical use of human embryonic stem cells—prudence (and the United States Food and Drug Administration [FDA]) are likely to require preclinical trials with the human cells in non-human animals. The result is likely to be a large number of human/non-human chimeras. When pluripotent embryonic stem cells are used instead of more differentiated stem cells, the concerns potentially become greater; a human embryonic stem cell, even if placed in the liver, might be able to become a neuron, a skin cell, or, ultimately, an egg or sperm.

Although Weissman has not performed the two experiments discussed in the report (he has continued some human/non-human chimera experiments), other researchers have continued to make human/non-human hybrids, in a wide variety of contexts, such as studying human tumor cells by transplanting them into mice. These chimeras receive little or no attention, but two

researchers have received some publicity for work with chimeras, one involving neural cells, one with liver cells.

A group of Yale University (New Haven, CT) researchers led by Dr. Eugene Redmond have been experimenting with transplanting immature human neural cells into the dopamine-producing regions of the brains of green vervet monkeys. Those regions are associated with Parkinson's disease, and Edmond and his group hope that their research may ultimately be useful in understanding and treating humans with Parkinson's disease (Redmond 2002; Shreeve 2005).

Meanwhile, at the University of Nevada (Reno, NV), Dr. Esmail Zanjani has produced chimeras by transplanting human stem cells, mainly human blood-forming stem cells, into sheep. Zanjani has claimed that these human cells have been transformed into a variety of cell types in the sheep, in at least one case producing a sheep with a liver with 40% of the liver cells derived from human cells. According to Zanjani, these livers contained characteristically human structures and produced fully human proteins. Zanjani's work stirred up controversy with reports that the chimeric sheep had been given to a University-owned ranch that let the naïve research sheep out to graze as if they had been raised on the ranch, resulting in dead sheep and happy coyotes (Mullen 2005).

Bioethics

At the time of our report to Weissman, there was effectively no bioethics literature on human/non-human chimeras. That began to change in 2003 with the publication in the *American Journal of Bioethics* of a target article by Jason Scott Robert and Françoise Baylis (Robert and Baylis 2003). Robert and Baylis argued for caution in the creation of human/non-human chimeras, based on the possibility of creating confusion about the moral status of the resulting organism. Their article attracted many comments, of which those by Greely, Streiffer, Cohen, and Karpowicz were particularly interesting (Cohen 2003; Greely 2003; Karpowicz 2003; Streiffer 2003).

Phillip Karpowicz, Cynthia Cohen, and Derek van der Kooy published a useful article in 2005, following up in more detail on a 2004 article (Karpowicz et al. 2004; Karpowicz et al. 2005). They analyze four arguments against human/non-human chimeras: moral taboo, species integrity, unnaturalness, and human dignity. They find only the last argument convincing, but only if the human cells,

> when transplanted into the prenatal mouse or monkey, were to proliferate and develop into a whole human-like brain and if human-like capacities associated with human dignity were to emerge in such animals to some degree . . . (Karpowicz 2005, 123–124).

The article continues to make specific recommendations for limiting chimera research to avoid the risks of "developing whole human-like brains" or "human-like capacities."

Also in 2005, a working group organized by Ruth Faden and Guy McKhann at Johns Hopkins (Baltimore, MD) reported its conclusions concerning transplanting human neural stem cells into the brains of non-human primates (Greene et al. 2005). This working group concluded that such research should try to minimize the risk that the resulting animal would have more human-like cognitive capacities. It argued that such experiments be subjected to special review and that the reviewing bodies should consider six factors:

> (i) the proportion of engrafted human cells, (ii) neural development, (iii) NHP [non-human primate] species, (iv) brain size, (v) site of integration, and (vi) brain pathology (Greene 2005, 386).

The Johns Hopkins working group recognized that there was "no simple relationship between these factors and, thus, no formula for making evaluative judgments." It did use the six factors to argue that grafting a large number of human cells into developing great apes would be more troubling than transplanting small numbers of human cells into the brains of healthy, adult monkeys most distant from humans.

Still later in 2005 Robert Streiffer took the position that the most important ethical considerations in such research should arise out of concern for the chimeras created by such research. He argued that such research *might* significantly enhance the moral status of those chimeras, which he sees as, on its face, good, but that the chimera will not be treated as its higher status demands. Recognizing the very substantial uncertainties about what qualities such research would create—and what ethical significance those qualities would have—he argues that, at this time, policies that require the early termination of such chimeras or that forbid the introduction of pluripotent stem cells into non-human primate blastocysts (the position taken by the National Academy of Sciences [NAS] Guidelines) are appropriate (Streiffer 2005).

Finally, in August 2006 a private organization called the Scottish Council for Human Bioethics published a report on "animal-human mixtures" (Scottish Council 2006). The report covered a wide range of ways in which human genes, cells, or reproductive processes might be mixed with those of non-humans. Two of this group's recommendations are particularly relevant:

> 11. The incorporation of human stem cells into post-natal animals should proceed with extreme caution. Moreover, such a procedure should only take place if it can be demonstrated that the cells cannot contribute to the germline or give rise to specifically human brain functions in the animals.
>
> 12. The incorporation of human stem cells into post-blastocyst stages of non-human embryos should only take place if it can be demonstrated that they cannot contribute to the germline or brain cells of the animal (Scottish Council 2006, 1).

The recommendation concerning embryos is more restrictive (cannot contribute to "brain cells" versus cannot contribute to "specifically human brain functions") because of greater concern about introducing cells at an earlier stage of development. The report makes these recommendations after reviewing many of the discussions of chimeras, which it says demonstrate the special sensitivity of possibly affecting a non-human's brain (or germ) cells.

Policy

The issue of human/non-human chimeras has led to at least two efforts to create guidelines or rules for this activity. The first comes from the National Research Council (NRC) and Institute of Medicine (IOM) in the United States. The second is legislation proposed in the United States. There may appear to be at least one more effort, as Canada prohibited the creation of "chimeras" in 2004 when it adopted its Assisted Human Reproduction Act. That act, however, defines chimeras only as *human* embryos into which cells from other human or non-human entities have been placed, and thus would not cover mice with human cells. (Assisted Human Reproduction Act 2004). (We have not tried to survey all legislation around the world in search of bans on chimeras; other such bans may exist.)

In April 2005 a committee created by the NRC and the IOM produced a report with guidelines for how to conduct human embryonic stem cell research (NRC 2005). The October 2004 meeting of this committee had included testimony from several scholars about the creation of chimeras, including Irving Weissman, David Garbers and Bridgid Hogan on the scientific issues and Henry Greely, Cynthia Cohen and William Hurlbut on the ethical issues (NRC 2005, Appendix C). These guidelines included the recommendation that an embryonic stem cell research oversight committee review and approve all research "involving the introduction of

hES [human embryonic stem] cells into nonhuman animals at any stage of embryonic, fetal, or post-natal development." The guidelines further urged that "particular attention should be paid to the probable pattern and effects of differentiation and integration of the human cells into the nonhuman animal tissues." The guidelines also stated that no animals in which human embryonic stem cells had been introduced should be allowed to breed and no such cells be introduced into the blastocysts of non-human primates (NRC 2005). The text of the report addressed specifically the issue of putting human embryonic stem cells into the brains of non-human animals:

> Perhaps no organ that could be exposed to hES cells raises more sensitive questions than the animal brain, whose biochemistry or architecture might be affected by the presence of human cells. Human diseases, such as Parkinson's disease, might be amenable to stem cell therapy and it is conceivable, although unlikely, that an animal's cognitive abilities could also be affected by such therapy . . . . Protocols should be reviewed to ensure that they take into account those sorts of possibilities and that they include ethically sensitive plans to manage them if they arise (NRC 2005, 50).

The NAS Guidelines and discussion dealt only with human *embryonic* stem cells, but the issues they raise apply to all human stem cells that can give rise to central nervous system cells.

It is not clear how widely the NAS Guidelines are being followed by United States institutions performing human embryonic stem cell research. Those following stem cell research generally believe that the NAS Guidelines are widely used, although firm evidence is lacking. In California, the NAS recommendations on embryonic stem cell research oversight committee review of chimeras have been largely adopted both as regulations by the California Institute for Regenerative Medicine, which manages the stem cell research funding provided by California's Proposition 71, and by the California Department of Health Services Advisory Committee on Human Stem Cell Research, which is charged with recommending guidelines for human embryonic stem cell research in California not funded by California Institute for Regenerative Medicine.

In early 2005, *The New Atlantis*, a conservative journal that calls itself "an effort to clarify the nation's moral and political understanding of all areas of technology," published an editorial entitled "The Bioethics Agenda and the Bush Second Term," in which it called for an aggressive legislative campaign both to ban the creation and destruction of human embryos for research purposes and to "defend and advance the dignity of human procreation and the human family" (Cohen 2005). In March 2005 Senator Sam Brownback (R-KS) proposed legislation that would largely implement the recommendations made by the editorial in pursuant of its second goal—and that would ban the creation of some forms of human/non-human chimeras.

The Human Chimera Prohibition Act of 2005, originally introduced in March 2005 and reintroduced that July as S. 1373, would make it a felony, punishable by 10 years in prison and a civil fine of at least $1 million, to create, attempt to create, transport, or receive for any purpose a "human chimera" (S. 1373, §302(2)). The Act defines "human chimera" in eight different ways, most of which appear to deal with hybrids of various sorts rather than chimeras (S. 1373, §301(1)). The eighth subsection appears to have been aimed directly at Dr. Weissman's proposed experiment:

> (H) a non-human life form engineered such that it contains a human brain or a brain derived wholly or predominantly from human neural tissues (S. 1373, §301(1)(H)).

(The Senator may have missed his target, as Weissman's most ambitious experiment, even if successful, would not affect the 90% of the mouse brain that was made up of glial cells instead of neurons.) It is this legislation that President Bush was endorsing in his 2006 State of the

Union address, legislation that has not been voted on in the Senate or the House of Representatives, or received committee hearings.

BACKGROUND TO THE WORKING GROUP REPORT

When the original report was written for Dr. Weissman, researchers had recently reported finding human brain stem cells—cells that can become all or most of the cell types found in the human brain, including neurons and glial cells. These cells, thought to be very infrequent in adults, were isolated from the brains of human fetuses. This discovery opened the possibility of studying human neurons *in vivo* in laboratory animals by creating mice whose brains were made up, in part or in whole, of human neurons.

Although such a "human neuron mouse" would not stand and talk like a cartoon character, its possible creation raises important and interesting ethical questions about research in human neuroscience. The next section lays the groundwork for evaluating these issues by discussing human brain stem cells, examining completed and planned experiments involving transplantation of these cells into mice, and finally by describing our working group and its general approach to the questions before it.

Human Brain Stem Cells

In 2000, researchers claimed to have isolated human brain stem cells from the brains of fetuses aborted after 12 weeks of development (Uchida 2000). Research with these cells *in vitro* showed that they could form many different kinds of human brain cells—not just neurons in their various types but also other cells that play essential roles in the brain, such as glial cells. They seem, therefore, to be multi-potent cells. The isolation of these cells opened the possibility of growing and transplanting mature brain cells, particularly neurons, into patients with such debilitating neural degenerative disorders as Parkinson's disease. In 2006, the FDA granted an investigative new drug exemption to one firm to perform such transplants for a rare childhood disease (Batten's disease); an institutional research board at the Oregon State Health University (Portland, OR) recently approved a trial of this approach (StemCells, Inc., Palo Alto, CA). Whether this kind of neural regenerative medicine will prove safe or effective remains, of course, unknown. Stem cell therapy with hematopoietic stem cells is regularly used, with frequent success, to build or rebuild a patient's blood and immune systems; it remains of speculative value in other contexts.

Dr. Irving Weissman at Stanford Medical School (Stanford, CA) was one of the researchers who helped isolate these brain stem cells. Weissman had long worked with stem cells and had been instrumental in the isolation of human hematopoietic stem cells. Working with those cells and other human tissues, in 1988 he and Dr. Joseph M. McCune created the so-called "SCID-hu" mouse. This work started with an inbred strain of mouse born with severe combined immune deficiency (SCID). These mice, as a result, had severely impaired immune systems. Weissman and McCune transplanted human hematopoietic cells (in later experiments, human hematopoietic *stem* cells) as well as the tissues that support for the formation of blood and cells of the immune system (human fetal bone, thymus and liver) into these SCID mice. The weak immune systems of the mice did not attack the human cells as alien and those cells were able to colonize the human fetal bone and liver, and later thymus, to create in them a human blood-forming and immune system. The result was a laboratory animal model of the human blood-forming and immune systems, on which experiments could be done that could not ethically be done with the only other creatures with an *in vivo* human immune system, living humans.

Using these mice the human hematopoietic stem cell was first isolated and gained FDA approval for trials that showed these cancer-free stem cells could regenerate the blood-forming and immune systems that had been depleted by cancer therapies. These animals were also used

to infect a human immune system with patient isolates of HIV, the first time one could show definitively that HIV caused the changes that characterize AIDS in humans.

The Mouse Transplant Experiments

As part of the research leading to the isolation of human brain stem cells, Weissman, Uchida and other colleagues at the firm StemCells Inc. began transplanting human brain stem cells into the brains of SCID mice with normal murine brains. (SCID mice were again used to avoid an immune system attack on the human cells.) The human brain stem cells were placed in a brain structure called the *lateral ventricle*, which, in mice, connects to their brains' quite large olfactory bulbs. Weissman's group was able to show that the human neuronal stem cells engrafted in a brain stem cell niche called the *subventricular zone*, near the injections. Those cells also migrated to a second niche, the dentate gyrus of the hippocampus. In these niches the human cells divided and many of them migrated toward the olfactory bulb (Tamaki et al. 2002; Uchida et al. 2000). Samples of the brains of these mice showed that the human neurons had survived and had connected to the mouse brain. Mouse brains have a (relatively) much larger olfactory bulb than human brains and new mouse neurons are regularly migrating to their olfactory bulbs; the human-derived cells did the same. Examination also showed that the human neurons had moved into other areas of the murine brains and made up more than 1% of the neurons in some regions. This research could not, however, determine whether the human neurons were actually functioning as part of the mouse brain, let alone whether they were functioning normally.

Weissman then wanted to do two other experiments transplanting human brain stem cells into mice. These proposed experiments were the subjects of our report.

The first proposed experiment would work with an inbred strain of mouse that lost all the neurons in its cerebellum several weeks after birth. In both mice and men, the cerebellum is involved in fine motor skills, coordination and balance. Other roles of the cerebellum, including any role in consciousness, are unknown. A mouse, or a person, without a functioning cerebellum can survive but with substantial motor deficits. Friedrich's ataxia is a human disease caused by the death of cerebellar cells; this strain of mice displays symptoms similar to those of humans with Friedrich's ataxia. Weissman wanted to transplant human brain stem cells into such a mouse just before its cerebellar neurons began to die. He hoped that the human cells would differentiate into neurons, would migrate into the mouse's cerebellum, and would begin to function. Unlike his earlier experiment with putting brain stem cells into the lateral ventricles of mice, this experiment would be able to determine whether the human neurons were not only surviving but were functioning, in part through seeing whether and to what extent the treated mouse showed signs of normal cerebellum activity. Based on Weissman's previous experiment, he also expected that the human cells would appear at low concentrations in other parts of the mouse's brain.

The second proposed experiment would use a different inbred strain of mice, developed by Fred Alt's laboratory. These mice form the beginnings of the nervous system, creating the structures that support the movement of early stage neural progenitors, but all these developing neurons die, leading to the death of the early stage fetuses. This mouse strain also has a mutation that makes them SCID mice, so that the resulting mice would not reject human cells. For this experiment Weissman planned to transplant the human brain stem cells *in utero* shortly before the murine neurons were expected to begin dying. He hoped that the human cells would differentiate, migrate to the places where the murine neurons are dying, and take their places. The result would be a mouse brain, the neurons of which were mainly human in origin. This experiment could have at least two different end points. In one version, the mice could be aborted as fetuses shortly before birth and have their brains examined on autopsy to see whether the human neurons had populated their brains and, if so, what kinds of brain structures—mouse,

human or mixed—they formed. Alternatively, the mice could be allowed to go to term and, in addition to examination of their brains, by neuroimaging while alive or by autopsy, their functioning and behavior could be observed for variations from the mouse norm. If the mice were viable, they might be the neuronal equivalent to the SCID-hu mouse in terms of being a laboratory animal that could be used for experiments on living, *in vivo*, human neurons.

Although, as subsequently described, the working group's report concluded that these experiments could be undertaken ethically, at this writing neither experiment has been performed. Weissman discovered that the Friedrich's ataxia model mouse did not, in fact, have the characteristics he needed for his experiment. One study, however, did follow up some of the questions involved in the cerebellar experiment. Dr. Fred Gage's laboratory reported in late 2005 that they had transplanted stem cells into the brains of rats and had been able, using patch clamping, to determine that the cells derived from the transplanted human cells were actually firing. The fact that these human-derived neurons were firing does not necessarily mean that those cells were functioning properly, either individually or as part of a larger unit, in the rat. But if it had been the case that the human-derived neurons were *never* firing, they clearly could not be functioning normally (Muotri et al. 2005).

As to the second experiment, there were problems with breeding the mouse strain with complete neuronal death. Weissman has also been occupied with other work, not only with other aspects of his own research but with administrative and advocacy work around human stem cell research. He also needed to find a graduate student or postdoctoral fellow interested in doing the work; the fellow who was interested at the time had gone on to other work. Weiss-man continues to say that he might try the second experiment, but he also from time to time refers to it as a "thought experiment." It is not clear to us, and perhaps not to him, whether or not he will return to this experiment.

The Working Group and Its Approach

Weissman was aware of the sensitivity of these planned experiments, both ethically and in terms of public reaction. He may well have had visions of a headline reading "Stanford Scientist Creates Mouse with Human Brain." As a result he asked one of the authors of this article (Greely) to consider putting together a group to examine the ethical issues in these proposed experiments. Greely pulled together this ad hoc group, with representation from several disciplines. We met several times during 2000 and 2001, interviewed Weiss-man, studied the scientific literature, and discussed the questions—and how we could approach those questions—at length. We concluded that the experiments did raise interesting and important, but manageable, ethical issues

In general, we approached the questions by asking about the potential benefits and the potential costs or risks of the proposed experiments. We first examined the costs to see if any of them might categorically rule out the experiments. We next considered ways in which the experiments might be undertaken to limit the costs of risks involved. We weighed the potential benefits of the research, with or without modifying conditions, against the potential costs or risks. We concluded that the experiments could proceed ethically, subject to careful staging and monitoring.

POTENTIAL BENEFITS

United States government regulations and international agreements on ethical research agree that research on human beings is only permissible if there are potential benefits, to applied or to basic science, from the research that outweigh the potential harms and risks. A similar, though weaker, standard applies in federal law to the use of many laboratory animals, including mice. Researchers obviously can do things to laboratory mice that they may not do to humans,

including routinely maiming or killing them. They may not, however, do such things without a good reason. Both because living animals were to be used and because of the nature of the human cells being used, Weissman's proposed experiments could be justified only if the experiments were likely to offer some benefits.

The most clear potential benefit is the creation of a non-human animal in which human neurons can be studied in a living brain. Many experiments on human neurons, and on the diseases of those neurons, cannot ethically be performed in humans. These experiments involve risks too high to be permissible for a human subject to bear or, in many cases, the killing of the human subject and the subsequent examination of his or her brain. Such research with human subjects is, of course, not morally acceptable.

This benefit, in effect, would come from the creation of a brain equivalent to the SCID-hu mouse. Thousands of SCID-hu mice have been used in research on the human immune system, particularly but not solely with respect to HIV infection. More than 100 grants from the National Institutes of Health (NIH) have involved the use of SCID-hu mice and, over the years, the NIH has contracted for the production of more than 1,200 SCID-hu mice.

Having a laboratory animal for studying human neurons might have substantial benefits, both for basic science and for clinical applications. For example, the methods by which various pathogens or exposures damage human neurons could be directly studied in a living brain without risking harm to a human subject. New drugs or other treatments could be first tested for their effects on human neurons in mice rather than in human subjects. Steps in the *in vivo* functioning of human neurons could be analyzed without risking harm to living people.

None of these benefits is assured. These experiments may fail, or, whether they fail or succeed, a human neuron mouse may prove impossible to create. Given the vast and thus far poorly understood number and type of interactions between cells that take place in the brain, we would be surprised if human neurons could function properly in all the roles necessary to create a properly working mouse brain. Even if a human neuron mouse proved possible, research with it might not be substantially better than existing alternatives. Studies of human neurons outside the brain through *in vitro* research or *in vivo* studies of mouse neurons in mouse brains might prove just as illuminating of human brain function as the study of human neurons in a mouse brain. Nonetheless, the potential for substantial scientific and even medical benefits seemed significant to us. Because of these anticipated benefits, the experiments seemed reasonable and, in the case of the experiment that could create a murine brain composed entirely of human cells, necessary steps to assess that potential.

RISKS AND COSTS

We identified five areas of concern that need to be examined and, if found significant, weighed against the potential benefits. These concerns include: 1) the sources of the human brain stem cells; 2) the potential for pain and suffering to the mice; 3) the propriety of this use of human tissues (particularly brain tissues); 4) the risks of possibly conferring some degree of humanity on another species; and 5) the risks to public support of science.

It is interesting, in retrospect, to compare those concerns with those subsequently expressed in the literature on human/non-human chimeras. Most of the issues that concerned us have been largely or entirely ignored in subsequent discussions. In one form or another, the question of "conferring humanity" has been the focus of the discussion, although generally expressed in terms of either human dignity (Karpowicz 2003) or avoiding moral confusion (Robert 2003). Streiffer's position is more complicated; he argues that the successful conferring of a higher moral status on a human-mouse chimera would not be wrong in itself, but would likely be wrong because we would not treat the chimera in a way consistent with that higher status

(Streiffer 2005). A little has been said on the other issues. The Johns Hopkins group on transplanting human neural tissue to non-human primates did discuss briefly the issue of harm to the subject animals (Greene 2005); Karpowicz did discuss and reject at least one form of the public relations argument (Karpowicz 2005).

We did not discuss in our report some of the moral taboo arguments rejected by Karpowicz, or integrity of species borders and unnaturalness arguments, rejected by both Karpowicz and the Johns Hopkins group. Our internal discussions had already considered and rejected all of those arguments and our report described only arguments we found potentially plausible.

Aborted Fetuses as the Source of the Human Brain Stem Cells

The human brain stem cells that Weissman uses were derived from the brains of human fetuses that had been intentionally aborted. Use of such tissue has been controversial in the United States because of its link to voluntary abortion. The issue of using human fetal tissue in research and medicine was discussed widely in the late 1980s, spurred in part by evidence that transplants of fetal brain tissue into the brains of people with Parkinson's disease could lead to improvement in their condition. (As it happens, this therapeutic application of human fetal tissue has since been shown, at least so far, to be neither safe nor effective.) For research and medical purposes, tissues from intentionally aborted fetuses were greatly preferred to tissues from spontaneous abortions or stillbirths because of the much greater risk that the cells and tissues from the latter had suffered from fatal genetic conditions, had been contaminated by pathogens, or had died in the long period between the *in utero* death of the fetus and the collection of the tissues.

In 1988 the Secretary of Health and Human Services imposed a moratorium on federal funding for research using human fetal tissue pending further consideration. Both government commissions and private commentators debated the morality of such use with an NIH advisory panel recommending in late 1989 that the moratorium be lifted subject to certain restrictions (Greely 1989). The first Bush Administration nonetheless extended the moratorium indefinitely. The Clinton Administration lifted the moratorium in January 1993. On February 1, 1993, the NIH adopted "interim policy guidance" that allowed the use of human fetal tissue in federally funded research under certain conditions (NIH 1993). This guidance was then superseded by very similar provisions in the NIH Revitalization Act of 1993 (NIH Revitalization Act 1993). The NIH conditions sought to ensure that the potential use of the tissue would not induce a woman to have an abortion that she otherwise would not have chosen. Note that at no time has there been a federal ban on the use of human fetal tissue in research *not* funded by the federal government. On the contrary, such research is not even limited by the conditions imposed first by the NIH and then by Congress.

Controversy over research and medical use of human fetal tissue from intentionally aborted fetuses continues in spite of the 1993 legislation. President Bush's August 9, 2001, decision concerning federal funding for human embryonic stem cells does not apply to the human brain stem cells, which are isolated from much older tissues, but it does reflect the continuing debate over the research use of fetal tissue (NIH 2001). The SCID-hu mouse itself has been the subject of a negative article in the conservative publication *Human Events*, focusing on the fact that its creation requires using live tissue from "a human child—and every child who donates tissue to create such mice is first killed by a medical doctor" (Jeffrey 2001, 1). In light of the continuing and high-profile controversy over human embryonic stem cells, it is perhaps surprising that the use of tissue from aborted fetuses has not reappeared as an issue in Congress or the Administration. Certainly, no one can guarantee that it will not return.

The derivation of human brain stem cells from intentional abortions did not raise substantial concerns for this working group, particularly if the tissue is donated in accordance with the

federal funding requirements. Abortion currently is a protected right in the United States and, even if some find that regrettable, the use of fetal tissue is unlikely to affect the number of abortions performed. Our group thus put to one side the issue of the morality of abortion. Nonetheless, we recognize that for some people this issue will be important. Others may and will take a different view. Should human brain stem cells of equal power become available from other sources about which less ethical controversy exists, such as adult humans or spontaneous abortions, researchers might prefer to use them.

Inhumane Treatment of the Mice

Both law and ethics require that laboratory animals not be used wantonly. They should only be used in risky or harmful experiments when the potential benefits outweigh the costs and with due regard to the pain they might experience. Laboratory animals may be killed painlessly if the experiment requires that result and its potential benefits justify the deaths. These animals should not normally be treated in a way that is painful unless both the need for the experiment and the justification for the pain are very strong. (Of course, some have stronger objections to the use of animals in experiments in ways that harm or disable them.)

That mice will be killed in this research, even if the deaths are painless, requires that the experiments have countervailing potential benefits. The effects of these experiments upon the mice, while alive, are, at this point, unknown. Mice killed *in utero* in the second experiment presumably would not experience significant pain. Otherwise, we have no way of knowing whether the mice in the first experiment, which might have cerebellums made of human neurons, or mice brought to term in the second experiment, which might have brains made entirely of human neurons, would feel pain as a result without actually doing the experiments. If the experiments resulted in, for example, constant painful seizures or apparently painful self-destructive behavior, then the continuation of the experiments would have to be reconsidered in light of that finding. (Of course, human consciousness trapped in a mouse's body would truly be cruel treatment, but, as discussed later in text, this possibility seems extremely unlikely.)

Respect for Human Tissue—Particularly Brain Tissue

A third concern arises from the fact that these experiments place living human cells inside a non-human animal. By so doing, some may argue that the researchers show insufficient respect for the human origin of the cells.

Both ethically and legally, we limit the potential uses of human tissues. Human remains are not normally displayed except as part of funeral services; most human organs cannot be sold; corpses and body parts must, by law, be disposed of in a respectful manner; and cannibalism is forbidden. It is not clear whether these prohibitions stem from respect for the individual whose body parts or tissues are involved or from a fear that such uses hold humanity itself in disrespect—and may, in time, lead to even more noxious disrespect for living human persons. Whatever its sources, the demand for respect for the bodies of the dead has deep roots in western culture—consider as one example Sophocles's play *Antigone*—and presumably in many other cultures as well.

In one respect, we do use human tissues for many purposes, at least with the relevant person's permission. Organs are used for transplantation and some human tissues, notably blood, semen and eggs, are bought and sold for medical purposes. Placentas have long been sold for various uses; more than one million each year are sold in the United States to firms that use them to make medications. Extracts from human placenta have even been used in cosmetics, notably skin creams. Corpses, skeletons and organs are used in medical training and in research. These uses are made not only for medical purposes but also for anthropological or historical ones,

including the display of human remains in museums. The line between the educational value of human tissues, for example, mummies displayed in museums, and their function as entertainment is, admittedly, a narrow one. In several places in Europe, that line seems to be fairly clearly crossed as old human skeletal remains are displayed as tourist attractions in catacombs. A newer version of this mixture of education and entertainment includes the touring "plastination" exhibits, which show, in dramatic poses, human corpses preserved through the infusion of plastics into their tissues (Bohannon 2003).

Our working group found little ethical discussion or scholarly literature on the appropriate treatment of human tissues. (Interestingly, there has been some public discussion about the proper uses of brains or brain tissue in two cases; the whole brain of Ishi, a much-studied California Native American who died in 1916 [Starn 2004] and sliced tissue samples from Albert Einstein's brain [Burrell 2004]). The type of tissue involved in these proposed experiments undoubtedly increases the stakes. Whereas both the heart and the liver have been viewed in different cultures as the location of a human's essence, there seems little doubt that in at least Western culture the brain holds a very special place as the seat of consciousness and, for many people, what they view as their souls. Transplanting human thymus or liver tissue into mice does not have the same overtones as transplanting neurons. Part of that heightened concern stems from the possibility of transmitting some human qualities to the mice, which is discussed later in text. But another part of this concern may stem from a sense of "sacredness" about the brain as the site of consciousness.

Our view was that several different considerations are important in analyzing the appropriate use of human tissues, including brain tissue. These considerations include whether the tissues are used with free consent of the proper person, the purposes for which they are used, whether they are treated in a respectful manner, and the tissue's "degree of humanness." After much discussion, we concluded that these considerations appear to support the use of human brain tissue in Weissman's proposed experiments. Human brain stem cells derived from aborted fetuses are not, obviously, used with the consent of the fetus but are used only with the consent of the woman who carried the fetus. No more appropriate source for consent seems plausible. In addition, existing federal law, although it only applies to tissue used in federally funded research, contains provisions to help ensure that the consent is freely given and that no one was coerced into or even influenced toward getting an abortion to acquire fetal tissue; in fact, the woman involved may not even know research use is a possibility before she has committed herself to the abortion (Department of Health and Human Services 2001). The purpose of this use of human tissue is research into the fundamental characteristics of human neurons and the prevention and treatment of neuronal diseases, which seems a worthy use. There seems no reason to believe that the small amounts of human tissue would be treated lightly or without respect.

As to the last point, the human tissues involved here, although they come from arguably the organ most tied to human identity, are small masses of disaggregated cells, suspended in fluid contained in vials. An outsider looking at them would have no idea what they were. They do not have the more obvious humanity of a severed head, a skull or a full skeleton. Human neurons are not human brains, but merely one of their constituent parts. It is not clear—indeed, one of the goals of the proposed experiments was to explore—whether the human neurons in a mouse brain would function in any meaningful way like human neurons rather than mouse neurons. Moreover, it is not obvious to us how "humanity" can be located in a cell or a body part; when we lose skin cells or even limbs, we do not use some of our humanity. If one somehow transplanted—or grew—a full and fully human brain into another animal, the objection about moving special human "tissue" would seem much stronger.

We recognize that the ethical discussion concerning the appropriate treatment of human tissues is not very fully developed and that reasonable people may well disagree with our report on this point. It is certainly clear that different cultures may have different views; respect for human body parts has increased markedly in European and European-derived cultures in the past few centuries as traitors' heads are no longer posted at city gates. At this point, however, our consideration of the apparently relevant factors leads us to conclude that proper respect for human brain tissues does not prohibit the appropriate use of these human brain stem cells in mouse transplantation experiments. (We have not considered how these concerns might ultimately affect successful therapies based on human brain stem cells.) Additional discussion of this issue seems appropriate.

One further issue concerns the appropriate disposition of the brains or bodies of any of the experimental mice after they have died or been killed. Zanjani was criticized for the disposition of his experimental sheep with partially human tissues, particularly heavily humanized livers. The sheep were given to a ranch, which treated them just like its regular sheep and put them out to graze. The research sheep, however, had no experience in the wild and quickly succumbed to its rigors, including coyotes. The press coverage centered on concern that putting these sheep in this situation was inhumane, which to us seems convincing (Mullen 2005). No one would plan to release human neuron mice into the wild, to be slaughtered by local cats. But there is another possible concern raised by the sheep example: it might be inappropriate for partly or fully humanized tissue to be eaten by other animals. For coyotes to eat partially ovine, partially human livers is certainly not cannibalism, but some might argue that, as most cultures strive to avoid letting human corpses be scavenged, we should do the same for human cells or tissues incorporated in non-human animals.

Of course, cultures that practice burial recognize that corpses are consumed, both by generically referenced "worms" as well as by bacteria and other microbes. The Parsi religion continues, at least in some locations, to dispose of their dead on "towers of silence," where the corpses are consumed by vultures (Dugger 2001). And, until now, no culture has had to determine what is the appropriate disposition for the bodies of animals that contain some human cells. In the context of the human neuron mouse, we believe this does not require any more than the treatment of the bodies and tissues of human neuron mice as medical waste, but this issue may need further discussion.

Conferring Humanity on Mice

In Kafka's *Metamorphosis*, Gregor Samsa was transformed into a cockroach; would these experiments, in any relevant way, transform a mouse into a man? Or, to be more precise, into a creature with some aspects of human consciousness or some distinctively human cognitive abilities? This result seems implausible, but we cannot rule it out on a priori grounds.

The mouse brain is significantly smaller than the human brain. In volume it is less than one-thousandth the size of the human brain. Even apart from their smaller size, mouse brains are organized differently from human brains. The proportion of a brain composed of the neocortex, the region most associated in human brains with consciousness, is hugely greater in humans than in mice. The brain is an incredibly complex network of connections. Neuroscientists believe that it is the architecture of the brain that produces consciousness, not the precise nature of the neurons that make it up. As an analogy, architecture determines whether a building is a cathedral or a garage, not whether the bricks used are red or gray. A mouse brain made up entirely of human neurons would still be a mouse brain, in size and architecture, and thus could not have human attributes, including consciousness.

This argument is extremely plausible but, to date, it has not been tested. At least one set of experiments has been done indicating that some behaviors might be transmitted between

species along with brain tissue. As early as 1988, scientists showed that by transplanting embryonic quail tissues, primarily tissues that give rise to the central nervous system, into embryonic chickens, they could produce chickens that exhibited quail-like behavior (Balaban et al. 1988). The resulting chicks "crowed" like baby quail rather than like baby chickens. These experiments involved the transplantation of large quantities of intact tissue, not disaggregated neurons, and much of that tissue remained homogeneously quail-derived in the chick's brains. Thus, the chicks' brains could be viewed as being one building with two different kinds of architecture as well as two different kinds of breaks. In this respect that experiment differs substantially from the experiments in question here. Given how little is still understood about consciousness and its sources, it is not clear whether it differs enough.

The quail-chicken experiments suggest that a crucial question for the human neuron mouse experiments is whether the human neurons become organized in the mouse brain in murine patterns or in human ones. The fact that the mice will have already constructed their own brains with murine neurons before the human cells are transplanted argues that the human cells would follow the existing murine structure, but without doing the experiment, that cannot be assumed. The cerebellar experiment will offer little information on this point. All mammalian cerebella are organized in generally identical, and relatively simple, structures. And, in any event, the cerebellum does not, at this time, appear to be significantly involved in human consciousness.

The whole-brain experiment, however, should offer many opportunities to see whether the brain organization is murine or human. Human and mouse brains are organized differently in many ways, at both large and small scales. The relative sizes of the various parts of the brain are one set of differences. The existence and nature of particular brain structures are others. For example, mouse brains have easily visible structures called "whisker barrels" in their cortexes that appear to receive and manipulate information from their whiskers. Each whisker reports to one and only one whisker barrel. Humans (even mustachioed men) do not have whisker barrels. In contrast, human brains (and other primate brains) have especially complicated visual centers with multiple layers of neurons involved in the processing of visual information. Mouse brains have few layers and less complexity in their primary visual area.

What we called "conferring humanity on mice" seems to be the main concern in the literature on chimeras and, presumably, is the main concern in the "brain clause" of the Brownback chimera bill. The authors have not used our language of "conferring humanity" on the transplanted animal, but the concerns each expresses seem equivalent to the concerns we encompassed in our term.

The Johns Hopkins group took the express position that human/non-human chimera experiments should try to minimize the risk that the resulting animal would have more human-like cognitive capacities. Robert and Baylis (2003) wrote of how chimeras might induce confusion about the moral status of the resulting creature, primarily as a result of the possibility that they would have some human cognitive capabilities.

Karpowicz et al. (2005) find that the only plausible argument against the creation of human/non-human chimeras is based on human dignity:

> By giving nonhumans some of the physical components necessary for development of the capacities associated with human dignity, and encasing these components in a nonhuman body where they would either not be able to function at all or function to a highly diminished degree, those who would create human-nonhuman chimeras would denigrate human dignity (Karpowicz et al. 2005, 121).

It is not clear to us that this is the case, but, in any event, the argument assumes, as the Karpowicz article later makes clear, that the recipient animal would, in fact, develop a "physical

component"—in this case, a brain—"necessary for the development of the capacities associated with human dignity." That would be an extremely unlikely result of these experiments.

And the NAS Guidelines noted the sensitive questions raised by the effects of stem cell transplants on an animal's brain, including the unlikely but conceivable possibility that the human cells might affect its cognitive abilities. The NAS urged embryonic stem cell research oversight committees to pay special attention to the possibilities that embryonic stem cell transplants might give the animal "characteristics that are valued as distinctly human" or "human characteristics that would be ethically unacceptable to find in an animal" (NRC 2005, 50).

We must mention one other way in which these experiments might confer some attributes of humanity on mice. Like hematopoietic stem cells, human brain stem cells are multi-potent. They make many different kinds of brain cells, including both neurons and non-neurons. Some much-contested recent research asserts that, in some circumstances, human hematopoietic stem cells can make cells from other tissues, such as the liver and the brain. If this turns out to be true, which many other researchers strongly dispute, it is conceivable that human brain stem cells could do the same. The possibility that the human neuron mouse would also have a liver or a kidney that was partially made up of human cells seems to add little, if anything, to concerns about its brain. It might be more troubling, though, if the human brain stem cells could become, in the mouse, germ cells—egg and sperm cells that contained wholly human genes.

This result seems almost impossible. Even if human brain stem cells can become germ cells, those cells (or, in the case of sperm, their progenitors) are formed early in mammalian development and would be created and in place long before the human cells arrived. Even if a mouse did produce human sperm or eggs, they could not fertilize or be fertilized by a mouse germ cell in a way that would lead to the production of an embryo. Humans normally have 23 pairs of chromosomes containing their DNA; mice have only 20. This difference, among many others, should forbid the production of any even transiently viable offspring. But, once again, it is difficult to make guarantees before the experiments are done.

This issue was discussed by the NAS guidelines on embryonic stem cell research (NRC 2005, 39–40). It concluded that no animals into which human embryonic stem cells had been transplanted should be allowed to breed. Given that the cells in Weissman's experiment would not be embryonic, and hence pluripotent, but only brain stem cells and thus presumably only able to make cells in the brain lineage, the guidelines would not apply to this research. In another respect, there seems no particular reason to breed human neuron mice; their progeny would not have human-derived brain neurons but regular mouse neurons.

One final issue about humanness is worth noting, even though it is not raised by these proposed experiments. Distinctive humanness does not just reside in the brain and the gonads. Although a chimpanzee with a human gall bladder or a human appendix would not be likely to raise grave concerns, a chimpanzee with a human face, a human skull or human hands and feet might. In addition to concerns about human brain functions and human gametes, giving non-human animals, in whole or in the part, the outward physical appearance of humans, could be deeply unsettling. Whether that is a moral argument or prudential one, such experiments should be undertaken, if at all, only for the most powerful reasons.

Public Reactions

Public reaction to unsettling scientific research has been called everything from "the yuck factor" to Leon Kass's "wisdom of repugnance." Based on our own reactions when we first heard of these experiments and from those of friends and colleagues with whom we have

discussed the experiments, we were confident that some people will have a strong initial reaction against this research. That reaction might be only a passing problem of public relations for the institutions where the research is performed. But it could also be a political problem if it undermined support for this and other useful biomedical research. And if one concluded that such research, aimed ultimately at the relief of human suffering, is not only ethically permissible but ethically compelled, doing experiments with a strong "yuck" factor may itself be unethical.

We could not, in 2002, confidently predict the public reaction to these experiments. Weissman had talked publicly about these experiments, including the completed experiment in mice with normal brains, and they had been discussed, to a very limited and brief extent, in the United States press (Krieger 2002). The news stories did not generate any substantial public reaction. In the United States arguments based on improving health have had great political power; to the extent the human neuron mouse is seen as likely to lead to improved treatments for human disease, we suspected it will not be enormously controversial here.

The British Isles, however, presented a different picture. Weissman's proposed experiments were covered by several prominent newspapers in the United Kingdom and Ireland, including the *Financial Times*, as well as the more populist *Daily Mail* and the *Mirror* (Beattie 2001; Financial Times 2001; Kendall 2001). The experiments were also featured in a small section of a British television documentary on mice in research (Colville 2004). Greater concern about this research in the United Kingdom and Ireland may have been the result of greater cultural concerns about various forms of genetic engineering, as seems to be the case with respect to genetically modified food. It could also stem, in part, from a stronger animal rights movement, particularly with respect to laboratory animals. Or it might just be the result of a more alert press.

In fact, human/non-human chimeras have generated more continued discussion in the United States than we would have expected, particularly in light of the relatively few dramatic cases of such chimeras. News stories have appeared regularly. The NAS guidelines' limited discussion of such chimeras seemed to get more attention than its much broader and more significant recommendations for controlling human embryonic stem cell research. And, in 2005 human/non-human chimeras were both singled out by "bioconservatives" as key part of a "bioethics agenda for the second Bush Administration" (Cohen 2004) and were the subject of Senator Brownback's anti-chimera bill, including a clause (S. 1373 §301(1)(H)), which seems aimed directly at the human neuron mouse. In spite of its endorsement by President Bush in his 2006 State of the Union address, no hearings have been held to date on the Brownback bill. Its chances for passage are uncertain at best. In addition, there seems to have been little attention in the United Kingdom or Ireland to such chimeras since 2003.

RECOMMENDATIONS

In 2002, we told Dr. Weissman that we believed that his two outlined experiments may ethically proceed, but we suggested certain safeguards to minimize any risks.

First, we argued that human brain stem cells only be used if they were obtained pursuant to the procedures required for fetal tissue that may be used with federal research funding. Those procedures help ensure that the donor's consent was freely given.

Second, we urged the experiments should be performed in stages and should be carefully monitored. Disquieting or disturbing results at one stage should lead to discontinuance of the experiments pending further review of the ethical implications of those results. Such results could include the infliction of pain on the mice receiving the transplants, the formation of human-like structures in the mouse brains, or odd and possibly human-like behaviors by the

mice. We believed the cerebellar experiment should be performed first as it seems to have the fewest implications for consciousness. If it proceeded without disturbing surprises, the next stage should be the whole-brain experiment in which the mice are aborted. The mouse brains could then be examined pathologically to determine both whether the experiment worked at all and whether the resulting brain structures were wholly murine, wholly human, or something in between. If the brain appeared functional and its structures appear clearly murine, the experiment could proceed to its next phase and the mice could be born, then observed for unusual behavior.

We recognized that, at each stage, distinguishing between normal and abnormal structures or behaviors might prove difficult. And, in ambiguous cases—for example, a mouse brain with distorted whisker barrels—the decision whether to proceed may prove quite difficult. If the results indicate human brain structures or human behaviors, or even significant ambiguity, the experiments should be stopped and reconsidered in light of the new information. We did not have recommendations about what any such reconsideration should conclude; we did urge that it proceed with great care.

Our third recommendation concerned the possible public reaction to these experiments. We recognized that our belief, based on our study, that these experiments are ethically appropriate did not mean that the public would take the same view. We recommended that these experiments be done in an open manner with information conveyed, when normally appropriate, to the press. The researchers should strive to provide background information about the experiments and the reasons for doing them so that the public's reaction to this work, positive or negative, can be better informed.

In retrospect, we would make two more recommendations for Dr. Weissman. First, the bodies or brains of the dead mice should be disposed of appropriately, such as through incineration as medical waste. Second, unless there is a clear and powerful scientific reason for it, these mice should not be allowed to breed. Although the risk that they would form human gametes seems extremely small, we can see no good reason to take that risk.

Our recommendations were different from those of Karpowicz et al. (2004), the Johns Hopkins working group, or the NAS, but they are consistent with each. Those groups' recommendations sought to avoid the same primary end—the creation of animals with some possibility of human-like cognitive abilities—but focused largely on what cells would be inserted into what creatures, when and how. The Johns Hopkins working group provided six factors to consider in minimizing those risks in experiments with non-human primates. Karpowicz et al. (2005) recommended that as few cells as possible be used in transplants into early non-human embryos, that animals closely related to humans should be avoided, and that dissociated cells be transplanted instead of chunks of human brain. The NAS pointed generally to avoiding the risk of developing human characteristics in the recipient animal and expressly proposed banning the transfer of human embryonic stem cells (the most potent) into blastocysts (the earliest stage) of non-human primates (our closest relatives).

The context for our report made it unnecessary for us to reach those conclusions. We had been asked to give an opinion on transplanting dissociated human brain stem cells into very young mice (the first experiment) or mice in their fetal stages (the second experiment). The Johns Hopkins non-human primate factors were not relevant to these mouse experiments. The proposed experiments met the only relevant Karpowicz guidelines (the second and third guidelines) (Karpowicz et al. 2005). And we believe our analysis was exactly the kind of analysis the NAS guidelines seek from the reviewing embryonic stem cell research oversight committees, an assessment that the experiments are unlikely to result in an animal with human characteristics.

CONCLUSION

This article, and the report it was based on, tried to describe and discuss the ethical issues raised by one narrow set of proposed experiments, but the analysis may have broader implications. Three points deserve special mention.

First, the discussion of the ethical significance of transferring some aspects of human consciousness or some human cognitive abilities clearly needs to be taken further. Our report, and this article, do not conclude that it would be a clearly bad act to confer such capabilities on non-humans. We conclude only that it needs further discussion. We can note that, as far as we can see, the concern must be about specific kinds of human characteristics. A mouse with the human brain's sense of vision does not seem particularly troubling. Even a mouse with a memory of human quality might not be a concern. But a mouse with human language capabilities or that seemed to have a human level of self-consciousness would be, at the least, troubling. The thought experiment of considering mice (or other animals) with specific kinds of human cognitive or emotional capacities may prove one useful way to explore these problems.

And we further note that this issue is not limited to the human neuron mouse or even to biology. Some of the same issues would be raised by the creation of machines, as computers or as androids, with something approaching human consciousness. The creation of non-biological human/non-human chimeras with human-like intelligence may well be much more realistic than biological chimeras; after all, computers already have some human cognitive abilities, including some abilities that exceed ours, such as chess playing. Work looking at both the biological and the non-biological contexts seems likely to be important.

Second, our discussion of the appropriate uses of human tissue noted that human brain tissues, and perhaps particularly neurons, raise special issues. Many believe the field of neuroscience is entering a golden age of increased understanding of brain function. The extent to which the brain or tissues from the brain are given special, quasi-sacred status may have major effects on brain research and treatment. This is a particularly ripe issue for consideration in neuroethics.

Finally, and most important, all the specific issues noted in this article need to be watched. We tried our best in our initial report to predict what would seem ethically important about the human neuron mouse experiments and, almost 5 years later, we think we were largely, but not entirely, right. In the coming years, we are confident that our predictions will, in still other ways, small or perhaps large, prove to be wrong. The results of the experiments, the ongoing ethical discussion, and the interactions of the two need to be monitored to make sure that what now appears to be ethically permissible remains so. For, as noted by Robert Burns in his poem, *To a Mouse on Turning Up Her Nest with the Plough*,

> But, Mousie, thou art no thy lane In proving foresight may be in vain: The best laid schemes o' mice an' men Gang aft agley, An' lea'e us naught but grief an' pain For promis'd joy.
>
> —Robert Burns, *To a Mouse on Turning Up Her Nest with the Plough* (1795)

Acknowledgements

The authors wish to thank Professor Bill Newsome, our colleague on the working group and an extremely valuable contributor to the initial report; Dr. Irving Weissman, for giving us this opportunity and for his subsequent comments; an anonymous (and kind) referee; and Professor Greely's research assistant, Sean Rodriguez.

References

Assisted Human Reproduction Act, Canada. 2004. Clauses 2 and 5(1)(i)).

Balaban E, Teillet MA, Le Douarin N. Application of the quail-chick chimera system to the study of brain development and behavior. Science 1988;241(4871):1339–1342. [PubMed: 3413496]

Beattie J. Man or mouse?: Scientists produce rodents with brains that are one quarter human. Mirror 2001 February 27;:17.

Bohannon J. Anatomy's full monty. Science 2003;301(5637):1172–1175. [PubMed: 12947174]

Brownback, S. Keynote address at the Harvard Law School Society for Law, Life & Religion 2005 Symposium. 2005 [accessed January 5, 2007]. Available at http://brownback.senate.gov/OriginalDocs/cultureoflife/stemcellresea-rch/Adult%20Stem%20Cell%20Update%2005-11.pdf

Burns, R. Poems. London, UK: Faber; 2001. To a mouse on turning up her nest with the plough.

Burrell, B. Postcards from the brain museum: The improbable search for meaning in the matter of famous minds. New York, NY: Broadway; 2004.

Bush, GW. State of the Union address. 2006 [accessed January 5, 2007]. Available at http://www.whitehouse.gov/stateoftheunion/2006/index.html

Cohen CB. Creating human-nonhuman chimeras: Of mice and men. American Journal of Bioethics 2003;3(3):W3–W5. [PubMed: 14594462]

Cohen E. The bioethics agenda and the Bush second term. The New Atlantis 2004;7:11–18. [PubMed: 15812948]

Colville, C. BBC Four Documentaries. Mutant mouse. [accessed January 5, 2007]. directorBroadcast November 22, 2004. Available at http://www.bbc.co.uk/bbcfour/documentaries/features/mutant-mouse.shtml

Deacon T, Schumacher J, Dinsmore J, et al. Histological evidence of fetal pig neural cell survival after transplantation into a patient with Parkinson's disease. Nature Medicine 1997;3:350–353.

Department of Health and Human Services. 2001. *Regulations: Research involving, after delivery, the placenta, the dead fetus or fetal material*. 45 C.F.R. § 4602.

DeWitt N. Biologists divided over proposal to create human-mouse embryos. Nature 2002;420(6913): 255. [PubMed: 12447402]

Dugger, CW. New York Times. 2001 March 1. Bombay journal: In death, the unlovely vulture is sorely missed; p. A4

Financial Times. 2001. Of mice and men. *Financial Times* February 21: 20.

Greely HT, Hamm T, Johnson R, Price CR, Weingarten R, Raffin T. The ethical use of human fetal tissue in medicine. New England Journal of Medicine 1989;320(16):1093–1096. [PubMed: 2535632]

Greely HT. Defining chimeras . . . and chimeric concerns. American Journal of Bioethics 2003;3(3):17–20. [PubMed: 14594472]

Greene M, Schill K, Takahashi S, et al. Moral issues of human-non-human primate neural grafting. Science 2005;309(5733):385–386. [PubMed: 16020716]

Jeffrey TP. NIH mass produces 'human' mouse. Human Events 57(5):1, 7.

Kafka, F. The Metamorphosis and other stories. Richard, Stokes, translator. London, UK: Hesperus; 2002.

Karpowicz P. In defense of stem cell chimeras: A response to "Crossing species boundaries". American Journal of Bioethics 2003;3(3):W17–W19. [PubMed: 14594471]

Karpowicz P, Cohen C, van der Kooy D. It is ethical to transplant human stem cells into nonhuman embryos. Nature Medicine 2004;10(4):331–335.

Karpowicz P, Cohen C, van der Kooy D. Developing human-nonhuman chimeras in human stem cell research: Ethical issues and boundaries. Kennedy Institute of Ethics Journal 2005;15(2):107–134. [PubMed: 16149204]

Kass, L. The New Republic. 1997 June 2. The wisdom of repugnance.

Kendall P. The brain of a mouse that is a quarter human: More developments in brave new world of medicine. Daily Mail 2001 February 26;:31.

Krieger LM. Scientists put a bit of man into a mouse—ethics issues in mixing brain cells. San Jose Mercury News 2002 December 9;:1A.

Krieger LM. Stanford gets OK to try human cells in brain: Ethics guidelines limit study. San Jose Mercury News 2005 February 14;:1B.

Muotri AR, Nakashima K, Toni N, Sandler VM, Gage FH. Development of functional human embryonic stem cell-derived neurons in mouse brain. Proceedings of the National Academy of Science USA 2005;102(51):18644–18648.

Mullen FX Jr. From research to waste? Reno Gazette-Journal 2005 March;30:1A.

National Institutes of Health. NIH interim guidelines for the support and conduct of therapeutic human fetal tissue transplantation research. NIH Guide. 1993 [accessed January 5, 2007]. p. PT 34Available at http://grants.nih.gov/grants/guide/notice-files/not93-091.html

National Institutes of Health. Policy statement: Update on existing human embryonic stem cells. 2001 [accessed January 5, 2007]. Available at http://stemcells.nih.gov/policy/statements/082701list.asp

National Institutes of Health Revitalization Act of 1993, codified at 42 U.S.C. §289, *et seq.*; 21 U.S.C. §395.

National Research Council, Committee on Guidelines for Human Embryonic Stem Cell Research. Guidelines for human embryonic stem cell research. Washington, DC: National Academies Press; 2004.

Redmond DE Jr. Cellular replacement therapy for Parkinson's disease—Where we are today? Neuroscientist 2002;8(5):457–488. [PubMed: 12374430]

Robert JS, Baylis F. Crossing species boundaries. American. Journal of Bioethics 2003;3(3):1–13.

S. 1373, 109th Congress. 2005. A bill to amend Title 18, United States Code, to prohibit human chimeras. Available at http://thomas.loc.gov/cgi-bin/bdquery/z?d109:s.01373: (accessed January 5, 2007).

Scottish Council on Human Bioethics, Embryonic, Fetal and Post-natal Animal-Human Mixtures: An ethical discussion. Ethics of animal-human mixtures. Available at http://www.schb.org.uk/publications/report%20-%20animal-human%20mixtures.htm (accessed January 5, 2007).

Shreeve J. The other stem-cell debate. New York Times Magazine 2005 April 10;:42–47. [PubMed: 16715571]

Sophocles. Antigone. A new translation and commentary by David Franklin and John Harrison. Cambridge, UK and New York, NY: Cam-bridge University Press; 2003.

Starn, O. Ishi's brain: In search of America's last "wild" Indian. New York, NY: W. W. Norton; 2004.

StemCells, Inc. 2006. *StemCells, Inc. receives IRB approval from Oregon Health & Science University to begin Phase I clinical trial in Batten Disease* [press release]. Available at http://www.stemcellsinc.com/news/060309.html (accessed January 5, 2007).

Streiffer R. In defense of the moral relevance of species boundaries. American Journal of Bioethics 2003;3 (3):37–38. [PubMed: 14594488]

Streiffer R. At the edge of humanity: Human stem cells, chimeras, and moral status. Kennedy Institute of Ethics Journal 2005;15(4):347–370. [PubMed: 16453949]

Tamaki S, Eckert K, He D, et al. Engraftment of sorted/expanded human central nervous system stem cells from fetal brain. Journal of Neuroscience Research 2002;69:976–986. [PubMed: 12205691]

Uchida N, Buck DW, He D, et al. Direct isolation of human central nervous system stem cells. Proceedings of the National Academy of Science USA 2000;97:14720–14725.

Wade N. Scientists draft rules on ethics for stem cells. New York Times 2005 April 27;:A1.

Weiss R. Of mice, men and in-between: Scientists debate blending of human, animal forms. Washington Post 2004 November 20;:A1. [PubMed: 15586940]

Greene et al.,

ETHICS: Moral Issues of Human-Non-Human Primate Neural Grafting,

SCIENCE, vol. 309, no. 5733 (July 15, 2005)

ETHICS

Moral Issues of Human–Non-Human Primate Neural Grafting

Mark Greene,[1] Kathryn Schill,[2] Shoji Takahashi,[3] Alison Bateman-House,[4] Tom Beauchamp,[5] Hilary Bok,[6,7] Dorothy Cheney,[8] Joseph Coyle,[9] Terrence Deacon,[10] Daniel Dennett,[11] Peter Donovan,[3] Owen Flanagan,[12] Steven Goldman,[13] Henry Greely,[14] Lee Martin,[3] Earl Miller,[15] Dawn Mueller,[16] Andrew Siegel,[7] Davor Solter,[17] John Gearhart,[3] Guy McKhann,[6] Ruth Faden[7]*

If human neural stem cells were implanted into the brains of other primates what might this do to the mind of the recipient? Could such grafting teach us anything of value for treatment of neurological injury and disease? Could we change the capacities of the engrafted animal in a way that leads us to reexamine its moral status? These questions have gained significance since publication of research involving grafting human neural stem cells into the brains of fetal monkeys (*1*). In 2004, we formed a multidisciplinary working group; two plenary meetings over 12 months provide the basis for this Policy Forum.

Some group members have serious ethical concerns over *any* use of nonhuman primates in invasive research. However, we set aside broader controversies to focus on ethical challenges specific to human–to–nonhuman primate (H-NHP) neural grafting. We did not take votes or seek consensus on all the questions raised.

There is considerable controversy (reflected within our group) over the likely value of interspecies stem cell work for progress toward therapies (*2*). We cannot graft human neural stem cells into human beings solely for experimental purposes, even if they will lead to human therapies. Group members arguing for the value of research on human cells in NHPs pointed out that, because the aim is to learn about human neural stem cells, it makes most sense to use human lines. The fact that available NHP lines are few and poorly characterized (*3*) is an additional reason to use human lines. Another consideration is the need to assess candidate human cell lines for viability, potential to differentiate, and safety with regard to such possibilities as tumor formation. NHPs may be appropriate for in vivo screening.

Skeptics argued that differences between humans and NHPs could render results uninterpretable and that the preferred path for many questions is to study NHP neural stem cells in NHPs. Assessments of the scientific merit of the research must form and develop along with the field itself.

We unanimously rejected ethical objections grounded on unnaturalness or crossing species boundaries (*4*). Whether it is possible to draw a meaningful distinction between the natural and the unnatural is a matter of dispute. However, stipulating that research is "unnatural" says nothing about its ethics. Much of modern medical practice involves tools, materials, and behaviors that cannot be found in nature but are not unethical as a consequence

Another concern is that H-NHP neural grafting is wrong because it transgresses species boundaries (*5*). However, as the recent National Academy report notes (*6*), the notion that there are fixed species boundaries is not well supported in science or philosophy. Moreover, human–nonhuman chimerism has already occurred through xenografting. For example, the safety and efficacy of engrafting fetal pig cells has been studied in people with Parkinson's disease and Huntington's disease without moral objection. Indeed, some have suggested that porcine sources may be less morally contentious than the use of human fetal tissue (*7*). Merely because something has been done does not prove it right. However, we, like the National Academy, see "no new ethical or regulatory issues regarding chimeras themselves" [(*6*), p. 33].

The central challenge is whether introducing human cells into NHP brains raises questions about moral status. A variety of reasons have been given for according different moral standing to humans and NHPs. In the Abrahamic traditions, humans are set apart by God as morally special and are given stewardship over other forms of life (Genesis 1:26–28). For Kantians, human capacities for rationality and autonomy demand that we be treated as ends in ourselves (*8*). Mill finds, in the richness of human mental life, an especially fecund source of utility (*9*). Singer, although strongly defending equal consideration of nonhuman interests, argues that self-awareness affects the ethically allowable treatment of a creature by changing the kinds of interests it can have (*10*).

Many of the most plausible and widely accepted candidates for determining moral status involve mental capacities such as the ability to feel pleasure and pain, language, rationality, and richness of relationships. To the extent that a NHP attains those capacities, that creature must be held in correspondingly high moral standing. There are those, including Singer and some of our working group, who believe that we already overestimate differences in relevant mental capacities, and thus of moral status, between humans and NHPs. But the issue here is the extent to which human/NHP neural grafting might change capacities in a way that changes moral status.

Although we cannot assess altered capacities by experiencing an animal's mental life from within, we can assess its performance on cognitive tasks and observe its behavior. Establishing whether and in what ways engrafted animals undergo cognitive or behavioral changes requires an understanding of what the normal range is for a particular NHP species. Unfortunately, our understanding of NHP cognitive capacities is patchy, data are tricky to gather and difficult to interpret [(*11*); see supplementary material]. Thus, even if we observe what appear to be more humanlike capacities in an engrafted animal, we may be unable either to establish whether the capacities are outside of the normal range for that species, or to interpret the moral meaning of observed changes.

One conceivable result of H-NHP neural grafting is that the resulting creature will develop humanlike cognitive capacities relevant to moral status. H-NHP neural grafting may not be unique in having the potential to alter the capacities of NHPs. Chimps reared with humans behave in a more humanlike way than chimps reared by chimps (*12*). Transfer between species of predispositions

[1]University of Delaware; [2]Case Western Reserve University; [3]School of Medicine, Johns Hopkins University; [4]Columbia University; [5]Georgetown University; [6]Johns Hopkins University; [7]Phoebe R. Berman Bioethics Institute, Johns Hopkins University; [8]University of Pennsylvania; [9]Harvard University; [10]University of California at Berkeley; [11]Tufts University; [12]Duke University; [13]University of Rochester; [14]Stanford University; [15]Massachusetts Institute of Technology; [16]University of Maryland; [17]Max Planck Institute of Immunobiology. [For complete addresses, see SOM.]

*To whom correspondence should be addressed: rfaden@jhsph.edu

relating to auditory perception was found after transplantation of already formed portions of brain tissue (*13*). Introduction of human neural progenitor cells into developing mouse brains resulted in widespread incorporation of human neural progenitor cells; but behavioral alterations were not reported (*14*). Although such results are not reasons to think it likely, one unanimous conclusion of our group is that we are unable to rule out the possibility of effects on cognition of the sort that matter to moral status.

One option is to treat any development of more humanlike cognitive capacities as a risk to avoid. Alternatively, it might be argued that the challenge is less to avoid a direct ethical ill and more to understand the mental capacities of engrafted animals and to treat them in a manner appropriate to their moral status. Indeed, it might even be argued that such changes constitute a potential benefit to the engrafted animal, insofar as the changes are viewed as enhancements of the sort we value for ourselves. However, these more humanlike capacities might also confer greater capacity for suffering that would add to existing concerns about the harms caused by inadequate conditions for NHPs in research.

We propose six factors that research oversight committees and other review groups should use as a starting framework. They are (i) proportion of engrafted human cells, (ii) neural development, (iii) NHP species, (iv) brain size, (v) site of integration, and (vi) brain pathology.

Though even a few engrafted cells may affect neural activity, we expect that a higher proportion of engrafted human cells relative to host cells will increase the prospect of more humanlike neural function and, thus, of more humanlike cognitive capacities. High proportions of engrafted cells are more likely to be achieved by implantation early in neural development.

We also expect that the potential for engrafted cells to have significant functional influence will be markedly greater for engraftment at very early stages of development than for engraftment into the established architecture of adult brains. Although neural progenitor cells engrafted into the neonatal primate brain disseminate widely and integrate throughout the brain (*1*), the mature primate brain tends to resist incorporation of engrafted cells (*15*).

A graft recipient's degree of relatedness to our own species may matter for several reasons. Genetics contribute to brain structure by providing the protein building blocks that shape neurons and their interconnections. Factors such as cell surface markers and the mechanisms of cellular signaling are more similar in our closer relations (*2*, *3*). Also, although the picture is complicated by lifestyle similarities that cut across phylogenetic groups, our closest relatives among NHPs tend to show greater neuroanatomic similarities to human brain structures (*16*).

Also related to recipient species is brain size. It is unlikely that the structural complexity needed for any significant degree of humanlike mental capacity can be achieved under tight size limitations. However, brain size influences the size of the developing cranium, an effect seen naturally in hydrocephalus. Thus, a fetal marmoset engrafted with human neural cells might, to some extent, develop a larger brain than is typical for the species.

The specific sites into which the human neural cells become integrated within the recipient brain is also of potential significance. Functional integration into the cerebrum, which is associated with higher brain functions, seems more likely to affect cognitive capacities than does integration into the cerebellum; although engrafted neural cells may migrate and project to disparate brain areas.

Overall, we think it unlikely that the grafting of human cells into healthy adult NHPs will result in significant changes in morally relevant mental capacities. However, in the case of NHP models of human neurological disease and injury, adult recipients of human neural cells may have extensive disruption to their neural structures that might allow greater scope for engrafted human neural cells to affect cognitive capacities. We do not consider this a strong possibility, because diseased or injured brains will be starting from an impaired state from which even a return to species' normal functional levels is unlikely. However, the therapeutic point is to reinstate lost function, and we cannot be certain that this will be the only functional result of interspecies neural grafting. Furthermore, some of the disorders likely to be of interest (such as Alzheimer's) involve higher-level cognitive capacities.

There is no simple relation between these factors and, thus, no formula for making evaluative judgments. Considering issues of moral status that go beyond the ethical challenges attending any invasive NHP work, our framework suggests that experiments of greatest concern are those in which human neural stem cells are engrafted into the developing brains of great apes and constitute a large proportion of the engrafted brain. On the basis of this concern, and on doubts about scientific merit, some of us believe that engraftment of human neural cells into great apes should not be permitted, particularly early in neural development. Others argue against outright prohibition on grounds that scientific justifications might be forthcoming as the field progresses. For example, if a useful great ape model of a neurological disease is developed, and a promising human neural stem cell line is ready for use, there might be reason to proceed with human–great ape work, rather than waiting to develop great ape lines. Our framework suggests that experiments involving engraftment into healthy adult brains of our most distant monkey relations, especially when the proportion of engrafted cells is small relative to host cells, are the least likely to raise concerns about significant cognitive effects. However, especially as we consider experiments involving implantation of relatively large numbers of human cells early in development, there is no present empirical basis on which to rule out changes that might implicate moral status, whether the engrafted NHPs are great apes or monkeys.

In view of the challenges arising from moral status, we support the National Academy's recommendation that H-NHP neural grafting experiments be subject to special review. We agree that such review should complement, not replace, current review by animal-use panels and institutional review boards. We further recommend that experiments involving H-NHP neural grafting be required, wherever possible, to look for and report changes in cognitive function. Explicit data collection on cognition and behavior will help to ensure that ethical guidelines can be developed appropriately as the field advances.

References and Notes

1. V. Ourednik *et al.*, *Science* **293**, 1820 (2001).
2. J. S. Robert, *Bioessays* **26**, 1005 (2004).
3. K.-Y. F. Pau, D. Wolf, *Reprod. Biol. Endocrinol.* **2**, 41 (2004).
4. P. Karpowicz, C. B. Cohen, D. van der Kooy, *Nat. Med.* **10**, 331 (2004).
5. F. Fukuyama, *Washington Post*, 15 February 2004, p. B04.
6. Committee on Guidelines for Human Embryonic Stem Cell Research, "Guidelines for Human Embryonic Stem Cell Research" (National Research Council, National Academy of Science, Washington, DC, 2005).
7. J. S. Fink *et al.*, *Cell Transplant.* **9**, 273, (2000).
8. I. Kant, in *Groundwork of the Metaphysics of Morals*, K. Ameriks, D. M. Clarke, Eds. (Cambridge Texts in the History of Philosophy, Cambridge Univ. Press, Cambridge, UK, 1998), pp. 37–38.
9. J. S. Mill, *Utilitarianism* (Prometheus Books, Buffalo, NY, 1987), 83 pp.
10. P. Singer, *Inquiry* **22**, 145 (1979).
11. M. Tomasello, J. Call, B. Hare, *Trends Cognit. Sci.* **7**, 153 (2003).
12. M. Tomasello, S. Savage-Rumbaugh, A. C. Kruger, *Child Dev.* **64**, 1688 (1993).
13. K. D. Long, G. Kennedy, E. Balaban, *Proc. Natl. Acad. Sci. U.S.A.* **98**, 5862 (2001).
14. O. Brustle *et al.*, *Nat. Biotechnol.* **16**, 1040.
15. P. Rakic, *Nature* **427**, 685 (2004).
16. C. E. Oxnard, *Int. J. Primatol.* **25**, 1127 (2004).

17. We thank the Greenwall Foundation for support.

Supporting Online Material

www.sciencemag.org/cgi/content/full/309/5733/385/DC1

10.1126/science.1112207

Knowles,

Ethics of Research Using Hybrids, Chimeras and Cytoplasmic Hybrids,

STEM CELL NETWORK (2003)

Stem Cell Network
Réseau de cellules souches

Ethics of Research Using Hybrids, Chimeras and Cytoplasmic Hybrids

By Lori P. Knowles

What are Chimeras, Hybrids and "Cybrids"?

In stem cell research, creating and using human and non-human animal hybrids, chimeras and cytoplasmic hybrids, or "cybrids" as they have been nicknamed, poses some of the most contentious and confusing ethical issues in stem cell science and policy. This confusion arises from different understandings of exactly what hybrids, chimeras and cybrids are. Clear definitions have yet to be firmly established although several authors have attempted to clarify the differences and even offer taxonomies with examples.[1]

In Greek mythology the chimera was described either as possessing the head of a lion, the body of a goat and the tail of a serpent, or as having three heads, one from each animal. Either way, the result was a deadly, repellant monster. This history sets the stage for understanding what a chimera is biologically speaking, and why people often react fearfully to discussions about creating chimeras. Biologically, a chimera is an organism with a mixture of cells from two or more genetically distinct species. Chimeras are mosaics at the cellular level; individual cells are derived from either the host or the donor but not both. Chimeras can be created through transgenesis, a process by which a gene from one species is isolated and inserted in the embryo of another species. Examples of chimeras include humans with pig heart valves, sheep with human liver cells and mice with human neural cells.

Hybrids are created by breeding across species. They are generally the result of combining an egg from one species with sperm from another to form a single embryo. Hybrids contain recombined genetic material throughout their genome and throughout all the tissues in their body. In agricultural experimentation, plant hybrids have been created for over a century through traditional fertilization techniques. The mule is an example of a non-human animal hybrid, being the result of a female horse reproducing sexually with a male donkey.

Cybrids, or cytoplasmic hybrids, are created by taking an egg from a non-human animal and removing the nuclear DNA. This leaves only the cytoplasm or ooplasm of the animal egg which contains a small amount of mitochondrial DNA. Human nuclear DNA or an entire human cell is fused with the enucleated egg to create a cybrid embryo. The resulting embryo possesses human nuclear DNA and animal mitochondrial DNA. The mitochondrial DNA is minute in comparison with the nuclear DNA – approximately 13 genes compared with 23,000 genes. Cybrid embryos are said to be 99.9 percent human, however, it is unclear what effects the mixture of DNA from two different species will have.

Stem cell research and the issue of creating chimeras have been linked for over 10 years. Shortly after the first announcements that human embryonic stem cells and germ cells had been isolated, Advanced Cell Technologies (ACT), a biotech company in Massachusetts, USA announced that it was considering fusing enucleated cow ova with human nuclear DNA to make human/non-human embryos as a cheaper and more ethical source

1 Greely, H.T., "Defining Chimeras ... and Chimeric Concerns" *Am. J. Bioethics* 2003, 3:17–19.

of stem cells than using human ova and embryos. In response, President W. Clinton asked his National Bioethics Advisory Committee to look into the mingling of human and non-human species, saying he was "deeply troubled" by the creation of part-cow, part-human embryos.[2] Although the experiments were not undertaken by ACT and little attention was paid to these inter-species mixes for several years, ten years later the issue of human/non-human animal mixtures in stem cell research has become one of the most current and controversial ethical and policy issues in stem cell science.

Ethical Issues Related to Use of Animals in Research

Most of the ethical issues related to chimeric research are not particular to stem cell science or research. There are experiments that use human/non-human animal chimeras and hybrids in many well-accepted practices. For example, for almost 30 years fertility specialists have been fertilizing hamster eggs with human sperm to test sperm motility. In some ways, chimeric research is an extension of current research in transgenesis to generate 'humanized' animal models for research. But it may also be understood as part of a continuum of techniques within developmental biology established over the past 150 years.[3]

Protocols for chimeric research are well established for embryonic, fetal and adult systems and hundreds of chimeric experiments have been undertaken. Two examples involving human stem cells include the transplantation of human neural cells into the forebrains of a developing monkey in order to assess human stem cell behaviour in monkey development[4] and the insertion of human embryonic stem cells into very young chick embryos to assess human stem cell differentiation in chick development.[5] These experiments are subject to ethical and legal guidelines involving the use of animals in research activities. There have been some stem cell experiments involving cybrids. For example, in 2003 China extracted stem cells from cybrids created using rabbit eggs and human sperm.

2 "Clinton Asks Study of Bid to Form Part-Human, Part-Cow Cells," Nicholas Wade, *New York Times*, November 15, 1998.

3 Robert, J.S. "Model systems in stem cell biology" *Bioessays* 2004, 26:1005-1012.

4 Ourednik et. al. "Segregation of Human Neural Stem Cells in the Developing Primate Forebrain," *Science* 7 September 2001: 293(5536): 1820-1824. DOI: 10.1126/science.1060580

5 Goldstein, R.S., "Transplantation of Human Embryonic Stem Cells to the Chick Embryo" in Human Embryonic Stem Cell Protocols, Turksen, K., ed., (Springer, 2006) at 137, as cited in Robert, J.S. "Model systems in stem cell biology" *Bioessays* 2004, 26:1005-1012.

Why use Chimeras, Hybrids and Cybrids?

The main rationale behind the creation of cybrids, hybrids and chimeras in stem cell research is the creation of a non-human model system. This system enables learning about basic developmental stem cell biology. In addition, one particularly promising avenue of research involves the creation of cybrids using DNA of patients with conditions such as Arterial Lateral Sclerosis (ALS) or other genetic diseases such as Alzheimer's and Parkinson's. These animal-human mixes thus provide an invaluable tool for studying the genetic basis and development of a disease and potentially what drugs or therapies might effectively combat that disease.

For many years animals have been used in research to aid human health and medicine. And, while animal models are an invaluable research tool in stem cell research, there are systemic and cellular differences between animal stem cells and human stem cells. The fact that chimeras or cybrids have human DNA means that they are closer to a human model system and therefore, research data should be more predictive and closer related than data in a pure animal model. While this is true, some have raised cautions about the extrapolation of data generated by animal, chimeric or hybrid models to human data, noting that stem cell biology and behavior between species can be very different.[6] In stem cell research much animal model research is conducted using mice. In December 2008 a team from California announced the isolation of stem cells from rats, which is viewed by researchers as a promising advance as rat stem cells provide a closer model to humans than mice.[7]

Of course the best model system for stem cell research and therapies ultimately aimed at human application would be a human model system. There are, however, research projects performed on animals that are ethically and legally prohibited from being performed on humans. Arguments for the creation of human/non-human animal embryos in stem cell research include the practical and ethical difficulties in obtaining human ova. Animal ova are not scarce or expensive and do not have the same ethical issues attendant. However, where production and procurement of animal ova is involved, issues about proper treatment of animals will apply. These issues are not distinct to stem cell research, but are the same in any research endeavour that uses animals as research tools.

6 Robert, J.S. "Model systems in stem cell biology" *Bioessays* 2004, 26:1005-1012.

7 Buehr, M., Meek, S., Ying Q., et al. "Capture of Authentic Embryonic Stem Cells from Rat Blastocysts" *Cell* 2008, 135(7): 1287-1298.

Use of animals and chimeras in research

In stem cell research non-human animals continue to be an important source of stem cells for scientific and medical research. In addition, the use of animals or animal/human "mixes" provides a way of conducting experiments that either cannot be performed ethically or legally on human research subjects or in which it is not practical to use humans. Most countries have human subjects research legislation that defines the circumstances in which it is permissible to use humans for research. In Canada, institutions that accept funding from the three federal research councils (health, natural and social sciences) or which decide to bind themselves are subject to the guidelines articulated in the *Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans,1998 (with updates of May 2000 and September 2002)*.[8] Great reliance is placed on institutional research review boards, in Canada called Research Ethics Boards or REBs. These boards are responsible for ensuring that research protocols are valid, appropriately designed, and do not pose inappropriate risks to human subjects. In addition, most countries also have legislation and regulations governing the appropriate use of animals in medical and scientific research. These regulations are aimed at ensuring that the use of animals is necessary for a valid scientific aim, and that animal suffering is minimized wherever possible. In Canada, the Canadian Council on the Care of Animals is a good resource for understanding oversight of animal use in experimentation.[9] In addition to legislation, research institutions also have Institutional Animal Care committees that ensure use of animals in research protocols are scientifically valid and adhere to ethical standards.[10]

For new areas of biological research, such as stem cell research, one of the challenges faced by governing bodies and animal researchers is ensuring that these new developments are adequately covered by existing policies and practices regarding humane animal experimentation. New research areas often develop experimental animal procedures that can introduce animal welfare concerns not covered by current policies and practices. In the case of genetically modified animal models, there has been an overall increase in numbers of animals used in research. This increase runs counter to previous successful efforts to reduce animal numbers – a goal of policies to ensure humane animal experimentation.[11]

Similar to other aspects of stem cell research, governing bodies must be sensitive to whether new developments in animal research captures the contemporary ethical and social concerns about animal use. Unfortunately we know very little about whether or not the use of animals in stem cell research does present new animal welfare challenges. Old research techniques, such as parabiosis (anatomical and physiological union of two organisms), are currently being used in stem cell research. This technique requires high levels of skill and is considered to be a severe procedure in terms of animal suffering.[12] Whether this technique is widely used is unknown. Governing bodies and scientists need to be vigilant to the impacts of their research on the welfare of animals, constantly adapting to new ethical challenges.[13]

In addition to the animal welfare issues, public conversations about animal-human mixes have indicated an ethical unease with these mixes that is reflected in policy. The International Society for Stem Cell Research has addressed some of these issues in its Guidelines for the Conduct of Human Embryonic Stem Cell Research. In particular, they note that the type of tissue that is being transferred (for example brain tissue) and the animal involved, especially other primates may be ethically relevant. The Society suggests that mixing animal and human gametes be carefully monitored. Particular concerns arise when

8 *Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans, 1998 (with updates of May 2000 and September 2002)* (Ottawa: Tri-Council, 1998), online: http://www.pre.ethics.gc.ca/english/policystatement/introduction.cfm

9 Use of animals in research falls under both provincial animal care legislation and under federal criminal prohibitions against cruelty and abuse of animals. While all provinces have animal care regulations in some form, only Alberta, Ontario, Manitoba, New Brunswick, Nova Scotia and Prince Edward Island have legislated with respect to the use of animals in research, teaching and testing. See http://www.ccac.ca/en/CCAC_Programs/ETCC/Module01/toc.html for more information.

10 See http://www.ccac.ca/en/CCAC_Programs/Guidelines_Policies/POLICIES/ETHICS.HTM

11 Ormandy, E.H., Schuppli, C.A. and Weary, D.M. *Worldwide trends in the use of animal research. Alternatives to Laboratory Animals*, In press.

12 LASA (Laboratory Animal Science Association) 1990. The assessment and control of the severity of scientific procedures on laboratory animals. Laboratory Animals 24: 97-130.

13 The author thanks Cathy Schuppi of the University of British Columbia for her assistance on these issues. See Schuppli, C. A., Fraser, D. & McDonald, M. (2004) "Expanding the 3Rs to meet new challenges in humane animal experimentation" *Alternatives to Laboratory Animals* 32, 525-532, and Buehr, M., Hjorth, P. J., Hansen, A. K. & Sandøe, P. (2003) Genetically modified laboratory animals – what welfare problems do they face? *Journal of Applied Animal Welfare Science* 6(4), 319-338.

experiments may transmit genetic changes through reproduction. The Society counsels that chimeric animals should typically not be permitted to breed.[14]

A number of countries have prohibitions on the creation of animal-human mixes including Canada. The *Assisted Human Reproduction Act* (2004, c.2) states in section 5 that it is prohibited to:

(i) create a chimera, or transplant a chimera into either a human being or a non-human life form; or

(j) create a hybrid for the purpose of reproduction, or transplant a hybrid into either a human being or a non-human life form.

The *Act* defines "chimera" as the insertion of any non-human animal cell into a human embryo. The *Act* does not, therefore, cover the creation of cybrids or chimeras in which a non-human animal has human genes or cells inserted.

The Canadian Institutes of Health Research Updated Guidelines for Human Pluripotent Stem Cell Research go further than the *Act* with respect to chimeric research. The Guidelines indicate that research in which pluripotent cells, including embryonic stem cells, of human or non-human animal are combined with a human embryo will contravene the Guidelines. In addition, the Guidelines indicate that research in which human ES cells or other pluripotent cells are combined with a non-human embryo is also not sanctioned.[15] It would seem therefore, that in Canada cybrids can be created using enucleated non-human animal eggs.

In September 2007, the British Human Fertilisation and Embryology Authority (the governing regulatory body that hands out licenses to researchers) allowed three licenses for the creation of cybrid embryos as a source of embryonic stem cells after public consultation on the issue. One of these licenses went to Ian Wilmut, creator of Dolly the cloned sheep, to create cybrids with the ALS gene. In 2008, Britain had a contentious public and parliamentary debate over the ethics of permitting mixtures of human and animal cells for research in a new Human Fertilisation and Embryology Bill. An open vote was held in May 2008 and the creation and use of human animal cybrids and hybrids was passed in the Parliament. This makes the United Kingdom one of the world's most liberal nations with regulatory approval of animal/human mixes. In January 2008, Singapore announced plans to hold a public consultation with a view to creating animal/human cybrids for research into specific diseases. Early results indicate that there is a sharp division in public sentiment. A report will be forthcoming later in 2009.

Why not use human ova and create human embryos as a research model?

Using human ova and creating human embryos for research would circumvent the scientific uncertainties about the translation of data generated in non-human animals to humans. These practices, however, raise serious ethical concerns. First, the number of human ova available for research is scarce. This scarcity is the result of the amount of time invested and physical discomfort that must be endured by a woman to produce enough eggs for retrieval from her body. In addition, the process of ova retrieval is onerous and risky. Women who wish to use their ova for IVF or to sell or donate them must undergo weeks of daily hormone injections to induce hyper-ovarian stimulation. They must be monitored daily as they get closer to the ova "ripening" and then undergo general anesthesia and extraction of the ripe eggs through the vaginal wall. These procedures are not without risks.

Hyper-ovarian stimulation and ova retrieval are usually undergone by women hoping to use their own eggs in a "reproductive project"– an attempt to get pregnant through IVF. In order to have human ova for stem cell research, women would have to donate their eggs for research rather than have them fertilized for future implantation. Some have suggested that an agreement to donate eggs for research could be encouraged by lowering prices of IVF treatments. This, however, is problematic since it requires that a woman give up some possible chances at getting pregnant. Others maintain that it affords women who could not otherwise afford IVF the opportunity to have a chance at a baby. These issues about markets in human tissue, including ova, are more fully discussed in Knowles L., "The Use of Human Embryos in Stem Cell Research" Stem Cell Network and Knowles L., "Commercialization and Stem Cell Research" Stem Cell Network.

14 International Society for Stem Cell Research, "Guidelines for the Conduct of Human Embryonic Stem Cell Research" Art. 10. http://www.isscr.org/guidelines/ISSCRhESCguidelines2006.pdf

15 The Canadian Institutes of Health Research Updated Guidelines for Human Pluripotent Stem Cell Research, June 29, 2007, Ss. 8.2.4-8.2.6 http://www.cihr-irsc.gc.ca/e/34460.html

Concerns about naturalness and crossing the species boundaries

In the last few years several authors have written about the ethics of using human/non-human animal mixtures in stem cell research. In those articles they examine arguments against mixing human DNA with non-human DNA. The arguments against the creation of the resulting living organisms range from arguments about whether humans ought to be creating living organisms not envisaged by God, to concerns about human dignity and moral confusion and also arguments that crossing the "species barrier" is in various ways morally repugnant and wrong.

In part, much of the controversy about creating admixtures comes from a view that mixing human and animal DNA upsets a natural order. That is, the products of this research are unnatural in morally relevant ways and/or the process of creating these entities is unnatural and therefore, should be foregone. These arguments are not new to bioethics. Arguments about the moral acceptability of creating unnatural entities (entities not found in natures) or doing unnatural things (things that do not naturally occur outside the laboratory) are found in criticisms of agricultural, animal, environmental and human biotechnology.

Many people express feelings of repugnance or wrongness toward cross-species hybrids. Intuitive negative feelings that some idea or practice is repugnant have been identified as a "yuck" factor.[16] The yuck factor is often used as evidence of the intrinsic moral wrongness of the practice. While the yuck factor has been called the wisdom of repugnance by some noteworthy bioethicists,[17] others caution against using such feelings of disgust as a moral barometer without an appeal to evidence or rational explanation of the wrongness of the practice.[18] Individual and societal concepts of disgust can change over time. Interracial marriage, women voting, and same-sex marriage are all examples of practices that have evoked feelings of repugnance in certain segments of society and have changed or are changing over time.

Before dismissing the yuck factor, however, the feelings need to be unpacked and analyzed to determine if there are compelling moral intuitions at work. First, the idea of creating an entity by interbreeding distinct species is morally repugnant to many. The term "crossing the species barrier" signals a world view in which each species is distinct and "walled off" from every other species by natural reproductive barriers. This "biological understanding of species" in which species are isolated from one another by an inability to reproduce across species lines is pervasive but not persuasive. It does not address the world's most numerous species – those that do not reproduce sexually. There are others notions of what compromises a species, including "natural kinds" or evolutionary lineages but no one definition is entirely compelling. Nonetheless, the biological understanding of species remains the most popular understanding of what categorizes one species from another.[19]

The biological understanding of species also grounds a religious objection to creating entities that were not envisaged or created by God. By creating new living species not found in nature, we move ourselves from "created" to "creator" and may step into the territory generally thought of as "divine providence." Other religious thinkers however, believe the scripture in the Bible that asserts human dominion over all living things entitles us to act as a creative force. The "playing God" argument however, is rarely used to oppose the thousands of hybridized plant species created by humans over several hundred years. Additionally, the use of life-saving xenotransplants in humans such as pig heart valves or the introduction of human DNA into sheep to produce life-saving insulin for diabetics does not occasion much opposition on these grounds. And so, one can suppose that there may be something else at work in these objections.

Still others find the alteration of natural physical characteristics the source of their unease or repugnance. This is especially true when a resulting hybrid or chimera does not fit comfortably into the known cluster of characteristics that we associate with a particular species. So, the introduction of a jelly fish gene into monkeys such that the monkeys glow in the dark is wrong to many people because it breaks the rule "monkeys do not glow in the dark." A reaction to sheep that produce human insulin may be less negative because the sheep still look like sheep. Alternatively, the goal of the research may be judged to fall below the threshold of importance needed to outweigh the costs of doing a very unnatural

16 Midgely, M., "Biotechnology and Monstrosity," *Hastings Center Report*, Sept-Oct 2000; 7-15.

17 Kass, Leon R. "The Wisdom of Repugnance." New Republic Vol. 216 Issue 22 (June 2, 1997).

18 Nussbaum, M.C., "Danger to Human Dignity: The Revival of Disgust and Shame in the Law" *The Chronicle of Higher Education*, August 6, 2004, B6-9.

19 Robert, JS., Baylis, F., "Crossing species boundaries" *Am. J. Bioethics* 2003; 3(3):1-13.

thing. In other words, if the goal of the unnatural process is immediate and life-saving therapy it might be more morally acceptable than remote laboratory research of some future indeterminate benefit.

Concerns About Human Dignity

Some objections to animal/human mixtures enlist notions of human dignity. One commentator articulates the connection between the yuck factor and notions of human dignity in this way, "...in this age in which our given human nature no longer commands respect.... [r]epugnance may be the only voice left that speaks up to define the central core of humanity." The existence of human dignity is a relatively uncontroversial concept in Canada (where it is invoked in the *Tri-Council Statement on Ethics in Research* and in the preamble to the *Assisted Human Reproduction Act*) and in Europe. By contrast it is rarely part of policy and ethics discourse in the United States, where it is often regarded as a fuzzy, ambiguous term. This is in part due to cultural differences which place an emphasis on societal welfare in Canada and Europe and an emphasis on individual autonomy in the United States, but also because human dignity is hard to define in a pluralistic society. At its core, human dignity is something unique and sacred to human identity and membership in the human community, and exists in a rights-based ethical framework. In part, those who argue that animal-human mixtures threaten human dignity are asserting either that human tissue is sacred or that unique and sacred human characteristics are threatened by theses mixtures.

This argument is not new to stem cell research, chimeras and cytoplasmic hybrids, but has been used in conjunction with any number of biotechnological alternations that have been made to the human body. A question that arises is whether creating humans with artificial parts or parts from animals somehow confuses their humanity or compromises their human dignity? So, in 1974 when Barney Clark received the first artificial heart, musings about whether his humanity was compromised and what artificial organ transplants meant for humans took place. Similar discussions occurred in 1984 when Baby Fae received the first xenotransplant heart from a non-human primate (something that is no longer considered medically appropriate). Over time, as these types of interventions become more common we, as a society, often change our views about what they mean for the human race. We have grown accustomed to people with artificial knees, hips and breasts. Similarly, we do not consider people with pig valves in their hearts to be less human or have less human dignity than any other human. There are however, certain hybrids or chimeras that many agree do have implications for human dignity.

Human-Mouse Neural Transplant Research

The question remains open as to whether there is any threshold level of xenotransplantation beyond which a transplant recipient's humanity would be in question. Similar questions exist if animals receive certain human tissue or DNA. As of yet, no animal has been the recipient of numerous human organs. If an animal received say, a human heart, human lungs and human kidneys would we still look upon that animal the same way? Should we? Would we think it was deserving of special respect? These sorts of questions have been raised in the context of experiments that anticipated implanting mouse (murine) brains with human brain (neural) stem cells. These experiments were proposed by Dr. Weissman at Stanford University to learn more about human brain trauma and to lead to potential clinical and pharmaceutical therapies.

Prior to the commencement of the experiments Dr. Weissman consulted with Stanford ethicists. These ethicists[20] and subsequent commentators[21] made the following observations. The type of human tissue involved in the creation of human and non-human animal chimeras is morally significant. The creation of animals with human genes is not novel, but chimeras and hybrids that involve transplantation of human neural tissue or use of human gametes are of particular ethical concern. In these cases it is important to be careful that any resulting animal chimeras not develop uniquely human characteristics such that it might lead to the conclusion that some "degree of humanity" or human dignity has been conferred on the resulting entity.

In part this responds to the same sort of concern about conferring unusual physical characteristics on animals (see above). If animal-human mixes were to exhibit human-like behaviors they would break our rules about characteristics that do and do not belong to distinct species. In other words, we do not want to see mice

20 Greely, HT, Cho, MK, Hogle, LF, Satz, DM "Thinking about the human neuron mouse" *Am J Bioethics* 2007; 7: 27–40.

21 Baylis, F., Robert, JS, "Part-Human Chimeras: Worrying the Facts, Probing the Ethics" *Am J Bioethics* 2007; 7: 41–45; Cohen, C., "Beyond the Human Neuron Mouse to the NAS Guidelines" *Am J Bioethics* 2007; 7: 46–49.

playing chess or exhibiting problem-solving behavior that we associate solely with humans. This would raise issues of the dignity of life these creatures possessed, whether they held some sort of intermediate human dignity and how, in light of the former answer, they were to be treated. Such a blurring between human and non-human animals might lead some to devalue characteristics thought to be sacred due to their uniquely human status and/or to something called "moral confusion."

Moral confusion

Although the argument is made that the blurring of lines between the human and non-human animal species may compromise human dignity, another perspective is that such blurring raises moral confusion. About this confusion Baylis and Roberts say, "When faced with the prospect of not knowing whether a creature before us is human and therefore entitled to all of the rights typically conferred on human beings, we are, as a people, baffled. One could argue further that we are not only baffled but indeed fearful."[22] We understand our world by classifications. Some categories are watertight. In the law all entities are either people or property and one cannot be the other. Where the two become blurred (patents on human genes) we run into controversy as to how and whether to proceed.[23] Humans are female or male but not both, and when the line gets blurred it causes us to feel discomfiture and an uncertainty as to how to categorize and treat the person who is both genders (transgender or hermaphrodite).

Likewise chimeras and hybrids raise issues of moral confusion. What is an animal that has human tissue? Do we need to treat it differently or dispose of it with the respect and ceremony normally due to humans? Does this blurring of the line between human and non-human animals somehow compromise our human dignity? In fact, the human-mouse neural transplant experiments did not go forward, but the discussion of the issues has informed how to move forward with care and forethought in this area. Where cytoplasmic hybrids are concerned, it is generally agreed that the resulting entities should not be allowed to breed and have offspring. Many have recommended that the hybrid embryos be destroyed at the standard regulatory 14 day limit. Additional limits can be found in the section on regulatory oversight, see Knowles L., "Canada's Regulatory Oversight of Stem Cell Research" Stem Cell Network. The public, policy and regulatory discussions and limits placed on the use of chimeras, hybrids and cybrids in stem cell research reflect the ethical issues of using animals in research and of creating new life forms. These issues will not disappear, but only grow more complicated. Continuing communication on why and how stem cell research impacts these issues is needed.

References

Bordet, S., Feldman, S., Knoppers, B.M. Legal Aspects of Animal-Human Combinations in Canada. (2007) 1:1 McGill Health Law Publication, 83.

Bubela, T., Caulfield, T. 2007 When human dignity is not enough: embryonic stem cell research and human cloning in Canada, in *Emerging Technologies: Hindsight and Foresight* ed. Einsiedel, E. (Vancouver: University of British Columbia Press, 2007).

Buehr, M., Meek, S., Ying, Q., et al. "Capture of Authentic Embryonic Stem Cells from Rat Blastocysts" Cell 2008, 135(7): 1287-1298.

Buehr, M., Hjorth, P. J., Hansen, A. K. & Sandøe, P. (2003) Genetically modified laboratory animals – what welfare problems do they face? Journal of Applied Animal Welfare Science 6(4), 319-338.

Caulfield, T., Chapman, A., Human dignity as a criterion for science policy, *PLoS Med.* 2005 Aug;2(8):e244. Epub 2005 Aug 2.

The Canadian Institutes of Health Research Updated Guidelines for Human Pluripotent Stem Cell Research, June 29, 2007 http://www.cihr-irsc.gc.ca/e/34460.html

Donnelley, S., The Brave new world of Animal Biotechology, 1994, Special Supplement, The Hastings Centre Report.

International Society for Stem Cell Research, "Guidelines for the Conduct of Human Embryonic Stem Cell Research" http://www.isscr.org/guidelines/ISSCRhESCguidelines2006.pdf

Kaebnick, G., "Are chimeric embryos natural? And does it matter?" Bioethics forum online, http://www.thehastingscenter.org/Bioethicsforum/Post.aspx?id=466

Karpowicz, P., Cohen, CB., van der Kooy, D., "It is ethical to transplant human stem cells into nonhuman embryos." *Nat Med.* 2004 Apr;10(4):331-5.

Karpowicz, P., Cohen, CB., van der Kooy, D., Developing human-nonhuman chimeras in human stem cell research: ethical issues and boundaries. *Kennedy Inst Ethics J.* 2005 Jun;15(2):107-34.

Robert, JS. 2004 Model systems in stem cell biology *Bioessays* 26 1005-1012.

Schuppli, CA., Fraser, D. & McDonald, M. (2004) "Expanding the 3Rs to meet new challenges in humane animal experimentation" *Alternatives to Laboratory Animals 32*, 525-532.

22 Baylis, F., Robert, JS, "Part-Human Chimeras: Worrying the Facts, Probing the Ethics" *Am J Bioethics* 2007; 7: 41-45.

23 Knowles, L., "Property, Progeny and Patents," *Hastings Center Report* 1999; 29(2).

Hunter,

Chimeras: The Ethics of Creating Human-Animal Interspecifics,

Dissertation (Jan. 20, 2009)

Chimeras

The Ethics of Creating Human-Animal Interspecifics

Inaugural-Dissertation zur Erlangung des Doktorgrades der Philosophie

an der Ludwig-Maximilians-Universität München

vorgelegt von Constanze Huther

(Doctoral thesis submitted to Ludwig-Maximilians-Universität Munich, Germany, in partial fulfilment of the requirements for the degree of Dr. phil.)

Referent: Prof. Dr. Wilhelm Vossenkuhl

Korreferent: PD Dr. Ulrich Metschl

Mündliche Prüfung: 20. 1. 2009

Contents

Preface ..... **v**

Acknowledgements ..... **viii**

Chapter 1: Biological Basics **1**

A. Naturally occurring chimeras and other mixtures 1

B. Human-made chimeras and other interspecifics 4

1. Roots of chimera research 4
2. Intraspecific animal-to-animal (and human-to-human) chimeras 7
3. Interspecific animal-to-animal chimeras 9
4. Human-to-animal chimeras 11
5. Animal-to-human chimeras 20
6. Transgenesis 24
7. Human-animal hybrids 29
8. Human-animal cybrids 32

C. Definitions 36

D. Excursus: Legal situation of human-animal interspecific research 41

Chapter 2: Arguments Against Creating Interspecifics **49**

A. An introduction to the debate: Sources 49

B. Intrinsic objections 52

1. Repugnance, the "Yuk Factor", and arguments from emotion 54
2. Religious and quasi-religious objections 63
3. The boundary between humans and nonhumans 69
4. Human dignity arguments 75
5. Intrinsic arguments: Conclusion 82

C. Direct consequence-based objections 83

1. The benefit side 83
2. Bad consequences for the entities created 85
3. Bad consequences for human populations: Health risks 98
4. Risk, uncertainty, and precaution 101

D. Confusion: Indirect consequence-based objections 106

1. Confusion as complicated determinability 106
2. Absolute confusion 110
3. Robert's and Baylis' argument from moral confusion 111

Chapter 3: Excursus on Moral Status and Speciesism **115**

A. The concept of moral status: General considerations 115

1. Defining moral status 115
2. Advantages of the moral status concept 118
3. Groups, members, and the kind paradigm 120

4. Variations of approaches to moral status 121
5. Moral status assignment: Caveat 123
6. General critique – and reality – of status assignment 126

B. Speciesism 128

1. The moral relevance problem....130
2. The marginal cases problem 132
3. The classification problem 133
4. The universalizability problem 137

Chapter 4: Should We Prohibit Interspecific Creation? 139

A. Concise analysis of arguments 139

B. Conclusions and recommendations 151

Epilogue 156

Photography and Illustration Credits 157

Bibliography 158

Zusammenfassung in deutscher Sprache 176

Lebenslauf 182

Preface

In summer 2004, I had the pleasure of attending a seminar by Julian Savulescu and Nick Bostrom about "Human Enhancement, Artificial Beings, and the Future of Humanity" at the University of Oxford. Among other quite exotic topics, chimeras were the subject matter of one of our seminar meetings. This was a concept that was completely new to me at the time and apparently denoted some kind of mixture between human and animal, which was to be used in biological research. What struck me about the seminar was the immediate, visceral and resolute reaction the topic aroused in many of my fellow students, at a time when none of us were actually very informed about chimeras. While the typical discussion in a philosophy seminar allows for grey areas, undecidedness and reserved interest, even regarding hotly debated issues like abortion or death penalty, the topic of mixing humans and animals elicited immediate rejection and concern in most of us – excluding Savulescu and Bostrom, who tried to frame the topic in neutral or positive terms. Since then, I have made a very similar observation in countless situations whenever casually introducing a colleague, friend or acquaintance to the subject of chimeras: most will have an immediate, strong and negative response to the idea of mixing animals with humans. Yet at the same time, few can produce arguments to support this knee-jerk reaction, even among bioethicists.

It was this discrepancy between a strong, unambiguous intuitive reaction to chimeras and the diffuseness and vagueness of the arguments brought forward that made me become (and stay) interested in the topic. In 2005, human-animal mixtures became relevant for my M.Phil. thesis, where I discussed whether the ethical position that being human makes a difference in regard to the moral status of a being ("speciesism") is defensible.[1] The notion that there could be beings in between human and animal, after all, should be thrilling for anyone who is concerned with the question of moral status difference between humans and animals. Accordingly, a short excursus in my M.Phil. thesis was devoted to human-animal chimeras: I argued that speciesist approaches had difficulties coping with species-ambiguous individuals, and that, in a nutshell, chimeras were a point in case for giving up

[1] In the following, "speciesism" will denote any *general attitude or approach* which assumes that being human makes a difference in regard to how we should treat an entity. The *ethical principle* of Speciesism and its different varieties will be properly introduced, defined and analyzed in chapter 3, section B.
The concept of moral status will also be used losely up until its explanation and definition in chapter 3, section A.

speciesism (I will come back to this question in chapter 3, section B below). The topic of chimeras remained in the focus of my philosophical work after my M.Phil. and I immediately settled on this area for my dissertation.

At the centre of this thesis is the question whether there is one, persuasive moral argument that can be used to veto the creation of (human-animal) chimeras or similar interspecifics. While responses to the issue of creating human-animal chimeras are almost univocally and strongly negative, at a second glance it seems at least extremely hard, if not impossible, to come up with such a fundamental argument against chimera creation – a result I hope to establish in chapter 2, where a variety of possible arguments are closely scrutinised.

Before working on moral aspects of chimeras, it is crucial to lay out the biological basics. A considerable portion of this dissertation is therefore devoted to making clear what chimeras actually are, what other interspecific constellations exist naturally, artificially and which interspecific entities might come to exist in the future. By this, I hope to avoid the allegation of writing about speculative, hypothetical Science Fiction. Also, I want to clear the path for philosophical discussion by visibly laying out what is at issue. In a debate as young as the one about chimeras, many philosophical problems are at risk of being obscured by conceptual vagueness or misunderstandings, e.g. about the concepts "chimera", "hybrid", "species membership" etc., but also about the actual research done and its motives. This is problematic not only because it leads to futile debates about non-topics, but also because there is actually urgent demand for ethical guidance and analysis in the field of interspecific research.

An issue that is a necessary corollary to the analysis of arguments against chimera creation is that of human-animal chimeras' moral status, and that of speciesism. Moral status will be discussed not only in the limited context of the question whether the creation of human-animal chimeras should be prohibited or allowed, but also from a more abstract point of view, regarding the advantages and disadvantages of using this concept. A connected question that I will look at in an in-depth excursus is that of speciesism, i.e. the idea that the moral status of humans is fundamentally different from that of nonhumans. I will show that the very idea of mixtures between humans and animals, and our reaction to this idea, tells us something meaningful about our understanding of the moral status of animals as opposed to that of humans. It questions and may even undermine our way of seeing the world in categories of "human" and "nonhuman". The question of defensibility of speciesism is, as I will show, at the bottom of several of the typical objections to chimera creation.

Scientists working in the various fields of research that involve the creation of human-animal mixtures have pointed out that they are in need of ethical ground rules, and, even more urgently, concepts and methods to work with when discussing the issue of human-animal mixtures from an ethical standpoint. So the issue of chimeras is directly, practically relevant in the sense that society will have to decide on whether and how to regulate or prohibit the creation of such beings, and needs toe-holds (and maybe whole new conceptual step irons) in order to enter an informed debate. I hope to deliver such starting points and contribute to this debate in a way that elucidates the ethical questions that arise from the creation of chimeras. Rather than persuading the reader of my specific personal views (although these will necessarily influence my analysis), I would mainly like to help them with reaching their own conclusions regarding this complex issue by giving an objective and detailed overview of the field.

In retrospect, the topic of chimeras has turned out to be an exciting, at times surprising, complex and often mind-blowing subject that kept me fascinated until the very last page of this dissertation. I hope that some of my enthusiasm for this area of bioethics will rub off on my readers.

Acknowledgements

I want to thank Wilhelm Vossenkuhl for his help and motivation with my dissertation, and for encouraging me to take on this project in the first place. I am grateful to Johannes Hübner and Gerhard Ernst, who greatly assisted me during various stages of my work. My graduate seminar colleagues were constant in their encouragement and enthusiasm for my project and their criticisms were always constructive and helpful.

I also want to thank the Universität Bayern e.V. for their generous financial support of this dissertation.

Finally, I am very indebted to David for countless hours of technical (and psychological) support and thorough proofreading.

Chapter 1: Biological Basics

This chapter will offer a comprehensive overview of chimeras and a whole array of other mixed organisms or entities. These preparations are necessary for a reasonable discussion of the "chimera" debate, which, as we will see, actually concerns only some types of chimeras, but includes several types of non-chimeric interspecific beings.

Section A will give an outline of natural occurrences of chimerism, distinguishing it from other forms of mixing, while section B will address artificial chimeras and mixtures of all kinds. The primary focus of this section will be on providing an insight into technical possibilities in experimental biology's employment of chimeras, and explaining the motives behind chimera and other interspecific research. In section C, I will assess currently used definitions of the term "chimera" and try to offer suitable concepts of "chimera" and "interspecific" for bioethical debate. Section D will give a short introduction to the legal and political situation of (human-animal) interspecific research, especially regarding embryonic chimeras and so-called cybrids.

A. Naturally occurring chimeras and other mixtures

Biological laymen understand the expression "chimera" to denote either figures of Greek mythology or phantasms and illusions.[2] In common usage, the term "chimera" denotes impossible beings which, by their very existence, disrupt categories.

In biology, "chimera" is a technical term.[3] It denotes, as we will see, not only creatures whose mixed and artificial nature is obvious, but also inconspicuous beings and entities that result from natural processes. The concept and use of "chimera" in biology is complex and multiple. Settling on a definition seems not advisable at this point. In this and the following section I will give an overview of possible mixtures, and explain what chimeras are *not* by distinguishing them from other mixtures – not all mixed beings are chimeras. By Section C, we will have an outline of this complex area at hand that should suffice for assessing possible definitions of "chimera" and for settling on the future use of "chimera" and related terms.

[2] In German, the latter meaning is distinguished from the former by a different spelling - "Schimäre" denotes the phantasm, "Chimäre" the mythological (or actual) being.
[3] The somewhat antiquated spelling "chimaera" is used rarely today. Confusingly, "chimaera" also denotes a type of cartilaginous fish (order Chimaeriformes) which is related to sharks.

As a crude first approximation, one can say that chimeras are organisms which are the product of mixing materials from two (or more) genetically different organisms. (As you may have noticed, this approximation includes all organisms that are a product of sexual reproduction, but let us set this objection aside for the moment.)

Rather, let us have a look at what biologists consider naturally occurring chimeric organisms: both in animals and humans, twin embryos often exchange cells in the womb, sometimes leading to intra-species chimeras whose chimerism usually goes undetected, only sometimes showing in the form of strange iris coloration or fur patterns. Chimerism, on a small level, also occurs when fetal cells enter the maternal organism ("fetomaternal microchimerism"). Twin embryos also sometimes fuse in the uterus. These cases of so-called "disappearing twins" can result in an adult that carries a "parasitic" twin in its body (which leads to strange results in blood tests); it can also in rarer cases lead to noticeable deformations like hermaphroditism or supernumerous limbs. Likewise, conjoined twins exhibit a composition resulting from a fusion of two embryos. They are not regarded as chimeras, though, because they are the product of identical twins' fusion: unlike normal twins, identical twins stem from one common zygote (fertilized egg).

Probably the most well-known and obvious chimeras within the human species are patients who have undergone transplantation of tissues, body parts or organs from other (deceased or alive) human beings. Human-to-human transplantation is nowadays so common that allocation of organs is almost the only ethical question discussed in this area (maybe with the exception of the transplantation of whole body parts, specifically the face, which raises other issues as well). By contrast, animal-to-human transplantation or xenotransplantation, which produces animal-to-human interspecific chimeras, is still regarded as highly controversial – apparently not only because of its medical riskiness (for an overview of xenotransplantation research, see B.5).

Note that genetically differing sets of cells are not only found in chimeric organisms. Chimerism should not be confused with mosaicism, i.e. organisms which have genetically distinct sets of cells, but whose differing cell populations originate from just one zygote. Mosaicism is normal in female mammals, where x-chromosome inactivation leads to an organism which partly consists of cells where, randomly, either the paternal or maternal X chromosome is inactivated. These two cell types are scattered over the female mammal's body (visibly so in female cats with tortoiseshell or calico fur patterns). Mosaicism does also occur when identical twins exchange cells in the womb.

We can note that chimeric organisms consist of cells that have differing genetic information. These genetically differing cells do not originate from one zygote, as is the case with female mosaicism and mosaicism in identical twins, but from two or more differing sources.

Picture 1: Chimera. Etruscan Bronze, 5th century BC.

In Greek mythology, the main characteristic of chimeras is their compositeness, or more specifically, that they are made from different species of animal, human or mythological creature. According to Homer, the original Chimera was slain by Bellerophon with the help of Pegasus (another chimeric creature!) in Asia Minor. The mythical monster consisted of lion, goat and dragon or snake.[4] A village on the south coast of turkey is still called "Chimaira" in honour of the mythical Chimera. The naturally occurring chimeras I mentioned above do not exhibit other species' characteristics because they are intraspecific chimeras, and, as such, wholly inconspicuous to layman observers (apart from conditions like hermaphroditism that are present in some of them). Are there naturally occurring mixtures between species at all? Some think that lichen can be regarded as the chimeric symbiosis of algae and fungus. And in plants, production of interspecific chimeric organisms is not restricted to high-tech laboratories: graftage of fruit trees is a low-tech, traditional method resulting in chimeric plants which – for example – lets us grow pears on one and apples on another branch of a tree, or different-coloured roses on one rose stem. The bioethical debate about chimeras is focused on animal chimerism, as we will see in the discussion below. So, do inter-species animal chimeras occur naturally, too?

There are, indeed, mixes between different species: interspecific hybrids. These hybrids – also called "cross" or "bastard" – result from sexual reproduction between individuals of different, but closely related species and are often (not always) sterile themselves. To give but two examples, mules are the offspring of female horses and male donkeys, ligers and tions result from crossing tigers with lions. Many undomesticated species produce hybrids in the wild, without human intervention. Hybrids are not regarded as chimeras because

[4] Homer, in the Illiad (VI. 179-182), describes the chimera as "lion-fronted and snake behind, a goat in the middle, and snorting out the breath of the terrible flame of bright fire." For a comprehensive overview of mythological chimeric creatures' appearance in art, see Mode (1974), Fabeltiere und Dämonen in der Kunst. Die fantastische Welt der Mischwesen.

they do not contain genetically distinct cell populations. Instead of consisting of inhomogeneous sets of genetically different cells, they are wholly composed of homogenous cells that are (genetically) intermediary in type. This is because they result from the fusion of an egg and a sperm of different species into a single zygote, from which all other cells of the hybrid organism originate. In hybrids, the mixture takes place on *inner-cell level*, typologically resulting in an animal which is *sui generis* but not a mixture on the cell or organ level, since all cells of the hybrid animal carry the same genetic fingerprint. In chimeras, the mixture takes place on the level of *cells*, resulting in an organism whose cells keep their disparate genetic identity. If we, because of this difference, exclude hybrids from the area of chimeric beings, it becomes apparent that interspecies animal chimeras exclusively come to exist through artificial means.

Artificial chimeras – especially animal-human chimeras resulting from manipulation and mixing of embryos and stem cells – are at the centre of the bioethical chimera debate. Why, how and under what circumstances those creatures are, today, produced and used in research laboratories all over the world will be described in the next section.

B. Human-made chimeras and other interspecifics

1. Roots of chimera research

What scientific roots did current chimera research, especially inter-species chimera research, emerge from, and what are the deeper motivations for today's experiments with human-nonhuman mixtures? One can subsume current chimera research under three areas of particular interest.

Firstly, researchers have been trying for several decades to create animal models for all kinds of diseases; i.e. animals in which human diseases can be emulated. Many of the chimera experiments that are done today, especially human-animal chimera experiments, are directed towards imitating human diseases in animals. One prominent example of this practice is the SCID-hu mouse, a scientific breakthrough of the 1980s, which is regarded as a cornerstone of immunology research. Researchers grafted human stem cells as well as human fetal liver cells, fetal thymus cells and bone marrow into immuno-deficient mice in order to "humanize" the animals. The resulting mice have a human immune system.[5] The SCID-hu model was necessary to isolate human hematopoietic stem cells that are now

[5] McCune, Namikawa, et al. (1988), "The SCID-hu mouse: murine model for the analysis of human hematolymphoid differentiation and function", Science, **241**(4873).

commonly used in therapies of leukaemia.[6] It is also still widely used in HIV and other immune system research.[7] Newer chimeric models are often used to emulate neurodegenerative and psychiatric diseases.[8] Creation of "humanized" disease models is also done with methods of genetic engineering – a prominent example is the Harvard OncoMouse, which was "genetically engineered to contain a human cancer-causing gene" in 1988.[9] Transgenically humanized animals – which are not chimeric beings – will be further discussed in section 6.a below.

A second strain of research that lead towards today's chimera experiments is that of developmental biology, which has, over the last 150 years, introduced methods of tissue transplantation in order to find out about various developmental phenomena.[10] Some chimera experiments continue this search for explanations of how and why different types of cells (e.g. varying somatic cells, precursor cells or stem cells) develop, fuse, aggregate, diversify, change their level of potency, develop anomalies, or are influenced by their microenvironment, offering a model for research which cannot be done in human beings for ethical reasons and is carried out in animals instead.[11]

Another motive for induction of chimerism in research is due to the fact that scientific consensus and regulation (e.g. by the United States Food and Drug Administration, FDA) requires that stem cell therapies, before being applied to human subjects, first be tested in animals. Such testing results in human-to-animal chimeras. This is specifically relevant for the development of treatments for neurodegenerative disorders.[12] Chimeras as assay systems, which are used to find out about tumorigenicity and to test stem cell applications with therapeutic potential, can be created in adult and fetal animals *in vivo*, but also in embryonic *in vitro* experiments. The chimeric subjects are usually euthanized after

---

[6] Greely, Cho, et al. (2007b), "Thinking About the Human Neuron Mouse", American Journal of Bioethics, **7**(5), p. 31.
[7] Ibid., p. 32.
[8] Cf. Muotri, Nakashima, et al. (2005), "Development of functional human embryonic stem cell-derived neurons in mouse brain", Proceedings of the National Academy of Sciences, **102**.
[9] Sagoff (2003), "Transgenic Chimeras", American Journal of Bioethics, **3**(3).
[10] Robert (2004), "Model Systems in Stem Cell Biology", Bioessays, **26**, p. 1010.
[11] Examples: Stern (1973), "Chimaeras obtained by aggregation of mouse eggs with rat eggs", Nature, **243**(5408); Fehilly, Willadsen, et al. (1984), "Interspecific chimaerism between sheep and goat", Nature, **307**; Brüstle, Choudhary, et al. (1998), "Chimeric brains generated by intraventricular transplantation of fetal human brain cells into embryonic rats", Nature Biotechnology, **16**; Ourednik, Ourednik, et al. (2001), "Segregation of human neural stem cells in the developing primate forebrain", Science, **293**.
[12] Baylis and Fenton (2007), "Chimera Research and Stem Cell Therapies for Human Neurodegenerative Disorders", Cambridge Quarterly of Healthcare Ethics, **16**(2), p. 196f; Greely, Cho, et al. (2007b), "Thinking About the Human Neuron Mouse", American Journal of Bioethics, **7**(5), p. 28.

transplantation of human cells and undergo histological or genetic analysis.[13] This motive of testing of possible hESC treatment methods can be subsumed under the second branch of finding out how human cells develop and differentiate in vivo.

The third root of chimera research prevalent today can be found in the search for substitute tissue or organs for patients in need of transplantation due to illness or injury. Although porcine heart valves are nowadays routinely used as replacement for human heart valves, the use of living animal organs and tissues, in the past, has not been as successful as one had hoped for, since animal organs, unfortunately, do not properly integrate into the human organism. Therefore, human-animal chimeras are created in order to grow human organs or tissues within animal organisms.[14] In the future this method could be used for more successful xenotransplantations due to a reduced immune response of the human host (for further discussion, see 5 below).

Accordingly, one can see current chimera research as contributing to three main projects: that of emulating human diseases in animals, that of finding out about (human) cell development in vivo without harming human beings, and that of producing human organs or tissue substitutes in vivo. These research interests frequently overlap: research with human stem cells introduced in injured animal organisms (e.g. in stroke-affected brains of mice[15] and damaged spinal cords of mice[16]) is as interesting from the viewpoint of stem cell development as it is from the viewpoint of replacing damaged tissue in human organisms in the future.

Let us now look at the different types of entities that are created in these branches of research. They will be arranged in order of the direction of the chimeric manipulation (i.e. animal-to-animal, animal-to-human, or human-to-animal) and the developmental stage of the recipient. After analyzing chimeras, we will also have a look at non-chimeric animal and human-animal interspecifics, including hybrids and "transgenic chimeras".

---

[13] Robert (2006), "The science and ethics of making part-human chimeras in stem cell biology", Journal of the Federation of American Societies for Experimental Biology, **20** p. 840.

[14] Bianco and Robey (2001), "Stem cells in tissue engineering", Nature, **414**; Dekel, Burakova, et al. (2003), "Human and porcine early kidney precursors as a new source for transplantation", Nature Medicine, **9**; Almeida-Porada, Porada, et al. (2004), "Formation of human hepatocytes by human hematopoietic stem cells in sheep", Blood, **104**(8); Taylor, Cowin, et al. (2006), "Formation of human prostate tissue from embryonic stem cells", Nature Methods, **3**(3).

[15] Kelly, Bliss, et al. (2004), "Transplanted human fetal neural stem cells survive, migrate, and differentiate in ischemic rat cerebral cortex", Proceedings of the National Academy of Sciences, **101**(32).

[16] Cummings, Uchida, et al. (2004), "Behavioral improvement, differentiation, and immuno-electron microscopy of human central nervous system stem cells in spinal cord injured NOD-Scid and NOD-Scid/Shiverer mice", Society For Neuroscience Abstracts.

2. Intraspecific animal-to-animal (and human-to-human) chimeras

The beginnings of animal-to-animal chimera research within species (intraspecific animal-to-animal chimeras) are in the grafting experiments of Murray and Huxley, in the 1920s,[17] and the first embryonic mouse chimeras, created by Andrzej Tarkowski[18] and Beatrice Mintz in 1960s.[19] Intraspecific mouse chimeras were made by fusing two mouse embryos.[20] More elaborate techniques allowed not only the combination of two embryos, but also the combination of embryos with embryonic cells from a later stage (e.g. inner cell mass cells), cells from embryonic carcinoma, embryonic stem cells and embryonic germ cells.[21] Modern techniques produce chimeras by "sandwiching" cells of different provenience in layers.[22] Many of these experiments do not only result in chimeric blastocysts or embryos, but also in viable adult chimeric mice; they were also carried out in animals other than mice, such as rats, sheep and bovines.[23]

While the focus of the first intraspecific chimera experiments was on studying normal early development of cells and on finding out about phenomena such as hermaphroditism (which is sometimes based on intraspecific chimerism), intraspecific chimeras today often have a different role: transgenic germ line chimeras are used as carriers in the production of genetically modified animals. Manipulated embryonic stem cells are transplanted into host embryos which incorporate them into their germ line, producing genetically modified gametes.[24]

This is by far not the only area of chimeric intra-species experimentation. To give another example for the utility of animal-to-animal chimerism in research, British scientists transplanted retina cells from a particular ontogenetic stage in murine fetal development ("photo receptor precursors") to the retinae of blind adult mice in 2006 in an effort to advance the possible treatments for blindness. The cells apparently integrated into the adult mouse organism, enabling the transfer of information to nerve tissue and, accordingly, the

---

[17] Murray and Huxley (1925), "Self-differentiation in the grafted limb bud of the chick", Journal of Anatomy, **59**.
[18] Tarkowski (1961), "Mouse chimaeras developed from fused eggs", Nature, **190**.
[19] Mintz (1962), "Formation of genetically mosaic mouse embryos", American Zoologist, **2**.
[20] Tarkowski (1998), "Mouse chimaeras revisited: recollections and reflections", International Journal of Developmental Biology, **42**.
[21] Ibid., p. 904.
[22] Ibid.
[23] Ibid.
[24] Ibid., p. 906f. For an example of transgenic pig germ line chimeras, see Piedrahita, Moore, et al. (1998), "Generation of Transgenic Porcine Chimeras Using Primordinal Germ-Cell Derived Colonies", Biology of Reproduction, **58**.

mouse brain.[25] By determining the particular stage in development at which precursor cells succeed at integrating into the alien organism, the scientists hope to find out at which point in development stem-cell generated human cells should be transferred to blind patients' eyes (this would constitute a human-to-human, intraspecific chimera).[26]

These are all quite foreign procedures for the non-bioengineer, and some might think that chimeras, apparently, are something that one rarely encounters as a layman. This is misleading: As mentioned above, there are also intraspecific human-to-human chimeras among us whose existence is well-known even to the non-expert. Allotransplantation, be it cardiac, renal, or hepatic, be it from a living or a deceased donor, leads to a human being whose cells are partly of a different genetic set-up. These cases of intraspecific chimerism within our own species lead to particular ethical problems, mainly, the problem of organ allocation, which shall not interest us here because it is not a corollary of chimerism as such but a matter of the scarcity of donor organs. Apart from allocation problems, human-to-human transplantation nowadays rarely leads to reactions of horror or moral indignation. An exception to this rule might be face transplantation. The case of the first face transplant carried out on a Frenchwoman in 2005[27] demonstrates that, ultimately, loss of identity of the recipient or an inadvertent transfer of social identity of the deceased donor were not the central problems. The question whether informed consent actually took place or whether the patient was used as a guinea pig for not yet perfected therapeutic methods played a much greater role in this case.[28]

Another spectacular case of allotransplantation practice that typically elicits ethical concerns is that of therapeutic use of human fetal tissue – e.g. transplants of fetal brain tissue into the brains of Parkinson's patients[29] – which have, so far, been unsuccessful and even detrimental to the patients' health. One problematic aspect of this method is the use of human fetuses: it is feared that these fetuses could be reduced to their role as raw material for drugs, or that, in the case of scientific success, increasing demand could lead to induction of pregnancies for the sake of producing fetal material. There is a general debate around the propriety of use of fetal tissue in research, which also comes up regarding

---

[25] Die Zeit (2006), "Erforscht und Erfunden: Blinde Mäuse", 2006/11/09; MacLaren, Pearson, et al. (2006), "Retinal repair by transplantation of photoreceptor precursors", Nature, **444**.
[26] MacLaren, Pearson, et al. (2006), "Retinal repair by transplantation of photoreceptor precursors", Nature, **444**, p. 207.
[27] BBC News (2005), "Woman has first face transplant", 2005/11/30.
[28] For a discussion of ethical and psychological problems surrounding face transplantation, see Jungblut (2005), "Gesichtstransplantation - Ärztlicher Ehrgeiz oder Interesse des Patienten", ZeitWissen **2005** (2).
[29] Freed, Greene, et al. (2001), "Transplantation of Embryonic Dopamine Neurons for Severe Parkinson's Disease", New England Journal of Medicine, **344**(10).

interspecies xenografts (i.e. injection of fetal material into nonhuman materials) – for some comments on this problem, see chapter 2, section C.2.b below. In Germany, the ZEB (Zentrale Ethikkommission bei der Bundesärztekammer) dismissed therapeutic use of fetal/embryonic tissues in Parkinson's patients as ethically dubious out of a combination of numerous reasons and voiced a square refusal to such practices in 1998.[30] The transfer of material from one organism to another (i.e. the causation of chimerism per se) was not an issue in the moral concerns regarding neural transplants; the debate focused on the proper management of health risks.

My focus in this work will, as I have previously pointed out, be on interspecific chimeras, i.e. a type of creature where the individual being contains live material from two or more species. Let us first have a look at animal-to-animal chimeras which do not involve human material and then, in sections 4 and 5 below, at chimeras between humans and animals.

3. Interspecific animal-to-animal chimeras

Since the 1970s, numerous experiments have been carried out that resulted in interspecific chimeras. One of the first interspecific chimeras was brought about by M. Susan Stern, who created a chimeric rat-mouse blastocyst in 1973.[31] Many interspecific chimeras have been created since then; many of them reached adulthood and some were even fertile.

Picture 2: Sheep-goat chimera

One experiment of this kind which gives a very tangible illustration of chimerism was the sheep-goat chimera (see picture 2). In 1984 such an animal was created by artificially fusing a sheep and a goat embryo, which was then brought to term.[32] The creature, which is sometimes called "geep", displays characteristics of both sheep

[30] Zentrale Ethikkommisson bei der Bundesärztekammer (1998), "Übertragung von Nervenzellen in das Gehirn von Menschen."
[31] Stern (1973), "Chimaeras obtained by aggregation of mouse eggs with rat eggs", Nature, **243**(5408).
[32] Fehilly, Willadsen, et al. (1984), "Interspecific chimaerism between sheep and goat", Nature, **307**.

and goat, but these are not evenly distributed, resulting in an intermediate type (as would be the case in a sheep-goat hybrid). Instead, they are scattered, puzzle-like, over the animal's body depending on where in the organism sheep cells or goat cells prevailed. Thus, a geep has sheep parts which are woolly (or display other sheep characteristics) and goat parts that are hairy (or display other goat characteristics).

The creation of interspecific chimeras which live to later embryonic or even adult stages does not work between randomly selected species. Veteran chimerism researcher Andrzej Tarkowski notes that the attempt of a colleague at creating sheep-cow chimeras resulted in severely malformed lambs, and that reabsorption of implanted, non-viable chimeric blastocysts is a very common occurrence.[33] It soon became clear that the viability of such interspecific chimeric embryos depends mainly on whether the two species are closely genetically related.[34]

A notable step in chimera research – which might be especially interesting in regard to ethical questions – was the creation of "quail-brained chicken" by Balaban, Teillet and Le Douarin in 1988.[35] Parts of the neural tube of quail embryos (the structure that later develops into the central nervous system) were implanted in chick embryos. This resulted in chicks whose behaviour indicated a transfer of species-specific inborn properties: The quail-chick chimeras crowed similarly to quails. The extent of this chicken-atypical behaviour depended on how extensive the insertion of quail cells had been. This was "the first demonstration of cross-species behavioral transfer brought about by neuronal transplantation."[36] A transfer of "inborn auditory perceptual preference"– i.e. response to species-specific maternal calls – in the brain-transplanted chicks was observed in later experiments.[37] The example of the quail-brained chicken is often used to demonstrate that a transfer of behavioural characteristics from one species to another is possible, in principle.

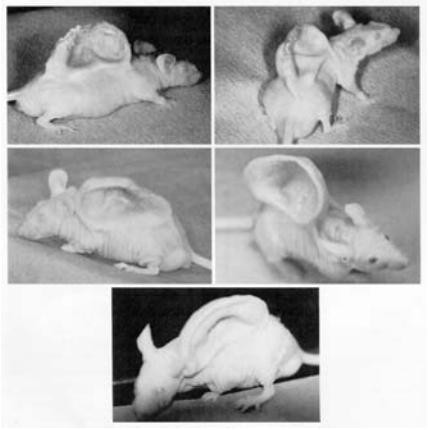
---

[33] Tarkowski (1998), "Mouse chimaeras revisited: recollections and reflections", International Journal of Developmental Biology, **42**, p. 905.
[34] Ibid.
[35] Balaban, Teillet, et al. (1988), "Application of the quail-chick chimera system to the study of brain development and behavior", Science, **241**.
[36] Ibid., p. 1341.
[37] Long, Kennedy, et al. (2001), "Transferring an inborn auditory perceptual preference with interspecies brain transplants", Proceedings of the National Academy of Sciences, **98**.

4. Human-to-animal chimeras

The development and possibilities of artificial manipulation of diverse human cells, above all, stem cells, is of great interest to researchers. Because there obviously are ethical limits regarding the study of such cells within the human body, many scientists have seized the opportunity to create human-to-animal chimeras – i.e. chimeras consisting of an adult, fetal or embryonic animal host into which genetically human parts of cells, unconnected single cells or cell structures/tissues are artificially introduced.

a. Human-to-animal chimeras (adult recipient)



Picture 3: "Vacanti Mouse"

An (alleged) example of this development which stays in collective memory was the infamous "earmouse", a naked mouse with an ear-like structure on its back, created by Charles Vacanti and Linda Griffith-Cima in 1997.[38] Iconic pictures of the "earmouse" (see picture 3) were publicized widely via the internet, allegedly symbolizing the horrors of "genetic manipulation". How and why did this strange creature come into being? Vacanti and Griffith-Cima seeded a scaffold of biodegradable polymer with cartilage cells and transplanted it onto the back of an immunodeficient mouse, whose organism then nurtured the auricle. Their research was aimed at the future possibility of re-growing ears or other cartilage structures in vitro, or even directly on human patients who need such a substitute because of accidents or genetic defects. Charles Vacanti is still working on making this "tissue engineering" approach ready for application in humans. Because the host, in the case of Vacanti's and Griffith-Cima's mouse, was an adult individual, this kind of chimera would be called an adult chimera. It was not a human-to-animal chimera, though. Despite its appearance, the ear on the mouse's back did not contain human, but bovine cartilage cells. The iconic image of the "earmouse" may be a powerful symbol for human-animal mixing, but the creature in question did not even contain human material.

Experiments resulting in actual human-to-animal adult chimeras employ techniques that differ from Vacanti's tissue engineering approach. Human material is introduced in animal organisms, but instead of somatic human cells, researchers use precursor cells or stem

[38] Cao, Vacanti, et al. (1997), "Transplantation of Chondrocytes Utilizing a Polymer-Cell Construct to Produce Tissue-Engineered Cartilage in the Shape of a Human Ear." Plastic & Reconstructive Surgery, **100**(2).

cells.[39] Let me first give some examples for the use of human precursor cells in transplantations to nonhuman hosts:

In 2002, Benjamin Dekel and colleagues from the Weizman Institute in Israel succeeded in inducing the growth of miniature kidneys in mice by transplantation of kidney precursor cells taken from human and pig embryos.[40] This experiment ultimately aims at the production of substitute organs for humans in need of transplantation, and it was a main point of interest for researchers to find out at what point in time kidney precursor cells are best transplanted to the alien organism in order to flourish.

To give another example of this kind of research, Angioi and colleagues transferred embryonic human stomachs, tracheas, intestine and lungs into adult mice in 2002, which led to the development of functional "micro-organs".[41]

Another human-to-animal chimerism experiment in which precursor cells were used focused on growing human prostrate tissue in mice by implanting specially manipulated human embryonic stem cells ("prostate tissue precursor cells"). This experiment was carried out by Renea Taylor and Prue Cowin in Melbourne in 2005.[42] Here, the focus was on finding out how benign prostate disease (BPH) and prostate cancer develops in order to be able to treat it more successfully in the future.

Similar research has also been carried out in Germany. Scientists at the Max Delbrück Center for Molecular Medicine (Berlin) transplanted liver cells derived from human embryonic stem cells into mice with partially damaged livers. Among other objectives, the researchers wanted to find out whether transplantations of liver cells prepared in this manner could be used for liver regeneration therapy in human patients.[43]

Ahmed Mansouri at the Max Planck Institute for Biophysical Chemistry (Göttingen) obtained a licence to conduct similar research in 2003. The MPIbpc project involved the

---

[39] Stem cells are less developed than progenitor cells and have greater potential for differentiation. In technical terms, progenitor cells are "multipotent" (can create only some kinds of cells), while stem cells are "pluripotent" (can develop into all kinds of cells). "Precursor cell" is a generic term for both "stem cells" and "progenitor cells", used in cases where it is not clear whether the cells at issue have stem cell or progenitor cell properties, i.e. are pluripotent or multipotent, which can be hard or impossible to ascertain. For a detailed explanation of terminology and an overview of current stem cell research, see Kempermann (2008), Neue Zellen braucht der Mensch: Die Stammzellforschung und die Revolution der Medizin.

[40] Dekel, Burakova, et al. (2003), "Human and porcine early kidney precursors as a new source for transplantation", Nature Medicine, **9**.

[41] Angioi, Hatier, et al. (2002), "Xenografted Human Whole Embryonic and Fetal Entoblastic Organs Develop and Become Functional Adult-Like Micro-Organs", Journal Of Surgical Research, **102**.

[42] Taylor, Cowin, et al. (2006), "Formation of human prostate tissue from embryonic stem cells", Nature Methods, **3**(3).

[43] Robert-Koch-Institut (2004), "7. Genehmigung nach dem Stammzellgesetz (erteilt am 21.10.2004)", Register genehmigter Anträge nach §11 Stammzellengesetz.

implantation of dopamine-producing human neural precursor cells obtained from human embryonic stem cells in fetal rats' brains (for this part of the project, done by Oliver Brüstle, see p. 16), and implantation of similar human cells into marmoset monkeys' brains which have been manipulated to mimic Parkinson's.[44] A somewhat sensational report[45] on these experiments (describing them as injection of human embryonic stem cells, while actually only differentiated cells were transplanted, and mentioning the startled and appalled reaction of the president of the "Nationaler Ethikrat" to these allegations) was vehemently disputed by the MPIbpc.[46] The institution's assertion that only blastocysts fused with alien cells lead to chimerism while the experiments discussed were "just transplantations"[47] is not without controversy: common definitions of "chimera" would include the creatures created in the MPIbpc experiments (cf. section C below).

Researchers also make use of chimeras to test stem cell-based therapies for diabetes – one U.S. research team based in San Diego derived a cell type from human embryonic stem cells that was capable of synthesizing pancreatic hormones, such as insulin. These insulin-expressing cells were implanted into mice with diabetes and damaged kidneys, leading to improved blood sugar levels – it is suspected that this was caused by the human stem cells integrating into and thereby repairing the mouse kidneys.[48] Similar experiments were carried out by a team of researchers at Tulane University, who used multipotent human stem cells derived from bone marrow which they injected in diabetic mice.[49] Diabetes researchers hope that in the future, cells derived from the patients' own bone marrow could be used to treat diabetes.[50]

Regarding spinal cord injuries (the cause of paraplegia), Cummings, Uchida et al., transplanted human stem cells to the injured portion of a mouse's spinal cord in order to

---

[44] See Robert-Koch-Institut (2003), "5. Genehmigung nach dem Stammzellgesetz (erteilt am 27.10.2003)", Register genehmigter Anträge nach §11 Stammzellengesetz.
[45] Traufetter (2005), "Der Mensch im Tier", Der Spiegel, 2005/05/02. On the events that followed the SPIEGEL article, see also Löhr (2005), "Chimären aus dem Labor", die tageszeitung, 2005/05/06.
[46] Max-Planck-Institut für biophysikalische Chemie (MPIbpc) (2005), "Richtigstellung und Stellungnahme - Informationen zum SPIEGEL-Artikel 'Der Mensch im Tier' und zur dpa-Meldung 'Nationaler Ethikrat will sich mit Chimären-Experimenten befassen'."
[47] "Bei den genannten Versuchen handelt es sich keineswegs um die Generierung von Chimären, sondern lediglich um eine Transplantation. Chimären sind Organismen, deren Gewebe nach der Injektion von undifferenzierten Stammzellen in den frühen Embryo (Blastocyste) aus unterschiedlichem Erbgut zusammengesetzt sind." - Ibid., p. 2.
[48] D'Amour, Bang, et al. (2006), "Production of pancreatic hormone-expressing endocrine cells from human embryonic stem cells", Nature Biotechnology, **24**.
[49] Lee, Seo, et al. (2006), "Multipotent stromal cells from human marrow home to and promote repair of pancreatic islets and renal glomeruli in diabetic NOD/ scid mice", Proceedings of the National Academy of Sciences, **103**(46).
[50] BBC News (2006), "Stem cell cure hope for diabetes", 2006/11/12.

"repair" it in 2004. The transplanted material apparently differentiated and survived, and an improvement of the animal's ability to climb along a horizontal ladder could be observed.[51]

Stem Cells Inc., a company that contributed to this research, and its leading scientist Irving Weissman, were also involved in a project researching the integration of human neural stem cells in the ischemic (post-stroke) brain of rats; the ultimate aim of the investigations was the question whether the transplantation of human stem cells into patients' brains could be a therapeutic option for stroke in the future.[52]

Transplantation of human stem cells in adult animals' brains is not only done in mice, but also in primates: Yale psychiatrist Eugene Redmond hopes to contribute to finding a cure to Parkinson's by carrying out transplantations of human neural stem cells in adult African green monkeys' brains.[53] The stem cells are hoped to morph into dopamine-producing cells when implanted at the right place. Dopamine is a substance that Parkinson's-affected brains lack, and the procedure apparently leads to an improvement of Parkinsonism in animals.[54] Just like Mansouri's experiments, Weissman's and Redmond's neural stem- or precursor cell xenograft experiments have been discussed in the media[55] and were ethically controversial enough to trigger a general interest of ethics' commissions regarding the topic of chimera research.[56]

b. Human-to-animal chimeras (embryonic or fetal recipient)

Many chimera experiments described so far involve only "discrete functions and organs" of the (adult) host, as Robert and Baylis put it.[57] Such "old school" chimeras are basically just animals with a few human cells or humans with a few animal cells (even if these few cells are in the brain). When compared to the introduction of differentiated somatic cells, using human progenitor or stem cells as transplantation material leaves a much bigger margin for unforeseen reactions and interactions of the introduced cells. And as biotechnology

---

[51] Cummings, Uchida, et al. (2005), Human neural stem cells differentiate and promote locomotor recovery in spinal cord-injured mice", Proceedings of the National Academy of Sciences, **102** (39).
[52] Kelly, Bliss, et al. (2004), "Transplanted human fetal neural stem cells survive, migrate, and differentiate in ischemic rat cerebral cortex", Proceedings of the National Academy of Sciences, **101**(32).
[53] For coverage of Redmond's experiments, see Bearden (2005a), "Extendend Interview: Eugene Redmond", Online NewsHour - A NewsHour with Jim Lehrer Transcript; Shreeve (2005), "The Other Stem-Cell Debate", The New York Times Magazine, 2005/04/10.
[54] For background information on Redmond's approach, see Redmond (2002), "Cellular Replacement Therapy for Parkinson's Disease: Where We Are Today?" The Neuroscientist, **8**(5).
[55] See e.g. Bearden (2005b), "Extendend Interview: Irving Weissman", Online NewsHour - A NewsHour with Jim Lehrer Transcript; Shreeve (2005), "The Other Stem-Cell Debate", The New York Times Magazine, 2005/04/10; Traufetter (2005), "Der Mensch im Tier", Der Spiegel, 2005/05/02.
[56] Greene, Schill, et al. (2005), "The Working Group on the Criteria for Cell-Based Therapies, John Hopkins University: Moral Issues of Human-Non-Human Primate Neural Grafting", Science, **309**; Nationaler Ethikrat (2005), "Wortprotokoll - Niederschrift über den öffentlichen Teil der Sitzung am 25. August 2005", p. 7.
[57] Robert and Baylis (2003), "Crossing Species Boundaries", American Journal of Bioethics, **3**(3), p. 1.

develops over the years, even more intricate (and less controllable) mixtures are within reach. When alien cells or materials are introduced in a host organism that is not adult and differentiated, but still in early developmental stages itself – e.g. fetal, embryonic, zygote or even gamete –, the integration and influence of alien cells or materials on the novel organism brings pronounced uncertainties. The earlier alien materials are implanted, the bigger and harder to predict the potential consequences for the developing organism.[58] This point is particularly applicable in regard to pluripotent cells (i.e. some types of stem cells) that have the ability to differentiate into basically all kinds of cells.

Experiments where human cells were introduced in animal recipients in the fetal stage include Evan Snyder's 2001 project at Harvard University. Snyder's team implanted human neural stem cells into the brain of fetal bonnet monkeys. The scientists waited until the monkeys' cerebral cortex was developed and then carried out a histological examination of the fetal animals: the human cells had widely migrated, survived and integrated to great extent.[59] This experiment improved the prognosis for success of gene therapy or cell-substitution approaches via neural stem cell transplantation to the brain of large nonhuman primates or – as the ultimate goal – humans.

German stem cell pioneer Oliver Brüstle, working at the MPIbpc's project on differentiation of human embryonic stem cells and xenografts of dopamine-producing precursor cells into marmoset monkeys, and his colleague Ahmed Mansouri, obtained a licence for transplantation of human neural progenitor cells in fetal rat brains in 2003.[60]

In another experiment utilizing fetal chimeras, Esmail Zanjani of the University of Nevada and his research group implanted human hematopoietic stem cells, extracted from bone marrow or cord blood, in fetal sheep, during the stage of development where the immune system of the fetuses had not yet developed. This resulted in adult sheep whose livers contained up to 20% human cells.[61] While Zanjani was initially just interested in gene therapy of genetically defective (human) fetuses, he soon discovered that using animals to

---

[58] Greely (2003), "Defining Chimeras…and Chimeric Concerns", American Journal of Bioethics, **3**(3), p. 18.
[59] Ourednik, Ourednik, et al. (2001), "Segregation of human neural stem cells in the developing primate forebrain", Science, **293**; Robert and Baylis (2003), "Crossing Species Boundaries", American Journal of Bioethics, **3**(3), p. 1.
[60] Robert-Koch-Institut (2003), "5. Genehmigung nach dem Stammzellgesetz (erteilt am 27.10.2003)", Register genehmigter Anträge nach §11 Stammzellengesetz; Nationaler Ethikrat (2005), "Wortprotokoll - Niederschrift über den öffentlichen Teil der Sitzung am 25. August 2005", p. 7.
[61] Almeida-Porada, Porada, et al. (2004), "Formation of human hepatocytes by human hematopoietic stem cells in sheep", Blood, **104**(8).

grow human organs or tissues for transplantation might be very promising.[62] Zanjani's group also did similar work on the heart.[63]

Using even less developed recipients, scientists have transplanted human cells into animal embryos. Brüstle, Choudari and Karram, for example, created rats with chimeric brains by transplanting fetal human neural progenitor cells into embryonic rats in 1998.[64] This resulted in extensive integration of the human progenitor cells in the rats' brains, which were killed and examined after one to seven weeks, but not – as opposed to the behavioural transfer in the quail-chick chimeras described on p. 11 – in change of behaviour.[65] For researchers, it is highly interesting to see how human neural cells migrate and develop in a living organ, that is, an animal brain, and how they respond to the multiple developmental cues they are given by the host brain in order to be integrated in the cell structure. In a similar experiment, scientists of the University of Jerusalem implanted human embryonic stem cells in chick embryos in 2002, summing up:

> *"Our results show that human ES cells transplanted in ovo survive, divide, differentiate, and integrate with host tissues and that the host embryonic environment may modulate their differentiation. The chick embryo, therefore, may serve as an accessible and unique experimental system for the study of in vivo development of human ES cells."*[66]

In 2005, Fred H. Gage from the Salk Institute in La Jolla, California and Japanese collaborators injected 100000 human embryonic stem cells into the brain of 14-day-old mouse embryos. These chimeras were brought to term and contamination with genetically human neurons in the brains of the resulting mice amounted to 0.1%. Using patch clamping, it was shown that the human neurons inside the mouse brain were actually firing, which can be regarded as proof for (at least limited) function, rather than mere survival, of the neurons.[67] Apart from hopefully furthering fundamental knowledge of human neural development, the experiments are thought to contribute to the future creation of chimera

---

62 Pagán Westphal (2003), "'Humanised' organs can be grown in animals", The New Scientist, 2003/12/17.
63 Airey, Almeida-Porada, et al. (2004), "Human Mesenchymal Stem Cells Form Purkinje Fibers in Fetal Sheep Heart", Circulation, **109**.
64 Brüstle, Choudhary, et al. (1998), "Chimeric brains generated by intraventricular transplantation of fetal human brain cells into embryonic rats", Nature Biotechnology, **16**.
65 Greene, Schill, et al. (2005), "The Working Group on the Criteria for Cell-Based Therapies, John Hopkins University: Moral Issues of Human-Non-Human Primate Neural Grafting", Science, **309**, p. 386.
66 Goldstein, Drukker, et al. (2002), "Integration and differentiation of human embryonic stem cells transplanted to the chick embryo", Developmental Dynamics, **225**.
67 Muotri, Nakashima, et al. (2005), "Development of functional human embryonic stem cell-derived neurons in mouse brain", Proceedings of the National Academy of Sciences, **102**.

models for emulating human neurodegenerative and psychiatric diseases and for assessing the effectiveness of new drugs. Gage's work gained a lot of publicity.[68]

Similarly to Fred Gage, Irving Weissman is a scientist whose actual experiments, as well as possible research scenarios, have stirred up a lot of discussion. Involved in the research of the human lymphoid and hematopoietic system, Weissman helped develop the "SCID-hu mouse" in the 1980s (see p. 5). Experiments that were much more challenging from the bioethicist's standpoint were proposed by Weissman some years ago (but never actually implemented). Because of the apparent ethical import of the experiments he was considering, Weissman contacted Henry Greely of Stanford University Law School in order to find out whether what he was planning could be done ethically. Weissman's scenarios were discussed in 2002 by a working group assembled by Greely, resulting in a report analysing the ethical implications and possible problems of such research. The report remained unpublished, yet Weissman's research plans and the results of the working group were summed up (and updated) in an American Journal of Bioethics target article in 2007.[69] According to this source, Irving Weissman was confronted with the finding of human "brain stem cells" and their successful isolation from human fetuses. At this point, it must have seemed to be a tantalizing prospect to create a mouse model that could accommodate a human neuronal system (or even just some living human neurons): just as the SCID-hu model offers new possibilities of doing research on the immune system, such a "human neuron mouse" would enable research on living, in vivo human neurons that could otherwise not be done. Additionally, in 2003, it had been shown that human brain stem cells can survive, migrate and even connect in the (SCID) mouse brain.[70] So Weissman devised two setups that would go even further. In one scenario, he was planning to use a mouse strain whose cerebellum neurons had the propensity to die off some weeks after birth. The cerebellum is the part of the brain which is otherwise responsible for movement and coordination. Accordingly, the deficient mice show symptoms that closely resemble those of human patients who suffer from Friedrich's Ataxia, i.e. severe motor deficits. Shortly before the expected death of the mouse cerebellum neurons, Weissman would implant human brain stem cells (from aborted human fetuses) into this part of the

[68] Editors of the American Journal Of Bioethics (2005), "Of Mice and Men", Bioethics.net Blog 2005/12/13; Spiegel Online (2005), "Mausgehirn: Menschliche Stammzellen werden zu Neuronen", 2005/12/14; The New York Times (2005), "Trace of Human Stem Cells Put in Unborn Mice Brains", 2005/12/13; Weiss (2005), "Human Brain Cells Are Grown In Mice", Washington Post, 2005/12/13.
[69] Greely, Cho, et al. (2007b), "Thinking About the Human Neuron Mouse", American Journal of Bioethics, **7**(5).
[70] Tamaki, Eckert, et al. (2002), "Engraftment of sorted/expanded human central nervous system stem cells from fetal brain", Journal of Neuroscience Research, **69**.

mouse brain. By looking at the ensuing cerebellum activity, Weissman would then be able to see whether the implanted (human) cells actually functioned in the mouse brain (in this case, the ataxia symptoms of the mice would disappear or be alleviated). The experiment has not been done yet because the mouse strain proved unfit for this specific use.

A second proposed scenario would have made use of an even more deficient strain of mice, in which all neurons die off already during embryonic development. These missing cells would then be substituted by human neurons from brain stem cells. This model would allow for a functioning formation of human neurons on an animal organism substrate (analogously to the SCID-hu mouse which models the human immune system in a mouse organism). Such a model, Weissman hopes, could not only be used for studying the behaviour of brain stem cells, human neurons in general or human neurodegenerative diseases, but also, in the long run, for drug testing regarding agents' influence on living human neurons in an organism (which can hardly be done today because of ethical boundaries regarding experimentation in humans). This experiment has also not been carried out because, so far, a mouse strain with complete neuronal death could not been found. It remains unclear whether Weissman will return to trying to conduct these experiments in the future.[71] The second setup sounds particularly spectacular, but it would be inaccurate to call the resulting chimera a "mouse with a human brain". This is for two reasons: firstly, the brain does not only consist of neurons, but also of Glia cells, the structural cells of the brain which are a necessary substrate for the neurons. Glia cells would remain murine in Weissman's experiment and constitute up to 50% of the brain mass. Secondly, what makes a brain "human" is not the origin of the neurons in it, but rather the way they are assembled, i.e. their architecture. As long as a brain has a clearly murine architecture, in theory, it is not humanized and human attributes will not emerge. There is some scientific agreement regarding this architecture hypothesis, although it has, as Greely et al. point out, "not been tested."[72] But even if we remain sceptical regarding the attribute "human", it is clearly not true that Weissman's "takeover" mouse would have a "100% human" brain, as Jeremy Rifkin claimed in a 2005 article.[73]

Another chimera experiment involving embryonic animal recipients raised eyebrows in 2006: Ali Brinvalou at New York Rockefeller University implanted human embryonic stem cells into mouse blastocysts (i.e. mouse embryos at a very early stage of development,

[71] Greely, Cho, et al. (2007b), "Thinking About the Human Neuron Mouse", American Journal of Bioethics, **7**(5), p. 32.
[72] Ibid., p. 35.
[73] Rifkin (2005), "Are you a man or a mouse?" Guardian, 2005/03/15.

before the usual time of implantation). Brinvalou's team then went a step further and proceeded to implant the chimeric human-mouse blastocyst into the uterus of a mouse in order to test the pluripotency of stem cell lines, which is hard to ascertain otherwise (human blastocysts cannot be used for this "test" for ethical reasons). Brinvalou and his colleagues stated that "Embryonic chimeras generated in this way offer the opportunity to study the behavior of specialized human cell types in a nonhuman animal model."[74] Brivanlou's plans for "human-mouse embryos" received attention and criticism even well before they were actually created. The New York Times' Jamie Shreeve pointed out what he called the "gonad quandary". This problem, he mused, could arise when implanting human stem cells at early stages of development and then letting the resulting adult chimeras breed:

> *"If the experiment really works, the human cells should differentiate into all of the embryo's cell lineages, including the one that eventually forms the animal's reproductive cells. If the mouse were male, some of its sperm might thus be human, and if it were female, some of its eggs might be human eggs. If two such creatures were to mate, there would be a chance that a human embryo could be conceived and begin to grow in a mouse uterus – a sort of Stuart Little scenario, but in reverse and not so cute."*[75]

Brinvalou's plans had also met opposition in a 2002 forum of stem cell researchers, not only because of some scepticism concerning the transferability of results gathered in murine blastocysts to human environments, but also because of general concerns about the "ethical complexity" of such experiments. Some of Brinvalou's colleagues feared that the human-murine embryo would "provoke public disquiet, and could galvanize political opposition to all research involving human embryos."[76]

Recent successes in the field of chimera research have fired the imagination of the public. The visions evoked by Gage's, Weissman's, Brivanlou's and others' experiments are hardly ever utopian. Considering the rapid and complex developments of science regarding interspecies mixtures, some believe that scientists will soon be able and willing to create truly "humanized" chimeras. Such creatures could, hypothetically, be produced with similar methods as the "geep", e.g. by fusing a human and a chimpanzee embryo – which could result in "humanzees" or "chumans", chimeric mixtures of human and chimpanzee. The perceived threat of the "humanized chimera" motivated government advisor and biotech

[74] James, Noggle, et al. (2006), "Contribution of human embryonic stem cells to mouse blastocysts", Developmental Biology, **295**.
[75] Shreeve (2005), "The Other Stem-Cell Debate", The New York Times Magazine, 2005/04/10.
[76] DeWitt (2002), "Biologists Divided over Proposals to create human-mouse embryos", Nature, **420**.

critic Jeremy Rifkin and Stuart Newman, a cell biologist, to file two patent applications for "chimeric embryos and animals containing human cells" in 1997.[77] Rifkin and Newman wanted to keep scientists from creating any kind of mammal-human chimera by not giving out any licenses.[78] Both patents, one covering chimeric mixtures with primates, such as the "humanzee", the other regarding mixtures of human material with other animals, such as the alleged "human-brained mice", were turned down in August 2004 – U.S. law forbids the patenting of anything human, and the proposed patents would have resulted in something "too human", in this sense.[79] Though Rifkin hopes that, now that his applications have been turned down, the apparent lack of patentability will keep stem cell researchers from creating human-animal chimeras,[80] current developments seem to prove him wrong. On the other hand, the degree of humanization Rifkin fears[81] as a consequence of chimera research is nowhere near realistic today: there are no "mice who think like human beings", no mice who beget human beings, no "ideal laboratory research animals" in the form of "humanzees". Contrary to Rifkin's assertions, such scenarios still *are* Science Fiction today – albeit fiction that, some argue, has a chance of becoming reality in our lifetime unless we soon take care of installing rigorous regulation preventing such scenarios.

5. Animal-to-human chimeras

The novel creatures we have looked at so far were characterized by an animal recipient or host into whom human material was artificially introduced. Scientists have also done the reverse, namely introducing genetically nonhuman material, sometimes whole animal organs, into the human organism.

The prospect of using animal organs for substitution of defective human organs is quite promising, since it could solve (or at least reduce) the problem of organ scarcity and thereby prevent many deaths. Unfortunately, researchers of organ xenotransplantation have encountered severe difficulties in the last century. To begin with, immune rejection, which is the central problem of all transplantation ventures, is much stronger when using organs of alien species. Rejection of interspecies transplants cannot be controlled by the

[77] Newman (2002), "The Human Chimera Patent Initiative", Medical Ethics Newsletter (Lahey Clinic), **9**(4).
[78] Bailey (2003), "Shimmering Chimeras - Moving sheepishly toward the biotech future", Reason Magazine, 2003/12/24.
[79] Kittredge (2005), "A Question of Chimeras", The Scientist, **19**(7); Lamb (2005), "A Mix of Mice and Men", Christian Science Monitor, 2005/03/23.
[80] Kittredge (2005), "A Question of Chimeras", The Scientist, **19**(7).
[81] Rifkin (2005), "Are you a man or a mouse?" Guardian, 2005/03/15.

immunosuppressive means used in (human) allotransplants, but using stronger immunosuppression creates intolerable, fatal complications.[82] Another problem is the differing anatomy, size and functionality of animal organs. Although pigs are somewhat anatomically similar to humans, it is not clear whether organs such as the lung could accommodate to the vertical positioning of their human host over long periods of time; also, porcine tissue may react differently to hormones and other substances within the human body in the long term.

Apart from these problems of compatibility, introducing animal organs or tissues into humans increases the risk of zoonoses, i.e. infectious diseases that are transmitted from animals to humans. Some of the most dangerous diseases in humans result from infectious agents mutating and crossing over the species lines, under circumstances of close contact with infected animals – and introducing animal organs or tissue into immunosuppressed human organisms is probably the closest kind of "contact" imaginable. While most microorganisms can be eradicated from the source pigs, porcine endogenous retroviruses (PERVs) are apparently impossible to completely eliminate so far and could result in tumours and immune deficiency in the human host after transplantation.[83] (The potential risks of xenotransplantation will be further discussed in chapter 2, section C.3 below).

Despite the numerous problems it has to face until today, xenotransplantation has a long (and quite interesting) history – for more than a hundred years, the prospect of using animal material to help diseased humans has fascinated researchers.[84] Solid organ xenotransplantation in modern clinical settings dates back to Princeteau, who transplanted parts of rabbit kidneys in a girl in 1905;[85] and to Ernst Unger, who used a monkey kidney for implantation in 1909.[86] Keith Reemtsma's projects of the 1960s,[87] due to advances in immunosuppression techniques, were the starting point for more promising attempts at xenotransplantation. In 1963, he transplanted chimpanzee kidneys into humans – all but one of the fourteen recipients died within two months, one survived for 9 months. A

---

[82] Hammer and Thein (2003),"Xenotransplantation: Medizinische und ethische Fragen", p. 294, in: Oduncu, Schroth, et al. (eds.) Transplantation: Organgewinnung und -allokation.
[83] Denner (2002), "Fortschritte und Risiken bei der Xenotransplantation - Stellungnahme der GfV in Bezug auf Chancen und Risiken der Xenotransplantation."
[84] For introductions to the history of xenotransplantation, see: Reemtsma (1995), "Xenotransplantation: A Historical Perspective", Institute of Laboratory Animal Research Journal **37**(1); Deschamps, FA, et al. (2005), "History of Xenotransplantation", Xenotransplantation, **12**(2).
[85] Princeteau (1905), "Greffe rénale", Journal Medicine de Bordeaux, **26**.
[86] Unger (1910), "Nierentransplantationen", Klinische Wochenschrift, **47**.
[87] Reemtsma, McCracken, et al. (1964), "Heterotransplantation of the kidney: two clinical experiences", Science, **143**; Reemtsma (1969), "Renal heterotransplantation from nonhuman primates to man", Annals of the New York Academy of Sciences, **162**.

human recipient of a chimpanzee heart lived for two hours after transplantation in 1964. In 1984, Bailey succeeded in transplanting a baboon's heart into a newborn ("Baby Fae" lived for three weeks).[88] In 1992, Starzl and colleagues used baboon livers,[89] but the experiments were not very successful, just as Makowka's transplantations of pig liver and heart.[90] Generally speaking, whole organ xenotransplantation, which has been tried over 100 times with diverse organs,[91] has not been successful so far. Because of severe incompatibility problems, whole organ xenotransplantation will probably not catch on until organs can be sufficiently "humanized" via tissue engineering, transgenesis or chimerism.

Transplantation of animal cells and cell clusters (i.e. non-vasculated tissues), on the other hand, has been more successful. Animal (especially frog) skin grafts have been used as temporary adhesive and flexible covering of burn wounds for hundreds of years.[92] Since the 1960s, porcine skin xenografts were a common skin substitute for burn victims.[93] Pig and cow heart valves have been successfully used beginning with Binet's experiments in 1965,[94] resulting in what is today a standard procedure for replacing defective human valves. The animal valves are rendered biologically inert before implantation by a chemical tanning and fixation process and thus do not contain living cells. The same is true for porcine skin xenografts: they are basically dead tissue and are not vasculated during the healing process.[95] Note that therefore, a human being with a bioprosthetic heart valve, just as a burn victim whose wounds are dressed with porcine xenografts, would not qualify as an animal-to-human chimera under definitions of "chimera" that require the use of *live* alien material.

Other methods of xenotransfer did not stand the test of time. In the 1930s, it became therapeutic fashion to introduce live animal cells into the human body in order to generate effects of "revitalisation" – usually understood as pertaining to sexual function. Most famously among these early "endocrinotherapists" became Serge Voronoff, a Russian

---

88 Bailey, Nehlsen-Cannarella, et al. (1985), "Baboon-to-human cardiac xenotransplantation in a neonate", Journal of the American Medical Association, **254**.
89 Starzl, Marchioro, et al. (1964), "Renal heterotransplantation from baboon to man: experience with six cases", Transplantation, **2**.
90 Makowka, Cramer, et al. (1995), "The use of a pig liver xenograft for temporary support of a patient with fulminant hepatic failure", Transplantation **59**.
91 Hammer and Thein (2003),"Xenotransplantation: Medizinische und ethische Fragen", p. 300, in: Oduncu, Schroth, et al. (eds.) Transplantation: Organgewinnung und -allokation.
92 Hattermann (2003), "Risikoabschätzung von porzinen Circoviren in Bezug auf die Xenotransplantation", Fachbereich Veterinärmedizin, p. 14.
93 Demling and DeSanti (2005), "Managing the Burn Wound: Use of Skin Substitutes", Managing the Burn Wound.
94 Binet, Duran, et al. (1965), "Heterologous aortic valve transplantation", Lancet, **2**.
95 Demling and DeSanti (2005), "Managing the Burn Wound: Use of Skin Substitutes", Managing the Burn Wound.

working in Paris, who specialized in testicle grafts from chimpanzees and baboons to men, and of ape ovaries to women. He allegedly performed these procedures in 2000 patients.[96] Voronoff's work seems to have brought "relative success" in some patients – apparently the glands did not trigger massive immune reactions. Still, Voronoff lost all scientific and public reputation. The method of a certain Paul Niehans, who worked in Germany until well into the 1950s, was similarly unconventional: he injected crushed animal cells (usually from the thymus glands of lambs) to "rejuvenate" his patients. More than 30 of them died from severe immune reactions instead.[97] "Revitalisation" therapies involving gland xenografts and injection of live animal cells were never scientifically recognized and systematically studied; it remains unclear whether they ever resulted in animal-to-human chimeras with live animal cells integrating into the human organism.

A more modern, scientifically legitimate use of animal cells for therapeutic means is the external use of pig livers as temporary substitute for a failing human organ, i.e. "extracorporeal xenogeneic liver perfusion", which was first introduced in the 1960s. Today, scientists are testing transgenic porcine livers for perfusion applications, which apparently can work as a successful interim solution before allotransplantation.[98] Again, this technique would commonly not be considered to result in "chimerism" because alien material is not introduced into the body itself. The same applies to the extracorporeal use of bioreactors containing pig cells which are connected to patients with liver failure as temporary substitutes ("bioartificial liver devices" or BAL).[99]

There are some instances of successful transplants of animal tissue that actually lead to live animal-to-human chimerism in the patient. To give some examples from the 90s, scientists have used clusters of fetal porcine islet-like cells in diabetes therapy.[100] Pig cells have survived and produced insulin in the human organism for astounding periods of time, in one documented case, for 9.5 years.[101] Injections of fetal pig neural cells have been used to treat neurodegenerative diseases like Parkinson's and Huntington's – though the treatments

[96] Deschamps, FA, et al. (2005), "History of Xenotransplantation", Xenotransplantation, **12**(2), p. 95.
[97] Kempermann (2008), Neue Zellen braucht der Mensch: Die Stammzellforschung und die Revolution der Medizin, p. 37.
[98] Deschamps, FA, et al. (2005), "History of Xenotransplantation", Xenotransplantation, **12**(2), p. 100.
[99] Allen, Hassanein, et al. (2001), "Advances in Bioartificial Liver Devices", Hepatology, **34**(3).
[100] Andersson, Groth, et al. (1992), "Transplantation of porcine fetal islet-like cell clusters to three diabetic patients", Transplantation Proceedings, **24**.
[101] Elliott, Escobar, et al. (2007), "Live encapsulated porcine islets from a type 1 diabetic patient 9.5 yr after xenotransplantation", Xenotransplantation, **14**(157).

have not turned out to be very successful, alien neural cells survived in the host for prolonged periods of time.[102]

As we can see, animal-to-human chimeras are created exclusively in the adult recipient variety – this is because under most legislation, human embryos and fetuses cannot be subject to chimerism-inducing procedures. Induction of animal-to-human chimerism in adults suffering from degenerative diseases is evidently less controversial. This is not only because the introduction of animal material is justified by medical indications (as opposed to mere "experimentation"), but also because integrated xenografts in adult human recipients only affect discrete functions, rather than spreading within the body, which could be the consequence of xenografting during early stages of human development.

Xenotransplantation is currently at a crossroads. Its possibilities have fascinated researchers for almost one hundred years, yet it has never yielded mainstream applications. The use of animal tissues, maybe even whole solid organs, will probably increase and become more common once transgenesis and tissue engineering techniques are fully developed and animal materials can be manipulated in order to better adapt to transplantation purposes. As mentioned on page 16 above, researchers are already trying to grow biologically human organs in (chimeric) animals.[103] This seems like a promising outlook for transplantation medicine – another possible route is genetic manipulation of animal organs. Specialists of the field estimate that 2010 will see the first promising trials of transplantation of transgenic pig hearts into humans in the U.S.[104] "Humanization" of animal organs by means of genetic engineering is another branch of science where the line between human and nonhuman species is crossed by artificial means; I will look at transgenic "humanized" animals in the next section.

6. Transgenesis

Advances in genetic engineering have enabled scientists not only to interfere with the genetic information of a given species or individual (by "gene splicing") but also to transfer genetic information from one species to another (transgenesis).

---

[102] Deacon, Schumacher, et al. (1997), "Histological evidence of fetal pig neural cell survival after transplantation into a patient with Parkinson's Disease", Nature Medicine, **3**; Jacoby, Lindberg, et al. (1997), "Fetal pig neural cells as a restorative therapy for neurodegenerative disease", Artificial Organs, **21**(11); Fink, Schumacher, et al. (2000), "Porcine xenografts in Parkinson's disease and Huntington's disease patients: preliminary results", Cell Transplant, **9**(2).

[103] Cf. e.g.: Almeida-Porada, Porada, et al. (2004), "Formation of human hepatocytes by human hematopoietic stem cells in sheep", Blood, **104**(8).

[104] Glasmacher (2008), "Wann kann einem Menschen das erste Schweineherz transplantiert werden? Bericht vom 11. Minisymposium Xenotransplantation", idw online, 2008/06/06.

One technique that is commonly used in order to test methods of gene transfer is to introduce certain easily recognizable genes ("reporter genes") into mammals' gene sequences. Gerald Schatten and his team, for example, created the rhesus monkey "ANDi" in 2001.[105] ANDi (the acronym stands for "inserted DNA" read backwards) was manipulated in order to contain the fluorescent protein of a jellyfish (GFP), which results in a green glow of the animal under special lighting. Korean scientists created GFP transgenic pigs in 2006.[106] By using reporter genes like the GFP gene, scientists can make sure the transgenic organism actually expresses the introduced alien information, in preparation for introducing genes that have a more relevant effect (i.e., genes that cause disease). Another possible use of the GFP method could be in stem cell research, because fluorescent stem cells could be much more easily observed and tracked. Transgenesis experimentation is not limited to medical research: in 2000, Chicago artist Eduardo Kac had French scientists manipulate an albino rabbit ("Alba") in order to contain GFP, pronouncing this successful experiment and the ensuing public interest in the animal a "transgenic art project".[107]

a. Human-to-animal transgenesis

Transgenesis is also used to create human-animal interspecifics. One example for such transgenic human-animal interspecifics are animals that "model" or emulate specific human diseases. A relatively advanced way of bringing animal organisms to mimic a human disease is that of introducing certain genetic information – e.g., the gene(s) which triggers a certain disease in humans, or the gene(s) which make an organism susceptible to a certain virus – into animals' organisms in order to study the disease more closely and to be able to test possible therapies. Today, thousands of mouse models of human diseases are available – mice which are or become, by genetic disposition, immunodeficient, cancer-infested, above or below average size, naked, obese, sclerotic, diabetic or have chronic hypertension, cystic fibrosis or deficiencies regarding the production of a certain enzyme or hormone.[108] Often, these dispositions have been created by introducing human-typical genes into the mouse

[105] Chan, Chong, et al. (2001), "Transgenic Monkeys Produced by Retroviral Gene Transfer into Mature Oocytes", Science, **291**(5502).
[106] Hogg (2006), "Taiwan breeds green-glowing pigs", BBC News, 2006/01/12.
[107] Kac (2003), "GFP Bunny", Leonardo, **36**(2).
[108] Petters and Sommer (2000), "Transgenic animals as models for human disease", Transgenic Research, **9**(4-5); Herman (2002), "Mouse Models of Human Disease", Institute of Laboratory Animal Research Journal **43**(2).

organism. Usually this is done by using DNA microinjection or homologous recombination in embryonic stem cells.[109]

b. Animal-to-human transgenesis

The technology enabling scientists to create transgenic animals could be used to modify the genetic setup of human beings, as well. This is what scientists at Cornell University did in 2007: Zev Rosenwaks and his colleagues introduced GFP marker genes into a human blastocyst in order to find out whether the gene would spread over all the developing cells. And, in fact, all the newly developed cells in the embryo glowed.[110] The experiment was carried out on a non-viable embryo with a severe chromosomal deficiency, which was left to develop for only three days. Still, Rosenwaks' research stirred up controversy and was seen as an attempt to introduce "designer babies".[111] The "species-crossing" quality of his manipulation was apparently not seen as the main problem.

Apart from exceptions like Rosenwaks' experiments, transgenesis in human embryos is not a common field of research for scientists (and illegal in many countries). In an exploratory article, Oxford ethicist Julian Savulescu describes some scenarios in which introduction of animal genetic sequences into human genetic code, in his opinion, might not only be justified, but even advisable:

> *"Imagine that scientists discover that some species are resistant to HIV infection and that resistance is genetically encoded. Imagine that it becomes possible to introduce these gene sequences into the human genome in order to confer resistance to HIV. While this is speculative, it is not absurd."*[112]

Savulescu describes similar scenarios not only aimed at defeating diseases, but also concerning "enhancement" of human properties: e.g. transferring animal genes that lead to a longer life span, improved night vision or even to the emergence of new sensory abilities, such as sonar, in human beings.[113] It is unclear whether any of these scenarios will ever be within the bounds of scientific possibility; apart from this restriction, discussion of the moral advisability and implications of such plans would probably concentrate on the

---

[109] Petters and Sommer (2000), "Transgenic animals as models for human disease", Transgenic Research, **9**(4-5), p. 347.
[110] Zaninovica, Haoa, et al. (2007), "Genetic modification of preimplantation embryos and embryonic stem cells (ESC) by recombinant lentiviral vectors: efficient and stable method for creating transgenic embryos and ESC", Fertility and Sterility, **88**(Supplement 1).
[111] Pollack (2008), "Engineering by Scientists on Embryo Stirs Criticism", The New York Times, 2008/05/13.
[112] Savulescu (2003), "Human-Animal Transgenesis and Chimeras Might Be an Expression of Our Humanity", American Journal of Bioethics, **3**(3), p. 22.
[113] Ibid., p. 23.

question under which circumstances enhancement of human beings is morally advisable, and, above all, whether intrusion into the human germ line (which seems, at least under current conditions, to be an irreversible step) is such a good idea, in the first place. Confronted with these issues, the problem of the "species-crossing" quality of transgenic humans would probably take a back seat with bioethicists.

c. Massive human-animal transgenesis

Genetic manipulation across species can involve more than single genes – in the human-to-animal direction, for example, mice have been created that contain almost a complete copy of the human chromosome 21.[114] Critics of genetic manipulation fear that a massive introduction of human genes into animals or vice versa could lead to the scenario Sagoff describes in somewhat sensational tones:

> *"(…) a mad geneticist could produce a transgenic embryo, implant it in a surrogate mother, and bring to term a Caliban that is neither clearly animal nor clearly human."*[115]

Though Sagoff vehemently dismisses this as "too incredible for any but the most lurid cinema",[116] it is not an entirely invalid concern. Joshua Lederberg, geneticist and Nobel Prize laureate, noted as early as 1966 that "organisms whose karyotype is augmented by fragments of the human chromosome set", i.e. human-animal transgenic beings, might be more of an issue in future science than human cloning.[117] Lederberg's prognosis of the likely creation of "subhuman", human-animal beings by scientists was never realized, but the problem of "massive humanization" is still recognized as one. A report of the Academy of Medical Sciences in the UK, issued in 2007, pointed out that

> *"(…) it will be necessary to consider the appropriate conceptual and regulatory framework for transgenic and chimeric animals that contain significant amounts of human genetic material."*[118]

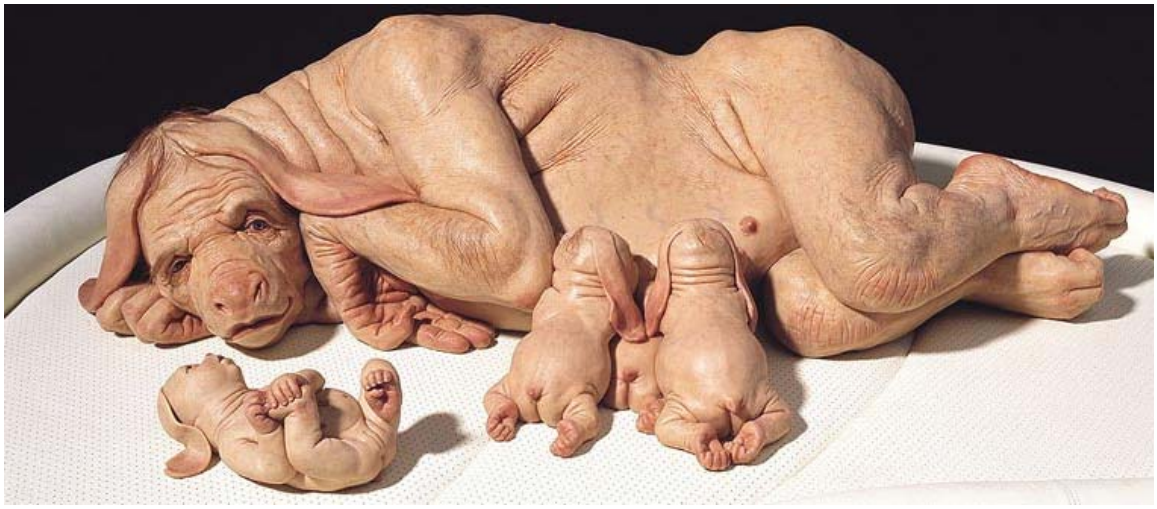
The image of a being that is a seamless fusion of human and animal, i.e. in which human and animal components fade into each other so much that one cannot say where one starts

---

[114] DeWitt (2007), "Animal-human chimeras: Summary of UK Academy of Medical Sciences Report", Nature Reports Stem Cells, **67**.
[115] Sagoff (2003), "Transgenic Chimeras", American Journal of Bioethics, **3**(3).
[116] Ibid.
[117] Lederberg (1966), "Experimental Genetics and Human Evolution", American Naturalist, **100**, p. 531.
[118] Academy of Medical Sciences (2007), "Inter-species embryos - A report by the Academy of Medical Sciences".

and the other ends, is powerful and iconic; maybe even more potent than that of the characteristically motley chimera found in the art of almost all ages and cultures.

Artist Patricia Piccinini has used such imagery in her work for the Australian pavilion at the Venice Biennale 2002 ("We are Family").[119] Among other exhibits, one especially striking live-sized sculpture titled "The Young Family" depicts a mother-creature – an eerily hyperrealistic mixture of human and pig – which idyllically suckles three demonstrably cute, pinkish human-pig babies (see Picture 4).



Picture 4: Patricia Piccinini, "The Young Family", sculpture created for Venice Biennale 2003

Piccinini's sculptures are evidently not realistic portrayals of what is done in today's genetics labs, but they tap deeply into the dream-like images terms like "transgenesis" conjure up in our minds. Current biotechnology has inspired Piccinini since the beginning of the 90s (when she worked on what she called "The Mutant Genome Project"); the catalogue essay accompanying the "human pig family" duly identifies Piccinini's creations as "transgenic".[120]

The ethical implications of "humanizing" animals (or "animalizing" humans) will be discussed later. The degree up to which transgenesis could actually lead to animals exhibiting human properties (or vice versa) remains unknown. Although popular culture sees genetic engineering as a singularly powerful, near-magical device – re-shuffling species seemingly without difficulty, with the help of all-determining, easily transferred genes – the reality of transgenic beings, as exemplified by the scenarios I described above, is considerably less flashy.

---

[119] Piccinini (2003), "We are Family", Sculpture in The Young Family, Australia Pavilion for Venice Biennale 2003.
[120] Michael (2003), "We Are Family (Catalogue Essay)."

The important point to take from this section is that not only chimerism but also transgenesis can cause creatures to stand between species lines. Transgenic animals like the ones described in this chapter are "interspecific" in the sense that their organisms express not only their own species-typical DNA, but also DNA that is typical for alien species. Mark Sagoff uses the term "chimera" in a somewhat confusing way when calling transgenic mice – such as the "Harvard OncoMouse", a mouse strain that contains a human-typical cancer-gene[121] –"transgenic chimeras" and implying they are "just [mice] with a few human cells."[122] Transgenic animals like the OncoMouse, "ANDi", or "Alba" are not chimeric, that is to say they do not contain "genetically human" cells, at all. Rather, they express one or several human-typical gene sequences in all or some of their cells.

7. Human-animal hybrids

We have, so far, looked at interspecifics that are a human-animal mixture on the cellular level (chimeras) and on the genetic level (transgenic organisms). But an interspecific mixture can also take place on a level that is, one could say, relatively "natural": on the level of egg and sperm (gametes).

A mixture of this kind between humans and animals is conceivable – even without highly complex means of contemporary biotech – regarding closely related species, i.e. other primates like chimpanzees, gorillas or orang-utans.

It is a little-known fact that the renowned Russian biologist and artificial insemination specialist Ilya Ivanov tried, with great effort and many supporters, to create human-ape-hybrids in the 1920s.[123] In a mission to Africa, supported by the Russian government, the Academy of Science and the Institut Pasteur in Paris, Ivanov went about this strange project by artificially fertilizing chimpanzee females with human sperm – without success, but this could just as well be due to the fact that the conditions under which the inseminations were carried out were quite adverse and that thus, in effect, no more than three attempts at artificial insemination were undertaken.[124] Back in Russia, Ivanov even tried to realise long-held plans for inseminating woman volunteers with orang-utan sperm

[121] For an example of this effect of transgenesis, see Leder, Kuo, et al. (1990), "v-Ha-ras transgene abrogates the initiation step in mouse skin tumorigenesis: effects of phorbol esters and retinoic acid", Proceedings of the National Academy of Sciences, **97**.
[122] Sagoff (2003), "Transgenic Chimeras", American Journal of Bioethics, **3**(3).
[123] For an extensive discussion of Ivanov's hybridisation experiments and their political, historical and social significance, see Rossiianov (2002), "Beyond Species: Il'ya Ivanov and His Experiments on Cross-Breeding Humans with Anthropoid Apes", Science in Context, **15**(02).
[124] Ibid., p. 297f.

– attempts to inseminate African women (without their knowledge and consent) had, fortunately, not worked out. Since Ivanov fell from grace with the Bolsheviks and ended up in a gulag, such experiments never took place.

Ivanov's curiosity concerning human-ape hybrids was not an isolated case: several biologists of his time wanted to try human-animal hybridization, mainly because of a strong interest in discovering human and other primates' phylogeny. Rossiianov, in his meticulously researched article on Ivanov's crossbreeding experiment, mentions Hermann Moens and Oscar Hermann Rohleder in this context.[125] Others locate the budding scientific interest in human-nonhuman primate hybridization in 19th century France, and cite, among others, Jean-Jacques Rousseau as advocating such experiments.[126] As late as 1971, Charles Remington, a Professor of Biology at Yale University, advocated and predicted human-primate hybridisation experiments, even working out a detailed plan on the raising of a human-chimpanzee hybrid in a primate laboratory,[127] noting dryly that "[t]he experiment's human interest value is too obvious to deserve much justification."[128]

Popular culture, from the 19th century on, seems to be obsessed with the topos of the human-ape hybrid: Gustave Flaubert wrote about Djalioh, product of a slave girl and an orang-utan, in 1837.[129] And fascination with human-ape hybrids remains vivid until today: Wikipedia contributors currently list more than a dozen examples of "human-ape hybrids" in contemporary culture,[130] from "Planet of the Apes" (1968) to Michael Crichton's novel "Next" (2006) – featuring the uncanny human-chimpanzee-hybrid "Dave" who, clean-shaven and well-behaved, certainly has come a long way from Flaubert's infanticidal, rapist Djalioh.

Scientific and popular fascination notwithstanding, the existence of a real human/nonhuman-primate hybrid has never been verified. Although recently, scientists have brought forward the hypothesis that hybridisation between early human and

---

125 Moens and Bernelot (1908), Truth: Experimental Researches about the Descent of Man; Rohleder (1918), Künstliche Zeugung und Anthropogenie.
126 Böhme (2002),"Monster im Schatten der Aufklärung", in: Raulff (ed.) Mensch und Tier. Eine paradoxe Beziehung.
127 Remington (1971),"An Experimental Study of Man's Genetic Relationship to Great Apes, By Means of Interspecific Hybridization", in Katz (ed.): Experimentation with Human Beings.
128 Remington apparently did not see any special ethical or legal problems concerning human-chimpanzee hybrids - they are to be treated just like "any other experimental mammals", not ruling out experimentation and "sacrifice for study"; Remington assumes that "contribution of one-half of the genetical material by *Homo*" would not constitute "legal humanness." Ibid., pp. 463-464.
129 Flaubert (1980),"Quidquid volueris (1837)", in: Jugendwerke. Erste Erzählungen. Aus dem Französischen von Trautgott König. For a discussion, see Böhme (2002),"Monster im Schatten der Aufklärung", in: Raulff (ed.): Mensch und Tier. Eine paradoxe Beziehung.
130 Wikipedia contributors (2008b), "Humanzee - Popular Culture", http://en.wikipedia.org/wiki/Humanzee#Popular_culture.

chimpanzee individuals was an important part of the speciesation process of *Homo sapiens*,[131] it remains unclear whether a hybridisation between modern homo-sapiens and other nonhuman primate could ever result in viable offspring.[132]

What about hybridisation of humans with non-primate animals? It is indisputable that such a mixture could not be arrived at by "natural" reproduction or the relatively low-tech means of artificial insemination, i.e. by a simple mixing of human and nonhuman gametes. There is one example that is, so to speak, on the brink of human-non-primate hybridisation: the "Hamster Test", a screening tool in reproductive medicine. In a "Sperm Penetration Assay", (SPA) human sperm fertility is tested on hamster ova.[133] The sperm quality is assumed to be sufficient if the sperm succeeds in permeating the hamster egg. If this does not work, this indicates that the sperm donor might be infertile. The resulting human-hamster hybrid embryo does not proceed beyond the two cell stage,[134] some state that "fertilization" does not even take place.[135]

Generally speaking, the less closely two species are related, the less likely it is that hybridisation between their gametes works. This definitely rules out that "simple" hybrids between humans and non-primates could develop into viable organisms. Additionally, there are no scientific reasons (excluding simple curiosity) that would make generating an (embryonic) hybrid between human and nonhuman appear sensible. This might change, though – as the 2007 report by the UK Academy of Medical Sciences points out, "given the speed of this field of research, the emergence of scientifically valid reasons in the future cannot be ruled out" and further, "the reasons for banning the creation of hybrid embryos for in vitro experimental use (…) are not clear to us (…)."[136] Abstract and tentative interest

---

131 Patterson, Richter, et al. (2006), "Genetic evidence for complex speciation of humans and chimpanzees", Nature, **441**.

132 One of the few expert opinions I could find regarding this somewhat unpopular question is by Michael Schwibbe of the German Center for Primate Resesarch, Göttingen, who stated that human-ape hybridisation is "conceivable" - ("Es ist denkbar, dass Mensch und Schimpanse gemeinsame Nachfahren zeugen können", sagt der Wissenschaftler, "denn genetisch gesehen ist die Distanz zwischen Esel und Pferd größer als zwischen Mensch und Großem Menschenaffen." Horaczek (2007), "Ein Affe will Rechte", Die Zeit, 2007/03/01.)

133 Kremer and Jager (1990), "The significance of the zona-free hamster oocyte test for the evaluation of male fertility." Fertility and Sterility, **54**(3).

134 Morriss (1998), "Blurred Boundaries", Inquiry, **40**, p. 259.

135, Petersen, et al. (undated), "Hamster Egg Penetration Test" (Patient Information Leaflet of the Utah Center for Reproductive Medicine). The leaflet states that "The human sperm does not fertilize the hamster eggs."

136 The authors of the AMS Report here point out what they regard as an inconsistency: the creation of other human-animal embryonic mixtures, such as "cybrids", transgenic, and chimeric embryos, is permitted in the UK, while true hybrid embryos are expressly banned. The Academy of Medical Sciences (2007), "Inter-species embryos - A report by the Academy of Medical Sciences", pp. 28, 38.

in human-animal hybrid embryos notwithstanding, the creation of fully grown "humanzees" certainly seems not to be what is at issue at the moment.

8. Human-animal cybrids

a. Technicalities and motives

Modern biotechnology offers new possibilities, and new motives, for creating human-animal mixtures. Since there is a growing demand for human embryonic stem cells, scientists are trying to find ways of easily obtaining such cells, or cell types with similar properties. An ideal stem cell source would be one that does not rely on human embryos or gametes which are hard to get hold of, and whose use can cause ethical concern. One alternative could be that of "reverting" human cells to embryonic cells by transferring a human cell nucleus to the enucleated egg of an animal. This technique – somatic nuclear transfer – is better known as "cloning": the creators of Dolly the sheep transferred the nucleus of an adult sheep cell into an enucleated sheep ovum which was reimplanted and brought to term. The clone has exactly the same genetic setup as the donor from whom the nucleus has been obtained. The enucleated cell only keeps some of its DNA in its mitochondriae (i.e. organelles that serve as "cellular power plant"). When this method is used on egg and nucleus of differing species, the resulting entity is called "nucleo-cytoplasmic hybrid" or "cybrid", for short.

Even among experts, there are differing views on how "cybrids" should be classified. Although they do not stem from different zygotes, some experts classify them as chimeras because they exhibit a "genetic mix" of differing mitochondrial and nuclear DNA.[137] Journalists often refer to them simply as "hybrid embryos". At the opening conference of CHIMBRIDS (an EU project on chimeras and other interspecifics), one expert declared cybrids "hybrids" while another classified them as "chimeras".[138] Cybrids are certainly not "true" hybrids, since their production does not involve the fusion of gametes of different species – hybrids are usually understood as products of sexual procreation.[139] As a matter of accuracy, cybrids created of human nuclei and animal eggs should be regarded as interspecifics *sui generis*.

---

[137] Jens Reich: "Auch das sind Chimären, und zwar, genetisch gesehen, mindestens wegen des Mitochondrienbesatzes. (…) Man beobachtet dabei einen zwischenartlichen Chimärismus." Nationaler Ethikrat (2005), "Wortprotokoll - Niederschrift über den öffentlichen Teil der Sitzung am 25. August 2005", p. 6.

[138] Weschka (2006), "Protocol of the CHIMBRIDS Opening Conference on 11/12 March 2006", p. 2.

[139] Jens Reich: "Der Hybrid ist ein einheitliches Lebewesen und er ist geschlechtlich entstanden, gezeugt worden."- see Nationaler Ethikrat (2005), "Wortprotokoll - Niederschrift über den öffentlichen Teil der Sitzung am 25. August 2005", p. 4.

The history of cybrid creation began in 1996, when Jose Cibelli and colleagues tried to apply the somatic nuclear transfer technique to cow eggs and human nuclei. The team claimed to have created cybrids, but the success of this experiment is doubted.[140] Some years later, a team of Chinese scientists led by Hui Zhen Sheng successfully employed the same approach to fuse human somatic nuclei with enucleated rabbit eggs.[141] As expected, the resulting embryos' DNA is predominantly human; with the exception of DNA which stems from the rabbit egg's mitochondriae. Resulting incompatibilities notably diminish the potential of this cybrid to grow into a viable organism – it remains unclear whether human-animal hybrids could, in theory, ever develop into an adult creature.[142] For the experiments at issue, this question is irrelevant, since the created cells are not expected to survive after the blastocyst stage. At that point, the inner cell mass of the embryo is removed to harvest the resulting nuclear transfer embryonic stem cells, or rather "stem-like" cells which are hoped to have the same (pluripotent) properties as stem cells created without the involvement of somatic cells. The Sheng group showed that the harvested "stem-like" cells are indeed capable of differentiation and self-renewal.[143]

b. Cybrids in the UK

From the perspective of bioethics, the question of human-animal cybrids was (alongside with Weissman's proposals) one of the most important condensation seeds of debate. The renewed bioethical interest in cybrids and interspecifics in general was triggered by the plans of several UK researchers to create human-animal cybrids. Lyle Armstrong of Newcastle University wanted to use cow eggs to develop stem cells for the treatment of diabetes and spinal paralysis; Stephen Minger, of King's College London, had plans to use human-cow cybrids to study degenerative neurological diseases, i.e. Parkinson's and Alzheimer's.[144] Chris Shaw of the Institute of Psychiatry, London, said he would need human-animal cybrids to study motor neuron disease. All three applied to the British

---

[140] McGee (1998), "Could the embryo be a new species?" Bioethics on MSNBC, 1998/11/13; Wade (1998), "Researchers Claim Embryonic Cell Mix Of Human and Cow", The New York Times, 1998/11/12; Weiss (2003), "Cloning Yields Human-Rabbit Hybrid Embryo", Washington Post, 2003/08/14.

[141] Dennis (2002), "Stem cells rise in the East", Nature, **419**; Chen, He, et al. (2003), "Embryonic stem cells generated by nuclear transfer of human somatic nuclei into rabbit oocytes", Cell Research, **13**(4); Weiss (2003), "Cloning Yields Human-Rabbit Hybrid Embryo", Washington Post, 2003/08/14; Cnn.com (2006), "Scientists seek Rabbit-Human Embryo", 2006/01/13; Highfield (2006), "Stem cell researchers plan to create rabbit-human embryos", news.telegraph.co.uk, 2006/02/13.

[142] For example, András Dinnyés, a Hungarian scientist working on the method of nuclear transfer in animal reproduction, stated that "the basic question whether a rabbit-human nuclear transfer embryo could develop into a human being remains unsolved." Weschka (2006), "Protocol of the CHIMBRIDS Opening Conference on 11/12 March 2006", p. 3.

[143] Chen, He, et al. (2003), "Embryonic stem cells generated by nuclear transfer of human somatic nuclei into rabbit oocytes", Cell Research, **13**(4), p. 263.

[144] Batty (2007), "Hybrid embryos get go-ahead", Guardian, 2007/05/17.

institution responsible for issuing licenses for research involving human embryos, the Human Fertilisation and Embryology Authority (HFEA) in November 2006.

A month later, a government white paper proposal was revealed which stood in clear opposition to the researchers' plans. This draft would have outlawed all kinds of interspecific beings in the UK.[145] Many scientists and patient organisations united in protest against these plans.[146]

The HFEA decided that before granting any licenses, a general licensing policy on creation of human-animal interspecifics should be agreed upon – a three-month process of public consultation followed. It was found that although initially most people were opposed to all interspecific beings, after some information and debate, a considerable majority of participants were in favour of creating cybrids. A quarter of the participants remained opposed to this type of research (scepticism remained much higher regarding chimeric embryos and true hybrids).[147]

A report of the House of Commons Science and Technology Committee opposed general legislative prohibition of cybrids and demanded "a greater role for the regulator within a broad permissive framework set out by the parliament" (Phil Willis, MP). The committee was also in favour of a free vote on the issue of interspecific research regulation.[148]

In a complete reversal from their previous position, the UK government issued a new, permissive draft bill in May 2007. This would allow for transgenesis, the creation of chimeras and cybrids involving human material, as long as the entities created would be destroyed after 14 days, and as long as no true human-animal hybrids were created.[149] Several ministers were opposed to this new bill – they were particularly critical of cybrid creation.[150] In September 2007, the HFEA announced that their consultation had not found fundamental arguments against cybrid experiments, and that specific committees would now look at the three license applications. Public reaction was immediate, worldwide, and mostly negative: e.g. several German church officials and politicians denounced the UK cybrid plans.[151]

---

[145] Department of Health (2006), "Review of the Human Fertilisation and Embryology Act: proposals for revised legislation (including establishment of the Regulatory Authority for Tissue and Embryos)".
[146] Janositz and Lüdemann (2007), "Britische Chimären", Tagesspiegel, 2007/09/06.
[147] HFEA (2007), "Hybrids and Chimeras - A report on the findings of the consultation."
[148] Kahn (2007), "Leave UK hybrid embryo decisions to experts: panel", reuters.com, 2007/07/31.
[149] Department of Health (2007), "Human Tissues and Embryo (Draft) Bill."
[150] Hinsliff (2008), "Brown faces deepening revolt over embryo bill", The Observer, 2008/03/23.
[151] Die Welt (2007), "Die Mensch-Tier-Embryonen bleiben umstritten", 2007/09/07.

In January 2008, Lyle Armstrong of Newcastle University and Stephen Minger from King's College were granted HFEA licenses for the creation of cybrids, even before the House of Commons had decided on the government's proposal. [152] The Newcastle team announced the successful creation of human-cow cybrids in April 2008.[153]

In May 2008, a strong majority of the House of Commons, in a free vote, decided in favour of the new permissive embryo bill, against criticism from several ministers and the Catholic Church.[154]

At the time of writing (July 2008), the HFEA has granted another one year license to scientists at Warwick Medical School who plan to create human-pig cybrid embryos in order to obtain stem cells which are then supposed to be differentiated into heart cells if the experiment works out as planned. To improve the cybrid procedure, the researchers around Justin St. John are planning to destroy all remaining (mitrochondrial) pig DNA in the cybrids: the resulting cells are supposed to be "the world's first human stem cells from embryos that are part human and part animal."[155] Removal of the mitochondrial pig DNA is supposed to improve the functions of the resulting cells. The ultimate goal is to create a human stem cell line with which to study cardiomyopathy (heart muscle disease).

What is particularly remarkable about the cybrid debate is that these entities seem extremely hard to grasp for laymen and even scientists. As we have seen, cytoplasmic hybrids defy old-fashioned modes of classification as "chimera" or "hybrid", but also unambiguous categorization as "human" or "nonhuman". They are also hard to grasp in a simpler sense: the entities involved are not accessible and well-known objects like human or animal bodies, but rather elusive, tiny microscopic cells. As John Burn, the head of the human genetics institute at Newcastle University put it, "We're talking about something that looks like sago under the microscope."[156] Maybe this elusiveness made the debate around cybrids so fervent and fruitful: as objects that are not easily imaginable, cybrids are the perfect blank screen on which intuitions about "human-animal mixing" in general can be projected. And so, one side comes up with comparisons like "sago" – implying that the mere idea of restricting such research could only have roots in silly, unjustified superstitions and myths – while the other side imagines something rather like Patricia Piccinini's human-animal abominations, or Frankensteinian procedures carried out on

[152] HFEA (2008), "HFEA Statement on licensing of applications to carry out research using human-animal cytoplasmic hybrid embryos."
[153] Spiegel Online (2008), "Forscher schaffen Hybrid-Embryo aus Mensch und Kuh", 2008/04/02.
[154] Koydl (2008), "Alles bleibt möglich", Süddeutsche Zeitung, 2008/05/21.
[155] Sample (2008), "Hybrid embryos: UK team plans stem cell first", Guardian, 2008/07/01.
[156] Batty (2007), "Hybrid embryos get go-ahead", Guardian, 2007/05/17.

babies, and is understandably up in arms. The intuitions of both sides clash violently and lead to the impression that compromise is impossible. After some consideration it seems that neither of these projections does justice to what is actually happening when cybrids are created. What we can learn from the debate around cybrids in the UK is that stepping back from knee-jerk reactions and analysing what, exactly, it is that makes us oppose (or welcome) the creation of interspecific beings is a necessary step when trying to find consensus on future policies regarding their creation: regarding interspecific beings, things are often not what they seem at first glance.

To come back to the original intent of this chapter, let us once more look at the precise definition of "chimeras" and other interspecific beings, which is the first stepping stone for any serious debate.

C. Definitions

As we have seen, the concept of "chimera" in biology is a plurivalent and complex one. There is no one authoritative definition of what "chimera" means in biology or bioethics. Let us, therefore, have a look at several approaches at defining chimeras before deciding on how to proceed.

Aiming at an all-encompassing taxonomy of chimeras, Henry Greely offers an extremely wide definition of chimeras. Under his definition, a chimera is "a single biological entity that is composed of a mixing of materials from two or more different organisms."[157] This is a suitable formulation for Greely's purpose (namely, giving a very wide taxonomy of interspecifics), but for a fixation of the meaning of "chimera" in bioethics, it seems too wide: after all, any animal (or human) that is a product of sexual reproduction would have to count as a chimera in that sense.

Jens Reich, in an introductory presentation on the subject of chimeras to the "Nationaler Ethikrat", gives a more restricted definition of chimeras as organisms that consist of genetically differing parts.[158] This would rule out organisms which consist of genetically identical parts (i.e. of cells which carry the same genetic fingerprint), and thereby not count usual outcomes of sexual reproduction. Reich's classical definition highlights the puzzle-like

[157] Greely (2003), "Defining Chimeras…and Chimeric Concerns", American Journal of Bioethics, **3**(3).
[158] Nationaler Ethikrat (2005), "Wortprotokoll - Niederschrift über den öffentlichen Teil der Sitzung am 25. August 2005", p. 3. In German, this passage reads: "Die biologische Definition von Chimäre besagt, dass es sich dabei um einen Organismus handelt, der aus unterschiedlichen Zellen oder Geweben oder Organen zusammengesetzt ist, unterschiedlich vor allen Dingen in ihrer genetischen Zusammensetzung."

quality of chimeric organisms on cell-level. Reich adds that chimeric organisms somehow stem from two source organisms, specifically, that chimeric organisms are "tetragametic": their DNA stems from four gametes, i.e. from two zygotes (fertilized eggs). Thus, a standard, general definition of chimera can be given as: "Animal that has two or more different populations of genetically distinct cells that originated in different zygotes."[159]

Some further qualifications or refinements seem advisable when defining "chimera". Firstly, as we have seen above, the hosts used for chimera research are not necessarily complete organisms. It seems advisable to describe the objects involved as "organisms and biological entities", since chimera production starts at the point of fused zygotes or other pre-organismal entities (e.g. cybrids).

Secondly, it might be advisable to restrict the definition of "chimera" to organisms which consist of genetically differing material which is alive. Greely mentions the example of the "man with the wooden leg".[160] When human beings with heart-valve implants of bovine or porcine origin are described as "chimeras", this is misleading: heart-valve implants are biologically dead, and their hosts are not animal-to-human chimeras, just as a man with a wooden prosthesis is not an oak-to-human chimera.

In a similar vein, it is questionable whether the use of extracorporeal bioreactors filled with porcine cells or extracorporeal xenoperfusion with animal organs (see p. 24) constitute instances of animal-to-human chimerism. A key factor of chimerism seems to be the mutual contact and influence of differing sets of cells. Extracorporeal bioprosthetics' mutual contact with the human organism and resulting feedback effects are very limited. In order not to blur the concept of chimera, it seems suitable to exclude extracorporeal and dead material that is brought in contact with the host from constituting chimerism.

Another problematic point that I already mentioned above is that of "transgenic chimerism". I would not find it advisable to subsume transgenic organisms under the concept of "chimera", even if they express DNA that is usually found in other species: such organisms do not exhibit the puzzle-like quality that is typical for chimeras, but are homogenous. The term "chimeric DNA", on the other hand, may be useful for describing genetic material that consists of sequences taken from different organisms.[161]

---

[159] Wikipedia contributors (2008a), "Chimera (genetics)", http://en.wikipedia.org/wiki/Chimera_(genetics).
[160] Greely (2003), "Defining Chimeras…and Chimeric Concerns", American Journal of Bioethics, **3**(3), p. 19.
[161] Karpowicz, Cohen, et al. (2004), "It is ethical to transplant human stem cells into nonhuman embryos", Nature Medicine, **10**(4), p. 331.

For the same reason, I think it would not be prudent to describe nucleo-cytoplasmic hybrids ("cybrids", see p. 32) as "chimeras" – if they would develop further, the resulting organisms would not consist of genetically differing sets of cells, but rather of homogenous cells whose origin from differing organisms and even species would only be evident on the inner-cell level.

Many contributors to the bioethical debate tend to restrict their discussion of ethical issues concerning chimeras to human-to-animal embryonic chimeras.[162] It is probably true that these particular creations raise more, and probably also more complex, ethical questions than, e.g., animal-to-animal adult chimeras. However, I will not employ this restricted use of "chimera", because I believe that it implies that chimeras, as such, are ethically problematic. Equalising "chimera" with "organism whose creation is ethically problematic and whose existence poses ethical problems or confusion" is not advisable because it might lead to ethically problematical non-chimeras being overlooked while ethically unproblematic creatures or experiments are scrutinized just because they involve chimerism. While it is unproblematic to limit discussion within a publication to human-to-animal embryonic chimeras and to call them "chimeras" for brevity's sake, it seems not advisable to extend this limited use of the term "chimera" to general discourse. "Chimera", therefore, should not be equated with "ethically problematic artificial being".

Concerning the definition of "chimera", another approach might be not to settle on one authoritative formula, but rather to point out that several definitions are in use. These definitions may differ, depending on the circumstances they are used in. A single, absolute definition seems not advisable to some: firstly, new types of organisms are created over the years. When sticking to traditional definitions of "chimera", one will have a hard time accommodating new types of beings like cybrids, fused embryos or transgenic beings. Secondly, different fields of expertise have differing requirements regarding the concept of "chimera". Karpowicz, Cohen and van der Kooy, commenting on the meaning of "chimera" and "hybrid" in the context of experimental biology, note that

---

[162] Cf. Bailey (2003), "Shimmering Chimeras - Moving sheepishly toward the biotech future", Reason Magazine, 2003/12/24; Karpowicz (2003), "In Defense of Stem Cell Chimeras: A Response to 'Crossing Species Boundaries'", American Journal of Bioethics, **3**(3); Robert and Baylis (2003), "Crossing Species Boundaries", American Journal of Bioethics, **3**(3); Karpowicz, Cohen, et al. (2005), "Developing human-nonhuman chimeras in human stem cell research: Ethical issues and boundaries", Kennedy Institute of Ethics Journal, **15**(2), p. 110; Max-Planck-Institut für biophysikalische Chemie (2005), "Richtigstellung und Stellungnahme - Informationen zum SPIEGEL-Artikel 'Der Mensch im Tier' und zur dpa-Meldung 'Nationaler Ethikrat will sich mit Chimären-Experimenten befassen'"; Sherringham (2008), "Mice, Men, and Monsters: Opposition To Chimera Research And The Scope Of Federal Regulation", California Law Review, **96**(765).

> *"For molecular biologists, chimeric DNA refers to sequences derived from two sources and combined into one; for cell biologists, there are nucleocytoplasmic hybrids involving somatic cell nuclear transfers (cloning) within or between species; for embryonologists, chimeras are prenatal combinations of cells derived from different zygotes, either intraspecies or interspecies; for geneticists, there are interspecies genetic hybrids such as the mule; and finally, there are interspecies xenografts of tissue into postnatal hosts."*[163]

We can see that the terms "chimera", "chimeric" and "hybrid" are sometimes used to denote not only creatures that are literal "chimeras", corresponding to the textbook definition given above, but to all kinds of biological entities that are a "mix" in the widest sense. When I use the term chimera, this will be in the – quite restrictive and biologically exact – meaning:

> ***Chimera*** *(Def.): Biological entity composed of genetically distinct living cellular material stemming from two or more different zygotes.*

This definition does not imply the artificiality of chimeras – it includes not only man-made novel creatures, but also natural occurrences like microchimerism. The definition also includes non-organisms (such as fused zygotes). It excludes some artificial interspecifics, such as cybrids and natural non-chimeric mixtures, such as hybrids, as well as creatures with transgenic ("chimeric") DNA.

Even if we have not yet discussed the specific ethical concerns regarding (some) chimeras and other novel creatures, it already seems quite clear that being a chimera as such does not make any ethical difference whatsoever. As Henry Greely puts it:

> *"As an ethical concern, chimerism per se might itself be 'an unfounded conception.' The fact that something is or isn't a chimera does not in itself raise ethical concerns. A new type of organism might raise concerns (…) whether or not it meets anyone's definition of chimera."*[164]

As I have mentioned above, many types of chimeras are plainly uninteresting for ethicists – take the odd bovine twin chimera or human microchimerism. On the other hand, and this is what Greely hints at, many non-chimeric interspecifics seem to be highly controversial – out of the same or very similar reasons that make some chimeras controversial.

---

163 Karpowicz, Cohen, et al. (2004), "It is ethical to transplant human stem cells into nonhuman embryos", Nature Medicine, **10**(4).
164 Greely (2003), "Defining Chimeras…and Chimeric Concerns", American Journal of Bioethics, **3**(3), pp. 19,20.

My further discussion will, therefore, usually not focus on "chimeras" or other tightly defined types of interspecies mixtures such as cybrids or transgenic beings, but rather refer to "interspecifics", defined as follows:

> ***Interspecific*** *(Def.): any organism or living biological entity which is the product of mixing of species, including but not limited to products of inter-species chimerism (as a result of embryonic injection, mixing, xenografting or xenotransplantation), products of inter-species transgenesis, products of inter-species hybridisation (sexual procreation between animals of different species), and inter-species nucleo-cytoplasmic hybrids ("cybrids").*

Usually, the interspecifics at issue in the bioethical context will be between human and nonhuman species (i.e. human-animal interspecifics). Similarly inclusive concepts are used, e.g., by David Castle, who also employs the term "human-nonhuman interspecifics (HNHIs)"[165] and by Jason Scott Robert, who refers to "part-human entities".[166]

In chapter 2 below I will spell out what types of ethical concerns are or could be caused by the creation or existence of chimeras and other interspecifics. At this point, it should become much clearer what kinds of interspecifics could be ethically problematic, in what sense, and why. Before this discussion of ethical implications, let me give a very short overview of the legal situation of chimera and other interspecific research involving human material.

---

[165] Castle (2003), "Hopes against Hopeful Monsters", American Journal of Bioethics, **3**(3).
[166] Robert (2006), "The science and ethics of making part-human chimeras in stem cell biology", Journal of the Federation of American Societies for Experimental Biology, **20** p. 839.

D. Excursus: Legal situation of human-animal interspecific research

A short overview of the regulatory situation regarding research with human-animal chimeras and other mixtures seems to be advisable for two reasons: firstly, the regulatory conditions surrounding this research influence what happens in research labs, determine where interspecific research will flourish or deteriorate, and what conditions researchers face concerning funding, licensing procedures and legal risks, and thereby helps us to gain deeper understanding of the situation of chimeric/interspecific research around the world which I presented in the previous sections. Secondly, an overview of the regulatory background and political positions on the subject indicates commonly held public or political attitudes towards interspecific research, which will be interesting in respect to chapter 2 below.

The legal and regulatory situation of human-animal interspecific research is closely tied to that of research with human stem cells, particularly human embryonic stem cells (hESC), since most chimeric/hybrid/cybrid research today involves the use of such materials – many instances of modern human-animal chimera research can be regarded as special cases of stem cell research. The status of hESC research is unclear in most countries: an international legislation database of the International Society for Stem Cell Research (ISSCR) lists policies, legislation and pending legislation regarding hESC research around the world, and shows that most countries do not have any explicit legislation of such research (much less on chimeras or cybrids produced involving hESC or other stem cells), be it restrictive or liberal.[167] There are also frequent changes in stem cell policies at the moment. The regulations that are in place on the national level regarding hESC research and chimeras vary wildly.

This underregulation, frequent change, and underlying discord about stem cell policies explains why there is no international legislation on this topic and much less on that of chimeras: international treaties claim a general "right to life" (e.g. The Universal Declaration of Human Rights, Art. 3), but it remains unclear from what point on human embryos are granted this right. Direct or uncontroversial conclusions in regard to interspecific human-nonhuman experimentation do not follow from a "right to life". Disagreement about the ethical permissibility of hESC (and chimera/cybrid) research is also common within the European Union and its member states. For example, the

[167] ISSCR (2007), "International Legislation on Human Embryonic Stem Cell Research."

European Parliament planned legislation on "Advanced Therapies" in 2007 which was meant to regulate and simplify the central licensing of medication and new therapies, among them hESC and chimera/cybrid-based methods. In the end, controversy concerning questions of authority and discord about ethical considerations led to an exclusion of all embryo related research (i.e. chimera and cybrid research, as well) from this EU regulation.[168] The European Union decided in 2006 to offer funding for stem cell research[169] (contrary to a German initiative for an EU-wide ban on stem cell research). Yet, definitive European legislation on permissibility of hESC research or chimera research seems unlikely to come to pass in the near future, because opinions are divided internationally and even within political camps.

Regarding regulations below the legislative level, there are international guidelines concerning hESC research, issued by the ISSCR (International Society for Stem Cell Research) in 2006.[170] These largely procedural guidelines recommend that "review, approval and ongoing monitoring by a special oversight mechanism" (SCRO – Stem Cell Research Oversight) should be maintained whenever "human totipotent or pluripotent cells" are incorporated into animal chimeras (8.1), and that in this process, "ethical permissibility and justification" should be factored in. At least some adult chimerism experiments would be exempt from this full-scale process (category 1 of the ISSCR guidelines entails "routine and standard research practice" such as assays of human tumour formation in SCID mice, 10.1), while embryonic chimeras would fall under full SCRO procedure (amount, point of introduction of cells, species, and affected organ would have to be considered here). Research that "should not be pursued" under ISSCR guidelines includes the cultivation of manipulated human embryos, or part-human structures with "human organismal potential" past 14 days or until formation of the primitive streak;[171] implantation of animal-human chimeras into an uterus; and breeding of human germline chimeras. The ISSCR guidelines are supposed to be incorporated by journal editors, who should prevent the publication of research that does not meet ISSCR standards. The ISSCR committee forum has also issued a report on "Ethical Standards for Human-to-Animal Chimera Experiments in Stem Cell

[168] Biotechnologie.de (2007), "EU-Parlament: Weg frei für die zentrale Zulassung neuartiger Therapien", 2007/05/22.
[169] Council of the European Union (2006), "Press Release: 2747th Council Meeting Competitiveness - (Internal Market, Industry and Research) - 11554/06 (Presse 215)", p. 7.
[170] ISSCR (2006), "Guidelines for the Conduct of Human Embryonic Stem Cell Research."
[171] This development marks the change from blastocyst stage to gastrulation, where the germ layers are established and the basic building plan of the organism is laid out.

research" which proposes more detailed standards for SCRO committees.[172] It is unclear to what extent scientists do actually follow these non-binding international guidelines or the ethical standards proposed.

Regarding regulation on the national level, in the U.S.A., the potential problematicity of human-animal interspecific research was already addressed by President Bill Clinton, who referred the "troubling" matter of "mingling of human and nonhuman species" to the National Bioethics Advisory Council in 1984.[173] No legislative action was undertaken back then. The U.S. National Academies of Sciences (NAS) issued guidelines for conducting human embryonic stem cell research in 2005,[174] which also include guidance for interspecific research: an additional SCRO review process is required for research involving introduction of hESC into animals. Also, patterns of integration into the animal organism should be closely watched – special attention should be paid to neural chimeras. NAS Guidelines prohibit the introduction of hESC into nonhuman primate blastocysts,[175] the introduction of human and nonhuman hESC into human blastocysts, breeding with human-to-animal chimeras and, finally, the cultivation of human-animal products of hESC research past 14 days/formation of the primitive streak. Although stem cell researchers claim to abide to the NAS guidelines, evidence for this claim is hard to come by.[176] State regulations in the U.S. concerning hESC and chimeric/cybrid research vary. California, for example, has adopted the NAS guidelines as state law. On the federal level, there is hardly any legislation regarding hESC research, although federal funding by the National Institutes of Health is limited to certain types of hESC research – expressly excluding at least some kinds of interspecific research.[177] A federal legislative initiative by four conservative U.S. senators, led by Sam Brownback, was started in 2005 to ban "human chimeras" altogether

[172] Hyun, Taylor, et al. (2007), "ISSCR: Committee Forum - Ethical Standards for Human-to-Animal Chimera Experiments in Stem Cell Research", Cell Stem Cell, **1**(2).
[173] National Bioethics Advisory Council (1998), "Discussion Transcript: President Clinton's Request Re:Embryonic Stem Cells ."
[174] Committee on Guidelines for Human Embryonic Stem Cell Research (2005), "Guidelines for Human Embryonic Stem Cell Research", National Research Council.
[175] Francoise Baylis discusses this specific policy in Baylis and Fenton (2007), "Chimera Research and Stem Cell Therapies for Human Neurodegenerative Disorders", Cambridge Quarterly of Healthcare Ethics, **16**(2).
[176] Greely, Cho, et al. (2007b), "Thinking About the Human Neuron Mouse", American Journal of Bioethics, **7**(5), p. 30.
[177] National Institutes of Health (2000), "National Institutes of Health Guidelines for Research Using Human Pluripotent Stem Cells", Federal Register, **65**(166), p. 51981. Research that is ineligible for NIH funding includes creation of human-animal hybrids and experiments involving cybrids (III D., E.) as well as " Research in which human pluripotent stem cells are combined with an animal embryo" (III F..), i.e. embryonic chimera creation.

("Human Chimera Prohibition Act").[178] The (unsuccessful) initiative was supported by President Bush, who openly criticized the creation of "animal-human hybrids" in his 2006 State of The Union Address as one of the "most egregious abuses of medical research."[179] A new, similar bill to "prohibit human-animal hybrids" was introduced in November 2007 by Brownback and 13 supporters, among them Republican presidential candidate John McCain.[180] The misleadingly named bill would apparently not only outlaw the creation of human-animal hybrids, but also the creation of cybrids and at least some human-animal chimeras: germline chimeras, nonhumans "with human brains" and, somewhat vaguely, "human embryo[s] into which a non-human cell or cells (or the component parts thereof) have been introduced to render the embryo's membership in the species *Homo sapiens* uncertain."[181]

In other leading research countries, the status of chimera research is under similar public and political scrutiny – in Great Britain, the possible ethical import of human-animal hybridisation was first mentioned in a 1984 report of the committee of Enquiry into Human Fertilisation and Embryology ("Warnock Report"), in the context of the "hamster egg test" mentioned in section B.7 above.[182] HESC research, in general, is allowed in the United Kingdom under quite liberal conditions (i.e. even creation of hESC for research purposes is allowed). As noted above, the Human Fertilisation and Embryology Authority, which is concerned with issuing the necessary licenses for such research, held a public consultation on human-animal chimeras and cybrids in 2007. The consultation's report came to the conclusion that cybrid experimentation could in principle be licensed in cases approved by the HFEA.[183] Three projects involving cybrids by UK researchers have been approved in November 2007, January 2008, and July 2008 respectively.[184] The legislation introduced in May 2008 is quite permissive, allowing for the creation of chimeras, cybrids,

---

[178] Congress of the United States of America (2005), "Human Chimera Prohibition Act (S. 659)", Introduced by Sam Brownback; Sherringham (2008), "Mice, Men, and Monsters: Opposition To Chimera Research And The Scope Of Federal Regulation", California Law Review, **96**(765), p. 13ff.

[179] "Tonight I ask you to pass legislation to prohibit the most egregious abuses of medical research: human cloning in all its forms, creating or implanting embryos for experiments, creating human-animal hybrids, and buying, selling, or patenting human embryos. Human life is a gift from our Creator – and that gift should never be discarded, devalued or put up for sale." Bush (2006), "State Of The Union Address By The President."

[180] Congress of the United States of America (2007), "Human-Animal Hybrid Prohibition Act of 2007 (S. 2358)", Introduced by Sam Brownback.

[181] §1131 (1), Ibid.

[182] Warnock (1985), A Question of Life: The Warnock Report, pp. 70,71.

[183] HFEA (2007), "Hybrids and Chimeras - A report on the findings of the consultation."

[184] HFEA (2008), "HFEA Statement on licensing of applications to carry out research using human-animal cytoplasmic hybrid embryos"; Sample (2008), "Hybrid embryos: UK team plans stem cell first", Guardian, 2008/07/01.

and transgenesis using human embryos.[185] What the new UK embryo bill prohibits are true human-animal hybrids and, similarly to NAS guidelines, implantation of manipulated embryos as well as cultivation for longer than a fortnight. All in all, Great Britain seems to remain on a relatively liberal course regarding interspecific research.

This is in contrast to the situation in Germany, where hESC research in general is handled rather restrictively. The Stammzellengesetz of 2002 used the cut-off date of January 1st, 2002 to determine which imported stem cell lines may be used; this legislation was reviewed in 2008, and the cut-off date was moved to May 1st, 2007. The parliamentary debates revealed deeply divided opinions on hESC research, ranging from demands to lift import restrictions to requests that hESC research should be stopped altogether. Accordingly, the future situation regarding chimeric research involving hESC remains quite unclear. The German licensing authority (Zentrale Ethikkommission für Stammzellenforschung, ZES) has, in fact, granted chimera experimentation with non-embryonic human stem cells in the past.[186] German law, while it may be hard on hESC researchers, does not prohibit the creation of embryonic chimeras – as long as the research does not involve the use of a human embryo, or totipotent parts of it, but disparate pluripotent embryonic cells.[187] German law does also, in principle, allow the creation of human-animal cybrids, experts on medical law confirm.[188] The legislative situation in Germany regarding interspecific research can currently be described as open-ended; hESC research as such is the problem dominating public and political discourse. Germany shows remarkably restrictive tendencies in this context compared, e.g., to the U.S. or UK. This indicates that extensive legislative restriction of chimera/cybrid research might be an issue in the future.

Apart from the U.S., the UK and Germany (which I have picked out as examples, since they are especially important research nations), the legislative/regulatory stances countries take regarding hESC and chimera research vary wildly. Some nations have decided to take a

---

185 Department of Health (2007), "Human Tissues and Embryo (Draft) Bill."
186 Zentrale Ethikkommission für Stammzellenforschung (2005), "Stellungnahme zur öffentlichen Debatte über die Chimären-Problematik."
187 The German licensing autority ZES has composed an explanatory interpretation of the Embryonenschutzgesetz specifically in regard to stem cell research - see Zentrale Ethikkommission für Stammzellenforschung (2007), "Stellungnahme der Zentralen Ethikkommission zur Stammzellenforschung: Die einfachgesetzliche Lage: Das Embryonenschutzgesetz."
188 Jochen Taupitz on the lawfulness of cybrid creation: "Derartige Experimente seien sogar in Deutschland möglich. Denn das Embryonenschutzgesetz sei in dieser Hinsicht lückenhaft. Es verbiete zwar die Schaffung von Schimären [sic] unter Verwendung von Embryonen unterschiedlicher Arten. Taupitz: 'In diesem Fall handelt es sich aber nicht um Embryonen, sondern um eine Körper- und eine Eizelle.'" Brüning (2007), "Eine Frage der Mischung", Berliner Zeitung, 2007/09/07.

restrictive position regarding the creation of human-animal interspecifics. For example, Australia, which is permissive regarding hESC research, adopted the "Prohibition of Human Cloning for Reproduction and the Regulation of Human Embryo Research Amendment Act" in 2006, which prohibits creating human-nonhuman chimeric and cybrid and hybrid embryos (except for human fertility testing, which can be carried out with a license under strict regulations).[189] The creation of cybrids and chimeric embryos involving human material is also forbidden in Canada, through the Assisted Human Reproduction Act of 2004 and regulations for state funding (which cover all research facilities since there are no private laboratories involved in chimera research in Canada).[190]

Other countries are particularly permissive of stem cell (and interspecific) research: at the forefront of this is China, which allows all kinds of chimera creation and even the introduction of human genetic material into nonhuman embryos.[191] Likewise, South Korea, Japan, and Singapore are relatively supportive of chimera/cybrid creation.[192]

Xenotransplantation, as we have seen, is another possible source of human-animal chimeras. The technique is closely regulated in many countries; the rare clinical trials that take place will usually have to be approved by oversight committees. Since the main problems associated with xenotransplantation are nowadays in the area of medical risk, i.e. tumorigenicity and virus transfer (rather than in the ethical problematicity of "species-crossing"), and also because xenotransplantation is currently not in the focus of research, I will not discuss these regulations in detail here.[193]

Summing up the legal and regulatory situation of chimera and other interspecific research, it has become clear that regulations vary wildly, and that the legal situation is unclear and/or currently changing in many regions. Additionally, as I pointed out, the legal and regulatory situation of *some* chimeric and interspecific research is complicated by their dependence on human embryonic stem cells, whose use in research is subject of public controversy around the world. Irrespective of these varying views on interspecific research

---

[189] Government of Australia (2006), "Prohibition of Human Cloning for Reproduction and the Regulation of Human Embryo Research Amendment Act 2006."
[190] Cf. Appendix C 2.4 in HFEA (2007), "Hybrids and Chimeras - A report on the findings of the consultation."
[191] See ISSCR report on legal situations regarding hESC research, http://www.isscr.org/public/regions/country.cfm?CountryID=52.
[192] For an overview of "legislation of countries with a permissive policy towards human embryo research" in regard to chimera and esp. cybrid creation ,see Appendix C 4 in HFEA (2007), "Hybrids and Chimeras - A report on the findings of the consultation."
[193] For an overview of xenotransplant regulation, see "Appendix: International Approaches to Xenotransplantation Regulation" in Toi Te Taiao (2005), " The Cultural, Spiritual and Ethical Aspects of Xenotransplantation: Animal-to-Human Transplantation", pp. 42-44.

and the overlapping controversies of chimeras/cybrids and hESC use, we can conclude from our look at the legal situation that some kinds of interspecific research are more likely to be forbidden than others.

Research that is most likely to raise controversy, and therefore to be regarded or declared as illegal in many countries, includes:

- Creation of *human-animal hybrids* (through fertilisation of human eggs with animal sperm, or animal eggs with human sperm). Human-animal hybridisation is a punishable offense in many legal systems and discouraged by both NAS and ISSCR research guidelines. Objections regarding the creation of "true hybrids" sometimes, but not always, extend to human-animal cybrids.
- Use of *whole human embryos as hosts* for chimera-creation is penalised by several laws, e.g. German Embryonenschutzgesetz, and restrictive U.S. draft bills. Using disparate hESCs in chimera creation is usually regarded as less problematic,
- *Use of hESC* for transfer into animals (while use of adult or somatic stem cells, or non-stem cells, is usually not regarded as extremely problematic)
- *Early transfer*, and
- *Transfer into especially relevant systems*, such as the neural system or gonads, is met with suspicion (cf. NAS guidelines).
- *Transfer into nonhuman primates* is regarded with more scepticism than transfer into not closely related animals, e.g. in the NAS guidelines.
- *Cultivation past two weeks* of cybrids, hybrids or chimerically manipulated human-animal embryos is often prohibited.
- *Implantation* into an animal or human uterus or otherwise *bringing to term* of human-animal interspecifics is regarded as problematic, and forbidden under many legislations/guidelines.
- *Breeding* with human-to-animal interspecific organisms is perceived as problematic.

These gradations in the legal/regulatory judgement of interspecific research should become much clearer once we look at the underlying ethical considerations concerning interspecific research. In the next part, we will therefore analyse the moral reasons and justifications brought forward for prohibition or strong(er) regulation of chimera/cybrid research. As

reflected by legal and other restrictions, many people think that creation of human-animal interspecifics is wrong – but why, exactly?

Chapter 2: Arguments Against Creating Interspecifics

So far, I have presented the natural occurrence and the artificial creation of chimeras and other interspecifics in a largely descriptive manner, and given a short overview of the legal situation of human-animal interspecific research. In the following sections, I will address the moral arguments that have been brought up regarding the creation of interspecifics, particularly human-animal interspecifics.

I will first give an introduction to the participants of the current debate around chimeras and other interspecifics, i.e. my main sources, in section A below. In the following sections, B, C, and D, I will present several types of arguments that experiments involving or resulting in chimeras and other interspecifics have given or could reasonably give rise to. This part will offer detailed descriptions of arguments against the creation of interspecifics, and a systematic classification of such arguments into different types. This taxonomy will be useful in making the terrain of argumentation against interspecific creation accessible for further analysis. That will be the task of chapter 4, where I will address the question central to this dissertation: Is there an argument that persuasively supports the general position that creating interspecifics (specifically: human-animal-interspecifics) is wrong and should be prohibited?

Before this concluding analysis, chapter 3 will offer an excursus to a closely related area, introducing the concept of "moral status" and discussing the question of the moral relevance of species membership ("Speciesism"); questions which will be relevant for the final analysis and conclusion in chapter 4.

A. An introduction to the debate: Sources

Who has contributed to the discussion of the ethical problems of creating chimeras, particularly human-animal chimeras or interspecifics, so far?

Many aspects and arguments discussed in chapter 2 of my dissertation are originally based on the American Journal of Bioethics' 2003 Target Article Collection "Crossing Species

Boundaries".[194] Numerous philosophers, bioethicists and scientists responded to Jason Scott Robert's and Francoise Baylis' assessment of the moral quandaries connected with the creation of human-to-animal interspecifics (specifically, human-to-animal embryonic chimeras). "Never", AJOB editor-in-chief Glenn McGee recalls, "has a Target Article collection published in *The American Journal of Bioethics* occasioned as much interest as 'Crossing Species Boundaries.' (…) Dozens more than we were able to publish wrote to suggest articles."[195] This might be because the AJOB chimera issue is one of the first instances of assessing the problem of human-nonhuman interspecifics – subjects that have gone more or less untouched, so far, invite discussion.[196] Secondly, this might be because the subject is deeply interesting and highlights aspects that are crucial for other ethical problems as well. Another selection of articles, this time on a specific form of animal-human chimeras (Weissman's "human neuron mouse" scenario) appeared in 2007, also in the AJOB.[197]

German and other Continental European philosophers have not extensively contributed to the chimera debate so far. There are some exceptions to this rule: Christoph Vallant's "Hybride, Klone und Chimären" (2008) is based on actual new developments regarding the creation of interspecific and other artificial beings, but does not strive for precision regarding biological terminology. Vallant aims for a sweeping analysis of big idea-historical connections rather than the analytical applied ethics approach I follow here. Irrgang, and Orland et al., in their 2005 discussion of posthuman perspectives, focus on and cyborgs or "enhanced" humans and mention the related, in some respects overlapping, field of interspecific beings or "chimeras" only in passing.[198] Eminent moral philosophers Robert

---

194 Robert and Baylis (2003), "Crossing Species Boundaries", American Journal of Bioethics, **3**(3).
195 McGee (2003b), "The Wisdom of Leon The Professional [Ethicist]", American Journal of Bioethics, **3**(3), p. vii.
196 Note that Peter Morriss offered an extensive and in-depth discussion of the human-animal interspecific (or "hybrid") problem already in 1998. The article did not stir much immediate reaction, the time for a "chimera debate" had apparently not yet come. Morriss (1998), "Blurred Boundaries", Inquiry, **40**.
197 Baylis and Robert (2007), "Part-Human Chimeras: Worrying the Facts, Probing the Ethics", American Journal of Bioethics, **7**(5); Cheshire (2007), "The Moral Musings of a Murine Chimera", American Journal of Bioethics, **7**(5); Cohen (2007), "Beyond the Human Neuron Mouse to the NAS Guideline", American Journal of Bioethics, **7**(5); Eberl (2007), "Creating Non-Human Persons: Might It Be Worth the Risk?" American Journal of Bioethics, **7**(5); Greely, Cho, et al. (2007b), "Thinking About the Human Neuron Mouse", American Journal of Bioethics, **7**(5); Greely, Cho, et al. (2007a), "Response to Open Peer Commentaries on 'Thinking about the Human Neuron Mouse'", American Journal of Bioethics, **7**(5); Lavieri (2007), "The Ethical Mouse: Be Not Like Icarus", American Journal of Bioethics, **7**(5); Rollin (2007), "Of Mice and Men", American Journal of Bioethics, **7**(5); Sagoff (2007), "Further Thoughts About the Human Neuron Mouse", American Journal of Bioethics, **7**(5).
198 Irrgang (2005), Posthumanes Menschsein? Künstliche Intelligenz, Cyberspace, Roboter, Cyborgs und Designer-Menschen - Anthropologie des künstlichen Menschen im 21. Jahrhundert; Orland, Ed. (2005), Artifizielle Körper - Lebendige Technik. Technische Modellierungen des Körpers in historischer Perspektive; Vallant (2008), Hybride, Klone und Chimären. Zur Transzendierung der Körper-, Art- und Gattungsgrenzen.

Spaemann and Julian Nida-Rümelin commented on the UK cybrid decision of 2008. Both apparently think that something is seriously wrong with creating human-animal interspecifics – Nida-Rümelin finds cybrids tolerable when they are just used as substitutes for human eggs, while he declares that the "'production' of human-animal-chimeras" would be a "crime against humanity" [transl. CH].[199] Spaemann speaks of "horrific visions of half-human chimeras" and notes that their creation is "one of the biggest crimes humans can commit, an opting out of Tao which denotes the immemorial frame of humanity" [transl. CH].[200] Apart from these passing remarks, Spaemann's and Nida-Rümelin's discussion of cybrids focuses on the general issue of using human embryos in stem cell research rather than on the specific aspect of mixing of human and nonhuman material.

An Ravelingien's thesis "Pig Tales, Human Chimeras and Man-Made Public Health Hazards" (2006) offers one of the few extensive philosophical academic discussions of the topic of mixing human and nonhuman.[201] While Ravelingien concentrates on the topic of xenotransplantation, some parts of her analysis overlap with the topic of this dissertation – particularly her chapter on human dignity argumentation.[202]

Journalists in the U.S., Great Britain, and Germany have extensively commented and reported on the subject of "interspecifics" in science – another important source for my typology of arguments. Irving Weissman's research plans spurned a first wave of debate around 2005, and the UK cybrid debate triggered an avalanche of journalistic commentary in 2007-2008.

Institutions responsible for bioethical counselling and research are discovering the subject all over the world: Germany's "Nationaler Ethikrat" was given a talk by its member, bioethicist and politician Jens Reich on "the question of cultivation of chimeras" in 2005.[203] Its institutional successor, the "Deutscher Ethikrat", discussed human-animal interspecifics in February 2008.[204] From 2005-2007, the EU funded an international project on "Chimeras and Hybrids in Comparative European and International Research" (CHIMBRIDS) at the Institute for Medical Law in Mannheim. As an interdisciplinary

---

[199] Nida-Rümelin (2008), "Neue Sachlichkeit - Über das Ende der Ideologie in der Stammzelldebatte", Cicero, Juli 2008.
[200] Spaemann (2008), "Jedes nach seiner Art", Cicero, (Mai 2008).
[201] Ravelingien (2006), "Pig Tales, Human Chimeras and Man-Made Public Health Hazards. An Ethical Analysis of Xenotransplant Benefits and Risks", Ghent University Faculty of Arts and Philosophy.
[202] See also Ravelingien, Braeckman, et al. (2006), "On the moral status of humanized chimeras and the concept of human dignity", Between the Species, **VI**.
[203] Nationaler Ethikrat (2005), "Wortprotokoll - Niederschrift über den öffentlichen Teil der Sitzung am 25. August 2005."
[204] Florian (2008), "Deutscher Ethikrat tagte erstmals öffentlich", Informationsdienst Wissenschaft, 2008/06/27.

project, one of the aims of CHIMBRIDS was to "enable a close, lasting and sustainable interrelation between the rapid progress in scientific research and basic ethical, philosophical and legal principles."[205] The final report had not been published at the time of writing (October 2008).[206]

The UK entered the debate about questions of interspecific research much earlier with the Warnock Report of 1984; more recently, the Embryology Authority HFEA carried out a public consultation on chimeras/cybrids and connected ethical questions,[207] which was extensively covered by the UK press. The topic of chimeras has probably received most attention in the U.S., culminating in the 2005 NAS guidelines and current legislative initiatives to regulate human chimera/interspecific research, which are backed by presidents and presidential candidates.

Human-animal interspecifics have become a mainstream subject of bioethics debate and public policy both in the U.S. and in Europe. The cultivation of human-animal embryonic chimeras and cybrids has triggered this discussion, but the arguments brought forward, as we will see, are rarely limited to these two particular kinds of interspecifics. Apart from some, very rare, exceptions,[208] commentators argue *against* chimera creation rather than defending it. Below, I have divided up possible objections into three types:[209] firstly, in section B, that of the intrinsic, "bioconservative" type; in section C, we will assess objections based on a fear of possible consequences of chimera production, and thirdly, in section D, indirect consequence-based objections based on the threat of "confusion" which is allegedly the consequence of creating interspecifics.

B. Intrinsic objections

Intrinsic arguments against creating chimeras are characterized by the implicit or explicit assumption that creating chimeras is wrong *as such*. Arguments of this type will not be brought forward by a consequentialist (i.e. someone who thinks that what makes an action wrong or right is its consequences), but rather by someone who believes in ethical

[205] www.chimbrids.org - Project Summary.
[206] Taupitz and Weschka, Eds. (forthcoming), CHIMBRIDS: Chimeras and hybrids in comparative European and international research – scientific, ethical, philosophical and legal aspects.
[207] HFEA (2007), "Hybrids and Chimeras - A report on the findings of the consultation."
[208] For authors that are not per se opposed to creation of interspecifics, even human-animal interspecifics, see: Morriss (1998), "Blurred Boundaries", Inquiry, **40**; Savulescu (2003), "Human-Animal Transgenesis and Chimeras Might Be an Expression of Our Humanity", American Journal of Bioethics, **3**(3).
[209] My distinction between "intrinsic" objections and objections concerning (bad) consequences is based on Bernard Rollin's typology of concern regarding genetic engineering and biotechnology, see Rollin (2003), "Ethics and Species Integrity", American Journal of Bioethics, **3**(3).

principles as guiding fundamental assumptions for his actions. The adherence or disobedience to the principle determines whether a certain action is morally right or wrong. For understanding and evaluating this kind of argument, we must find out what principles are brought forward against the creation of chimeras and whether they hold up under scrutiny (i.e. whether they are or will be violated by the creation of chimeras, and whether they are consistent).

Intrinsic arguments are, one might think in the first place, absolute arguments: once you have accepted such an objection, you are not going to change your mind just because (empirical) conditions in the world change. To take the example of chimera research: even if it turned out that, by means of painless experimentation on human-animal chimeras, scientists had found a way to cure cancer, a person who has intrinsic arguments against creating chimeras would still be opposed to such experimentation. Likewise, if it turned out that following his principle led to very adverse effects, this could not – in theory – lead to the intrinsic objector changing his mind on the matter. His objection against creating chimeras is not based on expected adverse consequences and he is therefore not fazed by a change in expectation (or actual outcome) regarding the action at hand.

This absolute view of intrinsic objections is doubted by Gregory Kaebnick: an intrinsic claim, he says, is not absolute and unchallengeable by changed expectations or outcomes. Rather than answering ethical questions once and for all from a standpoint that is independent of the world as it is, intrinsic arguments, specifically in bioethics, invoke a kind of "precautionary principle" and move us to adopt a "preservationist attitude".[210] Intrinsic and consequentialist arguments are not as intransigent or unconnected as it seems in the first place. Mary Midgley points out that the two are linked at a crucial point:

> *"Acts that are wrong in themselves can be expected to have bad effects of a particular kind that is not just accidental. Their badness follows from what is wrong in the act itself, so that there is a rational, conceptual link between them and their results."*[211]

Additionally, it can be noted that outlooks are imaginable where both intrinsic and consequentialistic aspects are considered and, only when taken together and balanced against each other, result in a position on a certain subject.

---

[210] Kaebnick (2000), "On the Sanctity of Nature", Hastings Center Report, **30**(5), p. 22. I will discuss the idea of a "precautionary principle" in regard to dealing with risk in chapter 2, section C.4 below.
[211] Midgley (2000), "Biotechnology and Monstrosity - Why We Should Pay Attention to the 'Yuk Factor'", Hastings Center Report, **30**(5).

I see a meaningful difference between intrinsic and consequentialistic argumentation mainly in their proponents' different argumentative focus or emphasis. Intrinsic arguments are open appeals to basic values or principles of the opponent or combatant. The empirical questions at hand are, in this type of argumentation, often taken for granted or not too closely considered. Consequentialistic arguments, on the other hand, give more attention to empirical questions (i.e.: "What will the consequences of the action really be?"). Yet, they also rely on basic principles – often in the form of a utilitarian approach – which are usually not discussed. Intrinsic and consequentialist argumentation overlap and, ultimately, can complement each other.

I do not want to give a general assessment of different types of ethical arguments or metaethical positions in this thesis. A question that should interest us more is: how convincing are the intrinsic arguments that are used to prove the wrongness of creating interspecifics? I have identified four approaches to why creating interspecifics could be intrinsically wrong. Some apply only to the mixing of human and animal, be it in the form of chimerism or hybridisation; others might, in theory, be applied to all kinds of artificial chimeras (even in plants). Firstly, let us have a look at arguments from "repugnance" or, more general, arguments from an intuitively negative emotional reaction to interspecifics.

1. Repugnance, the "Yuk Factor", and arguments from emotion

a. Leon Kass' "Wisdom of Repugnance"

The first kind of intrinsic argument that has attracted the interest of bioethicists is the so-called "Wisdom of Repugnance" argument. Leon Kass, a former chairman on President G.W. Bush's Council of Bioethics, developed this point in 1997,[212] noting that, though disgust is not an argument, as such, "in crucial cases (…) repugnance is the emotional expression of deep wisdom, beyond reason's power fully to articulate it." This alleged wisdom is there to "protect the central core of humanity". In his praise of "repugnance", Kass states:

> *"Indeed, in this age in which everything is held to be permissible so long as it is freely done, in which our given human nature no longer commands respect, in which our bodies are regarded as mere instruments of our autonomous rational wills, repugnance may be the only voice left that speaks up to defend the central*

[212] Kass (1997),"The Wisdom Of Repugnance", in: Kass and Wilson (eds.) The Ethics of Human Cloning; Kass and Wilson (1998), The Ethics of Human Cloning.

> *core of our humanity. Shallow are the souls that have forgotten how to shudder."*[213]

In 1997, this statement was meant as a response to the cloning of Dolly the sheep and the prospect of human cloning, but "repugnance" has since been used or at least alluded to in many areas of bioethics.

Some bioethicists answer Kass' argument with outright refusal or even ridicule. It is noted that intuitions or "knee-jerk reactions" have, in the past, been used to argue against morally neutral actions (e.g. interracial marriage, homosexuality), without any justification.[214] There are, arguably, some reactions based on repugnance that should have no moral consequences and which ought to be ignored or suppressed. Feelings of violent repugnance towards a very sick or disfigured person, a burn victim or a person suffering from a skin condition are common. How are we to tell that this repugnance caused by disease or unusual genetic variation in another person is not a "sign of wisdom" and that we should shun, avoid or punish all that are affected by such atypicalities? What is it that repugnance is telling us, if it tells us anything at all? David Castle notes that the type of argument Kass praises "is a viciously poor guide for channelling one's uneasy responses to people with severe disabilities or injuries."[215] Another problem that arises when trying to use the "repugnance" objection to the creation of chimeras is that, clearly, not all chimeras or even human-animal interspecifics look "yucky". Thus, the argument could probably not be used against not obviously suspicious cases like mice with a few human cells or genes, or against cybrids in early stages, or against human beings with (not directly visible) animal transplants. These are all quite pragmatic objections to or restrictions of the repugnance argument. Others attack Kass' type of argument from repugnance on much deeper grounds, stating that it lacks philosophical content, altogether. Science journalist Chris Mooney, in a critical assessment of Kass' career in US bioethics, ridicules his source of inspiration, Hans Jonas (whom he deems a "rather obscure German philosopher") and his "heuristics of fear"[216] as demagoguery, then accuses Kass of fear-mongering, and both of an utter lack of convincing ethical argument.[217] Glenn McGee (editor of the *American Journal of Bioethics*), in a similarly acidic tone, notes that the "rules for avoiding 'yuk'" are

---

213 Kass (1997),"The Wisdom Of Repugnance", in: Kass and Wilson (eds.) The Ethics of Human Cloning.
214 Cf. Karpowicz, Cohen, et al. (2004), "It is ethical to transplant human stem cells into nonhuman embryos", Nature Medicine, **10**(4).
215 Castle (2003), "Hopes against Hopeful Monsters", American Journal of Bioethics, **3**(3), p. 29.
216 Jonas' "heuristics of fear" can also be understood as a variation of the "precautionary principle", which, some argue, should guide our way of dealing with risk or at least certain types of risks. This type of argument will be discussed in chapter 2, section C.4 below.
217 Mooney (2001), "Irrationalist in Chief", The American Prospect, **12**(17), 2001/08/10.

completely arbitrary and that reference to "shared feelings of yuk" are just political tactics and part of a "flimsy new kind of neoconservative natural law theory."[218] Here, most of the objections to Kass are based on criticism of his role in (conservative) politics, rather than the philosophical content of his argument.

The most substantial objection to Kass-style arguments is that any moral intuition or emotion must be justified and defended as valid – intuition must be "legitimate". David Castle thinks that the Kass-style arguments mentioned by Robert and Baylis are

> *"so weak they can be toppled with pea shooters. Kass' 'wisdom of repugnance,' perhaps the most pernicious of the lot, puts typological reasoning to poor ends by backstopping claims about the legitimacy of moral intuitions."*[219]

It seems quite clear at this point that Kass' argument does not have many followers – particularly few, it seems, in contemporary U.S. bioethics – and does certainly not succeed in persuading adversaries.

b. Sub-argumentative references to emotion and intuition

Still, "repugnance" has been used by many objectors to creating chimeras – not necessarily as a free-standing argument, but in the description of typical "knee-jerk"-reactions to (human-animal) chimeras or other interspecifics. Most authors would not go as far as declaring their or the "typical" intuition regarding chimeras a fact that is directly morally relevant or decisive. Still, no author would go to great lengths at describing his or others' intuitive reaction to a phenomenon if he or she thought these reactions were entirely irrelevant.

Let us look at some examples of philosophers referring to or even elaborating on emotional reactions regarding chimeras or other interspecifics. Jeffrey Stout uses the example of a (hoax) cat/rabbit interspecific ("Cabbit"), shown on TV, for comments on the attribute "abominable". [220] He notes: "I have no objection in principle to cabbits. Yet the sight of a living cabbit did affect me. I found it revolting."[221] Seyfer, elsewhere raising religious concerns against creating chimeras (see section B.2 below), notes in the last sentence of his article that mixing humans and animals, (among other points) "(…) evokes a certain repugnance. Perhaps this repugnance is a sign of wisdom" – a direct reference to

[218] McGee (2003b), "The Wisdom of Leon The Professional [Ethicist]", American Journal of Bioethics, **3**(3).
[219] Castle (2003), "Hopes against Hopeful Monsters", American Journal of Bioethics, **3**(3), p. 29.
[220] Stout (1988), Ethics After Babel - The Language of Morals And Their Discontents, pp. 147.
[221] Stout (1988), Ethics after Babel – The Language of Morals And Their Discontents, Ch.7

Kass' Argument.[222] Mary Midgley defends a soft argument from revulsion concerning the advances of biotechnology: she thinks that there is a widespread feeling of revulsion regarding (trans-species) genetically manipulated plants and animals which should not be ridiculed but "spelled out" in the form of an argument.[223] Midgley's approach could just as well be applied to the question of artificial production of human-animal interspecifics – we will assess it more closely below. Physician, biologist and bioethicist William Hurlbut, interviewed by the New York Times' Jamie Shreeve, notes that "When we start to blend the edges of things, we're uneasy.(…) That's why chimeric creatures are monsters in mythology in the first place." He even offers an evolutionary explanation for this feeling of uneasiness, giving it the "justification" of being natural and useful: "Our minds have evolved to be hypersensitive to the borders between species, just as we see a rainbow as composed of six or seven distinct colors when it is really a continuum of wavelengths of light."[224] Morriss (who ultimately does not subscribe to an argument from revulsion) describes the typical reaction to human-animal hybrids like this: "We react with fright, with horror. This is not just the horror of ordinary physical fear, it is an existential horror – a metaphysical one. This sort of existential angst is a very powerful feeling (…)."[225] In the political sphere, U.S. President Clinton, according to a 1998 request regarding the bioethical assessment of the production of human-bovine cybrids to the National Bioethics Advisory Council, noted that he felt "(…) deeply troubled by this news of experiments involving the mingling of human and nonhuman species (…)."[226] In general, newspaper or magazine articles about interspecific research appeal to emotions of fear or horror in their titles with astonishing regularity.[227]

There are also more indirect forms of evocation of disagreeable feelings regarding interspecifics. The connection of interspecifics with the category of "monsters" is rooted in their name's ambiguity. As mentioned earlier, "Chimera" also denotes a monster of Ancient Greek mythology that is a composite of several animals (see picture 1, p. 4). This mythical connotation survives until today: Bruce Lehman, the U.S. commissioner of patents, calls

---

[222] Seyfer (2004), "The Ethics of Chimeras and Hybrids", Ethics and Medics, **29**(8).
[223] Midgley (2000), "Biotechnology and Monstrosity - Why We Should Pay Attention to the 'Yuk Factor'", Hastings Center Report, **30**(5).
[224] Shreeve (2005), "The Other Stem-Cell Debate", The New York Times Magazine, 2005/04/10.
[225] Morriss (1998), "Blurred Boundaries", Inquiry, **40**, p. 273.
[226] National Bioethics Advisory Council (1998), "Discussion Transcript: President Clinton's Request Re: Embryonic Stem Cells ."
[227] To give but some examples: Kastilan (2005), "Die Angst vor der Chimäre", Die Welt, 2005/05/02; Illinger (2006), "Die Angst vor der Chimäre", Süddeutsche Zeitung, 2006/11/08; Müller-Lissner (2007), "Angst vor der Chimäre", Tagesspiegel, 2007/09/10.

human-animal chimeras "monsters", outright.[228] So do others (not necessarily intrinsic objectors to creating chimeras): Bernard Rollin entitles one category of possible (consequentialist) objections "rampaging monsters",[229] Mark Sagoff mentions a "Caliban" (i.e. a villain) as one possible type of interspecifics one could create. Depiction of chimeras and other interspecifics, especially of the human-animal kind, as "monsters" is quite common, and undoubtedly this denotation provokes fear and disgust towards and exclusion of the creature referred to – it also to a certain degree predetermines the evaluative stance one will have towards creating such a being.

c. Can there be an "Argument from Emotion"?

We have seen that emotions of disgust, revulsion and repugnance play a role, be it a direct or an indirect one, in the debate around interspecifics. This type of objection – depending perhaps on the sensitivity of the person who uses it – can be directed against the creation of different kinds of interspecifics. Some apparently already feel revulsion when thinking about transgenic plants or animals, others deem an animal-to-animal chimera like the "geep" disgusting, less sensitive subjects feel revulsion only when considering "funny looking" chimeras, and for others, the line is crossed only if human material is involved.

There have not been many polls or similar inquiries into whether human-animal chimeras (and which kinds of them) do really stir the negative affective reactions cited by the bioethicists who make use of arguments from revulsion, although the HFEA report of 2007, which concluded a three-month public consultation process in the UK, notes:

> *"Certainly at the outset of the deliberative work, many of the participants expressed an initial repugnance in reaction to the suggestion of mixing human and animal material. Associations were drawn with incidents such as the Northwick Park drug trials, myths and legends, and the elephant man. However, when further factual information was provided and further discussion took place, the majority of participants became more at ease with the idea, although as one participant observed, 'The gut reaction is hard to overcome.'"*[230]

This gut reaction might not be as fixed and hard-wired as it seems, though. Morris points out that societies can be very different regarding their view of the inherent value of human-nonhuman boundaries, and that the portion of people who react with disaffection could be

[228] Dowie (2004), "Gods And Monsters", Mother Jones, (January/February 2004).
[229] Rollin (2003), "Ethics and Species Integrity", American Journal of Bioethics, **3**(3).
[230] HFEA (2007) "Hybrids and Chimeras - A report on the findings of the consultation", section 5.8.

"narrower than the human race".[231] It is very well imaginable, for example, that other cultures produce less horrendous, or even positive emotional reactions to mixed beings – analogously, intersexual persons evoke vastly different reactions in different cultures, ranging from disgust in (traditional, but also modern) western societies to spiritual worship: many non-western religions know intersexual – and interspecific – deities. The connotations of chimeric beings also seem to have changed within Europe's own cultural development. Ancient Greece, apart from frightening monsters like the Chimera and the Minotaur, also knew neutral or even "good" hybrid or chimeric creatures, such as the Centaurs (human-horse mixtures), Satyrs (often described as donkey- or goat-men), and Pegasus (a winged horse which helped slay the monstrous Chimera).

Apart from the varying prevalence of negative feelings raised by (human-animal) interspecifics, and apart from the question whether there are such feelings in all or the majority of the population: can direct or indirect reference to "emotions" be used as an argument in ethics, at all*?* Many have noted that emotion or intuition by themselves are not arguments: "If claims about repugnance are to have any moral force, the intuitions captured by the 'yuk' response must be clarified", Robert and Baylis state.[232] Much of the criticism Kass' and Kass-like arguments are met with is based on this point, and many philosophers forbid the use of "yuk factor" arguments because they are nothing more than thoughtstoppers.

But let us not prematurely discard this type of argument: what defences are there for the moral relevance of "repugnance"?

d. Defences of the "Yuk Factor"

Not all ethicists concerned with arguments of this kind immediately reject them as useless. Let us take an exemplary look at two defences of the "Yuk Factor", especially its use as an argument against biotechnological advances: Mary Midgley's (who is concerned with the progress of bio-engineering in general) and Robert Streiffer's (who specifically addresses the problem of human-animal chimeras).

In an article tellingly entitled "Biotechnology and Monstrosity – Why We Should Pay Attention to the 'Yuk Factor'"*,*[233] Midgley discusses arguments involving the "yuk factor" in a broader context, namely that of bioengineering in general. Aside from

---

[231] Morriss (1998), "Blurred Boundaries", Inquiry, **40**, p. 276.
[232] Robert and Baylis (2003), "Crossing Species Boundaries", American Journal of Bioethics, **3**(3), p. 7.
[233] Midgley (2000), "Biotechnology and Monstrosity - Why We Should Pay Attention to the 'Yuk Factor'", Hastings Center Report, **30**(5).

xenotransplantation and cross-species transgenesis, she mentions human enhancement and GM crops, and, generally, biotechnology that takes species not as fixed entities but as objects of our technical improvement or engineering. Undoubtedly, her reasoning could and would also be applied to the production of all kinds of artificial interspecifics. Midgley wants to make two points: she argues that we should take emotional objections seriously and try to understand or spell out what is behind them, because then we will see that they are not as "irrational and negative" as we took them to be. Secondly, we should recognize that pro-bioengineering positions are not as rational as they purport to be, but really the upshot of "algenic manifestos" and a general new "agenda" of biotechnology their supporters (unconsciously or consciously) subscribe to. I will not elaborate further on the second point here for it is a political or polemic one which does not really help us with the question whether emotional reactions can make (or at least support) valid arguments, in general. Let us focus on Midgley's first point instead: we should, in a nutshell, pay attention to the "yuk factor" because there *might be* a rationale behind the simple utterance of an adverse emotional impulse. Midgley goes further: in her opinion, in regard to bioengineering, there *is* such a rationale behind the "yuk". It is our responsibility to spell out and understand properly what an objector to bioengineering is actually saying behind his façade of seemingly "inarticulate disgust". And he or she is, in Midgleys words, really "objecting to the attacks on the concept of species". In Midgley's view, "there is good reason for that objection."[234] At this point, she goes on to spell out the rationale behind arguments that deem "unnatural" actions morally wrong. Is this a defence of the "yuk factor" or of an argument from repugnance? I think not, although Midgley tries hard to construct it as one. If an objector to bioengineering states that the mere thought of GM crops or animals fills him with disgust and revulsion, and that therefore one should forbid such advances of bioengineering, even with the most charitable interpretation, his or her statement cannot be understood as an argument drawing from the value of integrity of the species concept. It is true that "yuk factor" approaches can be beefed up and made persuasive by explaining what concepts or values are behind the emotional reaction reported, and that, in this context, the report and assessment of intuitions and knee-jerk reactions is helpful; but it remains true that the pure reference to the "yuk factor" is argumentatively void. It turns out that despite her express intentions, Midgley's argument is not one from the "yuk factor" but one from a quasi-religious view of species boundaries as

[234] Ibid., p. 9.

morally relevant. I will therefore assess Midgley's argument under the heading of "Religious and quasi-religious objections", below.

Robert Streiffer defends the view that the "yuk factor" could be used as a valid argument for objecting to the creation of human-animal chimeras. While he admits that such an action is not necessarily morally wrong just because it is "unnatural", and that the notion of moral wrongness qua "unnaturalness" is problematic, he notes that "proponents of the unnaturalness objection can insist that (…) they still know that crossing species boundaries is wrong."[235] (Karpowicz et al. (2005) do not defend, yet reconstruct a similar argument, referring to incest and cannibalism as abominations that might be comparable. They call this the "moral taboo argument".)[236] To support this, Streiffer identifies analogous cases in which moral wrongness cannot be further explained, but where we "know by just looking" that they are wrong: "Bestiality and pedophilia are wrong even when they cause no physical or psychological harm" and therefore: "Robert and Baylis' epistemological claim that intuitions must be justified if they are to 'have any moral force' is mistaken."[237] Thus, the "yuk factor" *could*, after all, be an argument against creating chimeras – though Streiffer does not decide on whether it is valid, he wants to hold on to the possibility of using arguments of this type. One could argue against Streiffer by doubting that his examples are convincing: for one, one could state that there is no "pedophilia" without harm done to the child (or rather, that mere "pedophilia" is not the problem – pedosexuality is, even if the two are often confused). One could also make the point that "bestiality" is not morally wrong in itself (as Peter Singer did, quite persuasively, in his review of Midas Dekker's book "Dearest Pet").[238] Some argue against the use of the "yuk factor" by pointing out that arguments of this kind have been used to support anti-miscegenation policies or other systems and structures now considered morally wrong. For the field of bioethics, note that blood transfusions and organ transplantation were, not too long ago, considered "abhorrent", and arguments were made against these new techniques based on these emotional or "taboo" responses.[239] Streiffer counters this somewhat weak objection by noting that, as with other types of arguments, it may be wrong for some, but right for other

---

[235] Streiffer (2003), "In Defense of the Moral Relevance of Species Boundaries", American Journal of Bioethics, **3**(3), p. 38.
[236] Karpowicz, Cohen, et al. (2005), "Developing human-nonhuman chimeras in human stem cell research: Ethical issues and boundaries", Kennedy Institute of Ethics Journal, **15**(2), p. 110ff.
[237] Streiffer (2003), "In Defense of the Moral Relevance of Species Boundaries", American Journal of Bioethics, **3**(3).
[238] Singer (2001), "Heavy Petting", nerve, 2001/01/03.
[239] Karpowicz, Cohen, et al. (2005), "Developing human-nonhuman chimeras in human stem cell research: Ethical issues and boundaries", Kennedy Institute of Ethics Journal, **15**(2), p. 112.

cases (his example are paternalistic arguments, which are today considered a mistaken approach when used concerning women, but appropriate when used concerning children). Yet finally, Streiffers' concluding remark regarding the repugnance argument shows the crucial problem of this kind of reasoning:

> *"Should the repugnance some feel at the crossing of species boundaries be dismissed (as the reaction of a racist should be), or does it constitute yet another intuition in a long line of intuitions where our difficulties in providing satisfactory theoretical explanations merely indicate theoretical inadequacy? Given the poor state of the arguments on both sides of the debate, it is too early to tell."*[240]

What Streiffer says is: we will know whether our argument from repugnance was right once we have worked out whether there are actual reasons that support it. The "argument", after all, is based on emotion and, as such, it is hugely influenced by our socialisation and cultural surroundings, and – most importantly – it cannot be used to convince other people who have different emotive responses. Therefore, it is useless in any ethical debate where there are conflicting emotive responses. Basically, Kass and Streiffer use emotions in a supporting role – as stand-ins for when they have run out of arguments. This does, in my opinion, not make their position more convincing. Karpowicz et al. (2005) offer a similar interpretation of what they call "taboo arguments":

> *"What makes such outrage justifiable, however, is not the emotion in itself, but the reasons why one responds with this emotion. We would be reluctant to accept ethical judgments based solely on emotions (…) for these can occur by chance and may be misplaced."*[241]

The authors extend this critique to arguments from "intuition" (as distinct from emotion) – though intuitions, in their view, "establish a prima facie case", they can still be conflicting and fallible, and "need to have the support of some form of reasoning that is intersubjectively available and can be followed by others."[242]

To sum up, the feelings or intuitions people have when confronted with novel beings such as chimeras are a valid object of research regarding their roots and the concepts behind them. They are useless as an argument and – at least this is true for emotions –, in my

---

[240] Streiffer (2003), "In Defense of the Moral Relevance of Species Boundaries", American Journal of Bioethics, **3**(3), p. 38.
[241] Karpowicz, Cohen, et al. (2005), "Developing human-nonhuman chimeras in human stem cell research: Ethical issues and boundaries", Kennedy Institute of Ethics Journal, **15**(2), p. 111.
[242] Ibid.

opinion, they should not even count as a vague indicator that "something is wrong". Karpowicz et al. arrive at a similar conclusion, finding that "taboo arguments" do "not provide an adequate basis for rejecting studies using human-nonhuman chimeras (…)." [243]

After this examination of the "yuk factor" or arguments from repugnance, let us look at other, more promising types of argument. As I mentioned above, it proves to be hard to draw lines between different types of intrinsic arguments, and also between intrinsic and consequentialist arguments; keeping a crude typology, however, seems advisable for the sake of a clear synopsis. The following two types of intrinsic argument are often constructed as explanations for (or reasons behind) the "yuk factor". Creating chimeras is deemed offensive and repugnant because it means "challenging God's existence".[244] Another type of argument highlights "boundaries" between species that are considered sacred or, as Robert and Baylis write, "inappropriate objects of human transgression". Transgression of such a boundary leads to the violation of a taboo which causes "instinctive and intense revulsion".[245] Are these objections to creating chimeras more convincing than the repugnance argument, or do they substantially improve its persuasiveness?

2. Religious and quasi-religious objections

It is not uncommon to explain revulsion or similar aversion to interspecifics by religious reasons. I will not dwell on this kind of argument for too long, but still mention some typical concerns. I will also have a look at what I call "quasi-religious" concerns: objections on the grounds of beliefs that are not necessarily religious in the traditional sense, but based on the belief in a higher order of some kind (e.g. the teleological belief that there is a sense or direction in nature which we should obey, or a sanctity that does not derive from specifically religious beliefs). These "quasi-religious" concerns are probably even more influential nowadays than religious concerns proper.

a. Christian attitudes towards the creation of interspecifics

Objections against the creation of chimeras, especially human-animal chimeras, can apparently be derived in a relatively direct fashion from the scripture: "bestiality", i.e. sexual

[243] Ibid., p. 113.
[244] Robert and Baylis (2003), "Crossing Species Boundaries", American Journal of Bioethics, **3**(3), p. 7. Robert and Baylis reconstruct but do not support this argument.
[245] Ibid., p. 2.

intercourse between human and nonhuman beings, is expressly forbidden.[246] Some infer from this that technically assisted "mixing" of humans and animals – especially in the quasi-sexual way of merging human and animal embryos or embryonic cells – would be highly problematic.[247]

A somewhat more abstract argument states that mixing one species with another species can be seen as meddling with the types of beings God has given us. Morriss reconstructs (but does not share) a view that sees the world as "complete" and any creation of novel creatures therefore necessarily as an insult to God.[248] In the same vein, a 1987 synod of the Evangelische Kirche Deutschland concerning genetic engineering and reproductive medicine referred to the "predetermined shape of creation", which would be "violated" by creation of chimeras and hybrids [transl. CH].[249] Mixing human with nonhuman beings would be regarded as an even graver offense, since humans are seen as a kind of being that has a special and unique connection with God. Human beings, according to Christian doctrine, belong to an altogether different category than nonhuman animals; God has created them in his image and correspondingly they carry an inherent value. Seyfer notes that, from the Christian viewpoint,

> *"Jesus Christ did not come as an animal, but specifically as a human being, in a human body. This bespeaks the dignity which God accords human beings and their bodies and how specially He views the human race. It thus seems to lead towards the blasphemous to purposefully combine the genetic or bodily material of a human being and an animal in a way that changes either of their identities. To mix the imago Dei with non-imago-Dei seems a violation, and evokes a certain repugnance. Perhaps this repugnance is a sign of wisdom."*[250]

Aside from this reference to Kass' argument from repugnance, the main part of the argument rests on the religiously grounded dignity of human beings and thereby the offensiveness of mixing human with nonhuman (for further discussion of "human dignity" in this context, see section B.4 below).

---

[246] See Leviticus 18:23: "And you shall not lie with any beast and defile yourself with it, neither shall any woman give herself to a beast to lie with it: it is a perversion." and Lev 20:15-16: "If a man lies with a beast, he shall be put to death; and you shall kill the beast. If a woman approaches any beast and lies with it, you shall kill the woman and the beast; they shall be put to death, their blood is upon them."

[247] Robert and Baylis (2003), p. 7, (though they find this reasoning mistaken), mention that: "Sexual intimacy between human and nonhuman animals typically is prohibited in law and custom, and some, no doubt, reason from the prohibition on the erotic mixing of human and nonhuman animals to a prohibition on the biotechnological mixing of human and nonhuman cellular or genetic material."

[248] Morriss (1998), "Blurred Boundaries", Inquiry, **40**, p. 279.

[249] Evangelische Kirche Deutschland (1987), "Zur Achtung vor dem Leben - Maßstäbe für Gentechnik und Fortpflanzungsmedizin. Kundgebung der Synode der EKD, 1987, Berlin."

[250] Seyfer (2004), "The Ethics of Chimeras and Hybrids", Ethics and Medics, **29**(8).

The overriding principle of Catholic Christian opinions regarding the creation of human-animal interspecifics is the priority of the doctrine of sanctity of human life from conception on. This results in staunch opposition to any research that involves the use of human embryos – including many types of interspecific research. The priority of the sanctity of life principle can lead to results that seem counterintuitive at first glance. Roman catholic bishops suggested in 2008 that, should human-animal embryonic chimeras be created (which they consider to be morally wrong), then there should be no legislation preventing the embryo from being implanted in the womb of the woman who donated the egg:

> *"Such a woman is the genetic mother, or partial mother, of the embryo; should she have a change of heart and wish to carry her child to term, she should not be prevented from doing so"*[251]

Sanctity of life considerations, in this point of view, clearly overrule other arguments that would make the bringing to term of such a hybrid being morally wrong.

Although the standard Christian reaction to hybridisation, chimerisation and transgenesis is negative, some argue that Christian viewpoints do not necessarily result in a firm opposition to the creation of interspecifics (even human-nonhuman mixtures). Theologian Daniel McGee points out that human life, though it has a supreme position in the hierarchy of Christian values, is not granted "absolute value or sacred status" in the Judeo-Christian tradition. Regarding chimeras, McGee reminds of Karl Rahner, who, in the 1960s, warned fellow Catholics not to absolutely dismiss new technologies for human manipulation: "He noted that Christians must recognise that such self-manipulation will contain the potential for both good and evil", McGee resumes, and that (even for faithful Christians) there is no one answer to the new complexities brought about by new developments of biotechnology such as chimera creation.[252] Robert and Baylis point out that

> *"Some would argue further that not only is it* not *wrong to play God, but rather this is exactly what God enjoins us to do. Proponents of this view maintain that God 'left the world in a state of imperfection so that we become His partners' – his co-creators."*[253]

---

[251] Gledhill (2007), "Human-animal hybrid embryos should be legal says Catholic Church ", Times Online, 2007/05/27.
[252] McGee (2003a), "Moral Ambiguity? Yes. Moral Confusion? No", American Journal of Bioethics, **3**(3).
[253] Robert and Baylis (2003), "Crossing Species Boundaries", American Journal of Bioethics, **3**(3). Citing Breitowitz (2002), "What's so bad about human cloning?" Kennedy Institute of Ethics Journal, **12**.

Let me close this incomplete and very tentative look at Christian views of chimera creation with the somewhat surprising result that the ethical classification of human-animal chimerism research is not a univocal one even within the bounds of Christian interpretation, at least not on the level of intrinsic objections.

b. A quasi-religious objection: Hubris

In the bulk of adverse reactions to biotechnology, there is one very common type of objection or concern which I would not call "religious" although it sometimes uses religious terminology. It is expressed in the formula that biotechnology, and particularly chimera creation, constitutes "Playing God" or "Meddling with Nature" and is, therefore, morally reprehensible or at least suspect.

In a 2005 interview with the Christian Science Monitor, Jason Scott Robert stated that "he's been struck by how 'even secular people, people who aren't of faith, nonetheless see the wisdom of the ›playing God‹ objection' to creating chimeras."[254] Robert Streiffer cites the U.S. Office of Technology Assessment's report on public perceptions of biotechnology as stating that concerns "about playing God and tampering with Nature" are quite prevalent in the (American) public.[255] Chakrabarty reports that "crossing the so-called evolutionary barrier through scientific interventions does not resonate well with most people; it is considered an overreach for scientists to play God."[256] Jeremy Rifkin, in an assessment of human-mouse xenograft experiments, states that such research will "stretch the limits of human tinkering with nature to the realm of the pathological" and he fears a "journey into a brave new world in which all of nature can be ruthlessly manipulated."[257]

I understand both the (non-religious) "Playing God" and the "Meddling With Nature" concerns to have one common root: the accusation of hubris. Stemming from Ancient Greek culture, this today denotes a combination of ignorance, arrogance and exaggerated pride of an agent which is typically followed by punishment by fate or higher powers. A typical kind of modern hubris view assumes that there are actions, or types of action, that are reserved for higher beings (God or Nature), and areas of life and nature which are inappropriate for human beings to interfere with. Robert Spaemann's reference to "opting

[254] Lamb (2005), "A Mix of Mice and Men", Christian Science Monitor, 2005/03/23.
[255] Streiffer (2003), "In Defense of the Moral Relevance of Species Boundaries", American Journal of Bioethics, **3**(3), p. 37.
[256] Chakrabarty (2003), "Crossing Species Boundaries and Making Human-Nonhuman Hybrids: Moral and Legal Ramifications", American Journal of Bioethics, **3**(3), p. 20.
[257] Rifkin (2005), "Are you a man or a mouse?" Guardian, 2005/03/15.

out of Tao" seems to fit this pattern;[258] others directly refer to the dangers of hubris concerning interspecific research.[259] Concerns of this type are not limited to the creation of human-animal interspecifics, they can be directed against the artificial creation of life, in general (e.g. in vitro fertilization), against the creation of manipulated life (manipulation of the genome, cloning, hybridisation), against the ending of life by man (suicide, assisted suicide, death penalty), against the undue prolonging of life by medical means, or even against contraception. Since it is assumed that a supernatural being (or "Nature") is in charge of creating categories of creatures, of giving life, creating life and taking it away as it sees fit, human interference with these responsibilities is seen as insolent and morally wrong.

Mary Midgley, whose "Biotechnology and Monstrosity" I introduced above as trying to defend "yuk factor" arguments, is one of the few to spell out this type of intrinsic objection. While her article addresses all kinds of manipulative biotechnologies, the paragraph entitled "How solid are Species?" focuses on beings that stand "between species" (she speaks of hybrids and, in her examples, of "novelties and monsters, chimeras and winged horses and three-headed dogs").[260] Can an argument be made from the statement that such beings are "unnatural" to the moral view that we shouldn't make them? Putting aside the assumption that everything that straddles species lines is somehow dangerous, Midgley admits that modern biology has uncovered that "species are not timeless essences – that they can be formed and can change and decay – and also that a few species hybridize and mingle at their borders."[261] Still, she indicates that, in modern (evolutionary) biology, the "evolutionary niche" has taken the place of the "species essence" – it is today what is believed to give "sharp edges" to the kinds of beings that can exist: "(…) actually very few evolutionary niches are available at any given time, and (…) these are normally far apart, accommodating only the rather widely varied creatures that now occupy them." Between the niches, nature is "inhabitable", Midgley states, and utterly inhospitable to beings like "mice with ears on their backs" or lion-tiger hybrids – "they could not survive in the wild." Midgley concludes: "Evolution (…) knows what it is about when it puts together the repertoire of characteristics that marks a species." I understand Midgley, from this analysis of the current state of nature, to conclude that "Nature Knows Best", and that creating beings that have no place in it is therefore demonstrably morally wrong. Species, then,

[258] Spaemann (2008), "Jedes nach seiner Art", Cicero, (Mai 2008).
[259] Wenzel (2007), "Rache der Chimären", Neue Zürcher Zeitung, 2007/09/06.
[260] Midgley (2000), "Biotechnology and Monstrosity - Why We Should Pay Attention to the 'Yuk Factor'", Hastings Center Report, **30**(5), p. 10.
[261] Ibid.

must be "taken seriously", because there is a principle telling us that (at least some) species characteristics "should not be moved".

Contrary to Midgley, I do not think that making use of modern evolutionary biology is of much help when defending the point that "Nature Knows Best". Firstly, I am troubled by Midgley's definition of "evolutionary niche". An evolutionary niche does not have to be in the wild. The niche concept can, more plausibly, be understood as meaning that all beings which are alive have, by virtue of being alive, and retaining the possibility of procreation, found their "evolutionary niche". This includes wild animals, but also pets which – from Chihuahua to Koi fish – could not survive in the wild (it also most likely includes a lot of human beings, e.g. short-sighted and/or weakish persons, like the majority of professional philosophers). This means that chimeras and other interspecifics, contrary to Midgley's assessment, *do* have an "evolutionary niche", even though they are not running wild in the woods like other participants in the competition of evolution: their niche is in labs and cages and in the willingness of humans to create and feed them. We can grant Midgley the point that interspecific chimeras do not live in the wild and do not occur without human intervention, yet the step from this assertion to the moral problematicity of their creation is hard to make. They are "unnatural" – probably yes, depending on your definition of "natural". But why this is an argument against their creation is not made clear by Midgley's argumentation. How can we make sense of her statements?

Midgley uses a telling metaphor when saying that "Evolution, in fact, knows what it is about (…)." In modern evolutionary biology, which Midgley stresses as her starting point in this paragraph, there is no way of saying that evolution "knows", "does", or "has in mind" anything at all. Behind this manner of speaking, there seems to be a view of Nature not as a random process, but as an almost personal being or "incorporated principle" that has aims and – most importantly – whose aims or intentions are morally relevant, *right* for us. I call this a "quasi-religious" view because Nature, crudely speaking, seems to take the place God occupies in other worldviews. It is this teleological view of nature which is the foundation for arguing against the creation of "unnatural" beings (i.e. beings that are openly at odds with the principle). Karpowicz et al. offer a similar interpretation of quasi-religious arguments (in their terminology: "The 'unnaturalness' argument"):

> *"This argument maintains that the operations of nature are to be understood and valued in terms of their purposes. It is indebted to Aristotelian thought, which asserts that every living thing has an inner tendency to reach its appropriate end or goal (telos) by exercising certain characteristic biological*

> *functions. According to traditional natural law theorists, the very fact that a living entity pursues a particular kind of life through certain biological processes is its own justification."*[262]

Once we assume such a teleological view of nature, it is possible that a promising intrinsic argument against the creation of chimeras could be made. In this sense, the "unnaturalness" argument need not immediately fail – natural features could then be regarded as having direct moral importance. Karpowicz et al. point out that the teleological kind of argument is far from helpful, though, mainly because we have no criterion for finding out when intervention is allowed and when it is against nature's aims, or which natural features or "aims" are morally relevant and which are not. Modern human life is basically identical with "intervening with nature"– it cannot be true, and it is probably not the claim proponents of this argument have in mind, that *all* interventions are wrong in themselves. The teleological route does not equip us with tools with which to find out which of them are. In any case, the road Midgley takes (i.e. via a teleological analysis of evolutionary biology) does not seem viable.

Another possibility for intrinsic objections to chimera creation, as we will see in the next section, is in a certain understanding of species boundaries.

3. The boundary between humans and nonhumans

The concept of a boundary between humans and nonhumans – and of the problematicity of crossing it by creating human-animal chimeras – is widespread in the discussion of interspecifics. Further analysis of this argument-type reveals that it would have to jump three hurdles by demonstrating that:

(I) There is a boundary between humans and nonhumans

(II) The boundary is morally relevant, i.e. there is a fundamental/categorical moral division between human and nonhuman beings

(III) Creating human-animal chimeras (or other interspecifics) constitutes a "crossing" or "violation" of the boundary

From these premises it would follow that creating human-animal chimeras or other interspecifics is morally wrong.

---

[262] Karpowicz, Cohen, et al. (2005), "Developing human-nonhuman chimeras in human stem cell research: Ethical issues and boundaries", Kennedy Institute of Ethics Journal, **15**(2), p. 113.

What is intrinsic about this type of argument is the assumption of a morally relevant boundary between humans and nonhumans – crossing the line, according to this approach, is wrong because of the innate sacredness of this boundary, not because of detrimental consequences its crossing might have. One can ask several central questions in this context:

Ad (I) What constitutes the "boundary" between humans and nonhumans? Is it genetic, or metaphysical (e.g. in the sense of humans and nonhumans belonging to different natural kinds)?

Ad (II) What confers moral relevance to the "boundary"? Why should there be a fundamental moral difference between humans and nonhumans?

Ad (III) In what way, at what point, is the boundary violated when someone creates an interspecific entity?

The first concept that comes to mind when thinking about what could constitute a proper "boundary" is that of (biological) species. Human beings, in contrast to other living beings, belong to the species *Homo sapiens*. To give a short glimpse of considerations to come: we will see below that the concept of species is today understood in a way that makes it difficult to accept it as the fundament of given, natural boundaries between kinds of beings (see chapter 3, section B.3.a below). But even if we did not have these problems with species concepts (which, for the sake of analysing this type of argument, I will assume for the duration of this chapter), we would need a good argument to explain why this biological categorisation should be deemed morally relevant for the question of chimera production, at all.

In their analysis of boundary arguments, Robert and Baylis refer to the notion of "fixed species boundaries" which are "inappropriate objects of human transgression".[263] They note that, far from concerning each and every biological species, only one particular species boundary is affected by this notion, namely that between human and nonhuman beings. Besides, the crossing of species lines cannot be immoral, as such, since it happens in nature (e.g. hybridisation of horse and donkey).[264] Robert and Baylis diligently consider the question of what could possibly fuel the moral power of this special boundary – and conclude that, in their opinion, there is no actual "human essence" or "species essence" that could be considered the root of a fixed boundary. Species boundaries are a "moral

[263] Robert and Baylis (2003), "Crossing Species Boundaries", American Journal of Bioethics, **3**(3), p. 2.
[264] Ibid.

construct",[265] and they reveal that worry about species boundaries is, in fact, a concern about something else. The human-animal boundary is a typical "taboo", they say – a social and moral shield held up against ambiguous things, because uncategorizable objects pose a danger to moral decision-making. Ultimately, Robert and Baylis find it unhelpful and mistaken to use the concept of "fixed boundaries" to argue against the creation of human-animal chimeras. The concern with human-animal chimeras, they say, is not really about a fixed boundary (because such a thing cannot be identified), but rather about "moral confusion" – i.e. an alleged consequence of chimera creation that will be discussed in detail in section D.3 below. Regarding my three-step analysis of boundary arguments, one could say that Robert and Baylis abandon this approach already at step (I): they believe that realism concerning the boundary is mistaken (because they think that boundary realism relies on species essentialism which they declare obsolete) – they also argue that, concerning step (III), it cannot be inherently wrong to "cross species boundaries" by, e.g., moving genes from one species to another, since such things also happen in nature. As we will see in more detail in chapter 2, section D below, Robert and Baylis are not prima facie disinclined to arguments belonging in the realm of step (II) (i.e. speciesism). Robert's and Baylis' influential discussion of the boundary argument was, as we have seen, not favourable.

On the other hand, there are several defenders of the view that there is a boundary between species (especially that between humans and animals) which is also a morally relevant border line. Mary Midgley, e.g., supports a kind of species realism which sees as the defining and determining element of species not a "hard essence", but rather the adaptedness of species to an evolutionary niche (Step I). Midgley does not explicitly state why violating the boundary would be morally problematic, but can be understood as using a teleological view of Nature (or "Evolution") to do so (Step II). I have made clear above that Midgley's route is not promising: because Midgley's account of evolutionary niches is mistaken, it fails to explain why chimeras violate the alleged boundaries between species (Step III), at all.

Robert Streiffer, to give another example, defends the moral relevance of species boundaries as at least possible. Regarding the question whether there is such a thing as "species boundaries", he states that just because there's disagreement about the boundary (i.e. the species concept), the concept isn't necessarily superfluous. He also doubts Robert's and Baylis' assumption that "crossing species boundaries" cannot be immoral as such since

[265] Ibid., p. 6.

it happens naturally: "(…) it can be natural for bacteria to move genes across species boundaries without it being natural for human beings to do so."[266] Streiffer does give some support to the concept of "species boundaries" – it might be useful and not necessarily superfluous for ethical debate. Still, his considerations do not, in my opinion, offer the "*Defense* of the Moral Relevance of Species Boundaries" the title promises, mainly because Streiffer's defence is grounded in a repugnance argument I do not find convincing (see chapter 2, section B.1.d above).

Louis Charland defends the species concept as such – claiming species can be understood as natural kinds – but he acknowledges that this doesn't do anything for explaining or erecting a morally relevant boundary (i.e., Step II): "None of this settles the question whether and how moral categories crosscut natural ones", he admits.[267] Charland does not address the questions subsumed under (II) or (III).

Cynthia Cohen[268] states that a species concept is *necessary* for keeping up the assertions Robert and Baylis make – we must first "understand which properties, features, characteristics and functions are distinctively and importantly human." – then we can decide in how far the created chimeras "have become human", i.e. "where the conceptual boundary between human beings and animals lies and when it has been crossed." In the following, she implies (or assumes) that "turning animals into human beings" is morally wrong, but gives no reason why it should be. Therefore, regarding step (III), Cohen's approach would supposedly be that creating chimeras is a transgression of the boundary if and in as far as it "turns animals into human beings." Similarly to Streiffer and Charland, Cohen defends the species concept in answering question (I), but shies away from giving answers to question (II) (i.e. the reasons for moral relevance of such a concept).

Like the commentators before him, Leo Zwanziger[269] defends the idea behind the species concept, stating that there is "significant and real, if not immutable, stability in Homo sapiens." Again, there is no mentioning of the question of moral relevance identified here as question (II).

At this point, we are confronted with commentators generally defending the usefulness of a concept of species boundaries or a firm boundary between human and nonhuman as such

[266] Streiffer (2003), "In Defense of the Moral Relevance of Species Boundaries", American Journal of Bioethics, **3**(3), p. 37.
[267] Charland (2003), "Are There Answers?" American Journal of Bioethics, **3**(3).
[268] Cohen (2003), "Creating Human-Nonhuman Chimeras: Of Mice and Men" American Journal of Bioethics, **3**(3).
[269] Zwanziger (2003), "Crossing Perspectival Chasms about Species", American Journal of Bioethics, **3**(3).

but shying away from the question whether and why it should be morally relevant, let alone the third step of formulating why and in what cases, exactly, creating human-animal mixtures constitutes a violation of the boundary defined. What could a successful boundary argument against the creation of human-animal interspecifics, specifically chimeras, look like?

Let me, again, resort to the crude outline of possible arguments described on p. 69-70. On the level of (I) – regarding the existence of a boundary – there are two roads one can take. One is an antirealist approach: "species", and, more specifically, the species boundary between humans and nonhumans, is understood as a mere "social construct" that has a value in as far as it prevents the occurrence of bad consequences. This is the road Robert and Baylis take in their article and which I will follow in section D. Such an approach would, however, leave the realm of intrinsic arguments that are the subject matter of this section. The other possible approach regarding question (I) is that of realism concerning species (at least in the sense of sticking to a classification into "human" or "nonhuman"). This is, basically, what defenders of the species concept like Charland and Zwanziger do. (We will see in chapter 3 that the problematicity of constructing an appropriate species concept, and more specifically, essentialism, are not only relevant for a discussion of human-animal chimeras, but also situated at the very centre of the speciesism debate).

Only at this point are we beginning to touch upon questions of morality, i.e. on questions of step (II). Why should species membership or, as it were, membership in a "natural kind", have moral relevance? One possibility here would be to assume that being a member of the natural kind "human being" confers the property of personhood or, more generally speaking, high moral status. The natural kind of "beings belonging to the species *Homo sapiens*" would be assumed to be coextensive with the natural kind of "beings deserving special moral consideration". This assumption is made by some who defend the moral privileges of members of our species as an ethical principle (i.e. by proponents of "Speciesism", see chapter 3, section B below). This should certainly be included in a typology of intrinsic arguments regarding chimeras, since it seems, at least prima facie, to do a satisfying job of connecting the realm of facts (existence of species) with that of morals (moral relevance of being a member of a species).

But does taking this route really help someone who intrinsically objects to the creation of (human-animal) chimeras? Let us recapitulate the course of the argument so far: the objector has stated that boundaries between species (especially between humans and nonhumans) do exist in reality/nature. Further, he has stated that these natural kinds are

relevant in a moral sense: beings which belong to the natural kind of "*Homo sapiens*" do also belong to the natural kind of "person". But there is a third hurdle to take. The objector must argue for the proposition that making a (human-animal) chimera constitutes a "violation" of the boundary he has identified.

Here, at step (III), my reconstruction encounters two problems: firstly, I cannot fathom how an objector to creating human-animal chimeras can take offense with mixing humans and animals, at all. A mixture in the sense of tainting the human essence with nonhuman parts (or vice versa) seems unimaginable in the conceptual framework presented. The natural kinds of "human/person" and "nonhuman/nonperson" must be mutually exclusive – otherwise, there would not be a clear boundary between them in the first place. Interspecifics in the sense we are discussing here – i.e. beings that are neither of the kind human/person nor of the kind nonhuman/nonperson (or part of both)[270] – seem conceptually impossible in such a view. Is that an argument against creating them – or rather, one against taking the route of declaring humans a natural kind?

Is there another rationale for declaring chimera creation a violation of boundaries? Putting aside the problematicity of natural kinds, one could, from the speciesist argument above, argue that crossing the human-animal boundary by "making an animal out of a human" (e.g. by injecting a huge amount of nonhuman stem cells in a human embryo) must be seen as morally wrong, since it destroys a person. This would not constitute a valid intrinsic argument against the creation of (human-animal) chimeras, though. For one, it would do nothing to explain why the opposite – namely, turning animals into human beings – should be deemed morally wrong (as is implied by, e.g., Cohen).[271] Considering that experiments done today are usually human-to-animal and not vice versa, this to me seems to be the bigger threat that is posed by chimerism experiments. With the suggested approach, such experiments might even be considered morally favourable – what, after all, could be wrong with creating a being that has the highest moral status (even if it is created from an animal)? This "uplift" scenario will be discussed further in section C.2.2 below.

Ultimately, it remains unclear what ethical principle an intrinsic boundary argument would be based on. What exactly is it that would make introducing living animal parts into human bodies (as in the case of chimeras), or alien genes (as in the case of transgenics) intrinsically

[270] This view, in an analogy to the doctrine of the two natures of Christ, is defended by DiSilvestro (2004), "A Neglected Solution To The Problem Of The Metaphysical And Moral Status Of The Human-Animal Chimera", Ethics and Medicine (Summer 2004).
[271] Cohen (2003), "Creating Human-Nonhuman Chimeras: Of Mice and Men", American Journal of Bioethics, **3**(3), p. W5.

wrong, while living together with nonhuman animals as pets, touching them, or even eating them, would *not* constitute such an intrinsically wrong "boundary crossing"?

4. Human dignity arguments

Several bioethicists analyse the creation of human-animal chimeras in relation to the notion of human dignity. Human dignity concerns are intrinsic concerns – they do not refer to specific interests of humans or animals or animal-human mixtures which are supposedly violated as a consequence of creating interspecifics, but to the general and abstract concept of "dignity" which makes, some argue, creation of interspecifics wrong in principle.

The most extensive discussions of dignity approaches are given by Johnston and Eliot (2003), Karpowicz, Cohen and Van der Kooy (2005), and Ravelingien, Braeckman, et al. (2006).[272] Others argue that "human dignity" is a nebulous and vague term, that there is no agreement on how it should be understood exactly, and that it adds nothing to the host of concerns for the wellbeing of humans which are brought forward without referring to the notion of "dignity", at all. Robert and Baylis,[273] for example, and with them the majority of authors in both the AJOB 2003 and 2007 issues concerned with chimeras, leave "human dignity" out of their analysis of chimera creation altogether; likewise, the CHIMBRIDS project opening discussion was highly sceptical of "human dignity" approaches.[274]

Nevertheless I think that the human dignity concept adds a perspective to the discussion that differs considerably from just stating that human beings might be treated inadequately. "Simple" concerns for the wellbeing of humans, as described in section C.2.b below, are based on the assumption that chimera creation might violate the interests or the rights of humans (or part-humans). Claiming that human dignity is being violated is a much stronger, and structurally different, claim – in particular because a genuine violation of human dignity is not justifiable by means of a cost-benefit analysis, which means that an

---

[272] Johnston and Eliot (2003), "Chimeras and "Human Dignity"", American Journal of Bioethics, **3**(3); Karpowicz, Cohen, et al. (2005), "Developing human-nonhuman chimeras in human stem cell research: Ethical issues and boundaries", Kennedy Institute of Ethics Journal, **15**(2); Ravelingien, Braeckman, et al. (2006), "On the moral status of humanized chimeras and the concept of human dignity", Between the Species, **VI**.

[273] Robert and Baylis (2003), "Crossing Species Boundaries", American Journal of Bioethics, **3**(3); Robert (2006), "The science and ethics of making part-human chimeras in stem cell biology", Journal of the Federation of American Societies for Experimental Biology, **20** ; Baylis and Robert (2007), "Part-Human Chimeras: Worrying the Facts, Probing the Ethics", American Journal of Bioethics, **7**(5).

[274] "The whole discussion reflected the general difficulty that even outside the ethical debate on chimeras and hybrids no consistent definition of human dignity has been found so far and that a consequent use of one specific concept of human dignity, which is convincing for one certain situation, might lead to unwanted conclusions in a different context." Weschka (2006), "Protocol of the CHIMBRIDS Opening Conference on 11/12 March 2006", p. 7.

argument from this approach would be much stronger than the consequence-based arguments discussed below. The statement that human dignity is "inviolable", in this sense, is a prescriptive one which means that violations of dignity cannot be justified or balanced out with other values (e.g. wellbeing).

Apart from concerns about outright violation of dignity, there are also concerns that chimera creation might lead to constrictions or limitations of human dignity. Such constrictions or limitations – "threats to human dignity" – might be somewhat more easily justifiable – they fuel consequence-based rather than intrinsic concerns. As Resnik puts it, "it is not reasonable to prevent all possible threats to human dignity, because this strategy would require societies to forego important opportunities or violate basic rights."[275] But still, such threats could be the basis for viable arguments against human-animal chimera creation: especially in the form of slippery slope arguments, they are influential in many bioethical debates. A variant of such concerns for a (indirect) threat to human dignity will be discussed in section D.3 below.

In the typical phrasing of "human dignity" approaches, human beings' *special characteristics* demand that they be treated as means, not only as ends. These characteristic(s) are defined in varying ways – as the ability to act in order to fulfil purposes (cf. Alan Gewirth),[276] as being created in the image of God (imago dei) (cf. Christian approaches), as a bundle or family of valuable capacities (cf. Karpowicz, Cohen, Van der Kooy),[277] or they can be found in the role of humans as moral subjects (cf. Kant).[278] Ravelingien's adaptionist approach to human dignity tries to identify uniquely human characteristics that are responsible for "human dignity" as "those adaptations that arose in response to the particular adaptive problems not shared by the ancestors of other species."[279]

Different interpretations of the "special characteristic" notwithstanding – what should be central to our analysis of human dignity concerns is the question of what, exactly, the violation, constriction or endangerment of human dignity consists in in the case of creation

---

[275] Resnik (2003), "Patents on Human-Animal Chimeras and Threats to Human Dignity", American Journal of Bioethics, **3**(3), p. 35.
[276] Gewirth (1992),"Human Dignity as the Basis of Rights", in: Meyer and Parent (eds.) The Constitution of Rights: Human Dignity and American Values.
[277] Karpowicz, Cohen, et al. (2005), "Developing human-nonhuman chimeras in human stem cell research: Ethical issues and boundaries", Kennedy Institute of Ethics Journal, **15**(2), p. 118 ff.
[278] "Allein der Mensch, als Person betrachtet, d. i. als Subject einer moralisch=praktischen Vernunft, ist über allen Preis erhaben; denn als ein solcher(…) ist er nicht blos als Mittel (…) sondern als Zweck an sich selbst zu schätzen d. i. er besitzt eine Würde (einen absoluten innern Werth) (…)", see Kant (1797/1968), Die Metaphysik der Sitten, pp. §11, 434-435.
[279] Ravelingien, Braeckman, et al. (2006), "On the moral status of humanized chimeras and the concept of human dignity", Between the Species, **VI**.

of human-animal chimeras or other interspecifics. Since not all types of interspecific-creation raise these issues (xenotransplantation of insulin-producing porcine cells, for example, is rarely mentioned in respect to dignity violations), there must be something about specific types of interspecific-creation which makes them problematic in this regard.

According to Karpowicz et al. (2005), there are different ways of violating human dignity:

(a) Human dignity is violated when individuals with valuable capacities are kept from exercising them. Examples for this are slavery or any kind of forcible coercion, which keeps humans from acting freely and deciding for themselves, which in turn makes their role as moral agents dubitable.

(b) Human dignity is even more severely violated when human beings which are in possession of valuable capacities are wilfully robbed of them. Murdering a human being is the worst possible case of this type, since it means denying a human all capacities; mutilation, in as far as it leads to a permanent diminishment of valuable capacities, is also regarded as an especially reprehensible violation of human dignity in this sense.

What, then, does the dignity violation consist of in the case of creation of human-animal interspecifics? The identification of a violation would presuppose that special characteristics/valuable capacities are affected – it is not conceivable, e.g., how the transfer of human muscle or renal cells into an animal could count as a violation of human dignity in these senses. Accordingly, Karpowicz et al. assume a transfer of those physical components that are necessary for "valuable capacities" from a human being to an animal host.

Let us look first for dignity violations of type (a), i.e. cases where beings are kept from exercising valuable capacities. One would find them in the (hypothetical) case of chimeras which do have valuable capacities, but which are prevented from exercising them because of the lab setting they are kept in. This is not a direct argument against the creation of human-animal-chimeras, but rather, parallel to inadequate treatment arguments (section C.2.c below), an indirect one. Assuming that the physical components transferred to the animal do *not* induce the development of valuable capacities, the chimera is not kept from exercising the latter and human dignity is not violated (at least not in the sense of type (a)).

For the sake of argument, Karpowicz et al. discuss the case of a whole-brain transfer from human to animal host and state:

> *"The development of such a chimera arbitrarily would limit the ways in which certain human characteristics and capacities associated with human dignity could be exercised in a nonhuman setting and therefore would contravene human dignity."*[280]

The situation of a whole-brain transfer into an animal seems, in this respect, to be similar to cases in which a human individual's body is mutilated or otherwise constricted (i.e. by drugging) in order not to exercise "valuable capacities". Accordingly, this would be a quite straightforward dignity violation of type (a). Such a procedure is wholly hypothetical, though, and certainly not what is currently understood by human-animal chimera creation.

As for dignity violations of type (b), it is doubtful here who is robbed of valuable capacities when physical components which are necessary for the former are removed and transferred to an animal host. The animal host organism cannot be the subject at issue, since it does not have valuable capacities before the transfer. If we, to follow Karpowicz et al., assume the transfer of big, undissociated portions of neuronal cells taken from an aborted fetus, we could prima facie understand this as "robbing" the fetus of something. But this approach raises several questions. Firstly, how could the fetus be robbed of valuable capacities it does not have (such as reason, being a moral agent, consciousness, etc.)? This could maybe be remedied with an argument from potential (although I am sceptical of such approaches). Secondly, and more importantly, it is to be assumed that *if* a human dignity violation takes place in the course of chimera creation with material taken from aborted fetuses, at all, it should be interpreted to take place in the act of abortion (or, in other scenarios, killing of the embryo which is created specifically for experimentation). This is the point at which the fetus can sensibly be regarded as being "robbed" of something. How its tissues are used afterwards, and, particularly, whether they are implanted into alien bodies, seems completely irrelevant regarding the primary violation that has already taken place. Finally, whether human tissue is transferred into a nonhuman body in this process, i.e. the "chimera creation" itself, does not play any role in whether we regard this as "dignity violation" regarding the embryo. Accordingly, I find it hard to identify a "dignity violation" in human-to-animal embryonic chimera creation itself – be it in sense (a) or in sense (b). If at all, this construction seems to work against all embryo-destructive research (and abortions), but is not conducive to arguing against chimera creation, in particular. Baylis, in her analysis of the prohibition of human-nonhuman primate blastocyst grafts, has similar

[280] Karpowicz, Cohen, et al. (2005), "Developing human-nonhuman chimeras in human stem cell research: Ethical issues and boundaries", Kennedy Institute of Ethics Journal, **15**(2), p. 123.

difficulties finding the point of violation of human dignity in such scenarios.[281] This critique does not rule out that a point of "dignity violation" could be identified in certain scenarios of human-animal interspecific creation in the future, but this point seems to require more clarification. Our difficulties in finding what the specific conditions of a dignity violation would be are similar to problems in the context of "boundary" arguments, where a specific, consistent principle stating what makes some kinds of species-crossing morally reprehensible while other types are neutral could not be given (see section B.3 above).

Additionally, the question may crop up whether human dignity talk is helpful, at all, when discussing human-animal chimera creation. What is so special about "human dignity"? The concept brings with it three characteristics that, I believe, are indispensable if one wants to take it seriously, at all:

(1) Firstly, human dignity is understood as something a subject either partakes in or does not partake in, i.e. an absolute value that is not doled out in degrees. Partakers in human dignity are not only different, but of a wholly different category than other beings.

(2) Secondly, a basic idea behind human dignity is the view that it does not depend on certain characteristics of the individual. The human individual does not have to jump any hurdles in order to gain this status – he or she has it, uncontestedly, in virtue of being human. Höffe, in his discussion of the concept, calls this aspect "Mitgiftwürde" (human dignity as an unmerited "dowry"), and observes that the contrasting aspect of "Leistungswürde" (human dignity as accomplishment) is, rather than being a precondition, just an appendix to this central characteristic.[282]

(3) Thirdly, human dignity approaches assume or imply speciesism, i.e. the position that human beings are fundamentally morally superior to nonhuman beings.

In addition to the assumed "inviolability" of human dignity mentioned at the beginning of this section, these three characteristics – being *absolute*, being *unconditional,* and *only applying to human beings* – are what distinguishes human dignity from other values, and therefore what distinguishes human dignity arguments from more straightforward or simple arguments that claim a plain violation of interests of living beings (i.e. arguments of the type spelled out in section C.2 below). I deem these characteristics to be indispensable, essential parts of the concept of human dignity. Yet, these very characteristics are also what could make

[281] Baylis and Robert (2007), "Part-Human Chimeras: Worrying the Facts, Probing the Ethics", American Journal of Bioethics, **7**(5), p. 202.
[282] Höffe (2002),"Menschenwürde als ethisches Prinzip", p. 132, in: Höffe, Honnefelder, et al. (eds.) Gentechnik und Menschenwürde - An den Grenzen von Ethik und Recht.

human dignity arguments problematic in the context of assessing the moral relevance of creating human-animal chimeras.

This is, firstly, because under some circumstances it might be hard to determine whether a being is human or nonhuman (think, e.g. about the humanness of the hypothetical case of a human-chimpanzee hybrid, or about the humanness of rabbit-human cybrids). Using species-membership as the determining factor for moral status, under these circumstances, might not be advisable. The assumption that every being falls clearly either into the "human" or the "nonhuman" category, and that this classification is central for the question of whether a being is accorded human dignity, is problematic. The part of "special characteristics" as preconditions for dignity, at this point, seems to be reduced to a mere appendix of the human dignity concept: If a being is human, it is accorded human dignity no questions asked, i.e. even if it does not exhibit any of the "special characteristics", or only exhibits them to a small degree. The most striking problem of using human dignity argumentation in the discussion of human-animal chimeras, then, is its inherent speciesism: Human dignity approaches assume fundamental human superiority. In the context of the animal rights debate, the assumption of "human dignity" (as opposed to any other kind of ethical value beings can have) is begging the question. The very term "*human* dignity" assumes and implies speciesism, i.e. a fundamental difference between humans and nonhumans. Baylis and Fenton identify the tension resulting from this connection in Karpowicz, Cohen et al. 2005, who work with the concept of "human dignity" and, according to their critics,

> *"want to both (a) value certain human functions and capacities for their own sake and not because they are human and (b) value certain human functions and capacities because they are human and not for their own sake. At the same time, both of these points in tension rely on an implicit appeal to a principle conferring intrinsic moral value on x if x belongs to a class A that contains members who manifest certain cognitive or emotional capacities, even if x herself does not. X is thus valued, or possesses moral significance, because x is a member of class A. In this case, the class is all humans."*[283]

That this connection between "human dignity" and "speciesism" is a necessary one is disputed by, e.g., Otfried Höffe, who states:

> *"Should there be beings with a similar capacity for reason on other planets of the universe, though, then these beings would deserve the same dignity. Arguing*

[283] Baylis and Fenton (2007), "Chimera Research and Stem Cell Therapies for Human Neurodegenerative Disorders", Cambridge Quarterly of Healthcare Ethics, **16**(2), p. 201.

> *against Peter Singer, therefore, this is not a case of morally disputable kind-egotism ('speciesism')."* [transl. CH][284]

This saving of human dignity approaches from the accusation of Speciesism does not work, in my opinion, because Höffe does not address Singer's (and other Anti-Speciesists') undoubtedly strongest argument, i.e. the Argument from Marginal Cases. A central characteristic of human dignity, for Höffe, is that it is accorded to all human beings, independently of merits or achievements. Human dignity is unconditional, innate, an unmerited "dowry" (Mitgiftwürde), and its sphere includes beings which cannot "answer for their own dignity" [transl. CH], such as babies, the mentally ill and slaves.[285] Nonhuman beings, on the other hand, are *excluded* from this sphere of beings that are accorded dignity although they also cannot "answer for their own dignity". This is because they do not belong to the biological species "human" which, according to Höffe, is not necessary but (in the case of beings who "cannot answer for their own dignity") *sufficient* for belonging to the sphere of carriers of dignity. Accordingly, human and nonhuman beings are measured by fundamentally different standards – if you are human, you are accorded dignity no matter what, if you are nonhuman, you must jump hurdles. So human dignity approaches *do* bring about speciesism. Whether that position is in fact "morally questionable", though, is a distinct issue that shall be discussed in chapter 3, section B below. Note that the success of dignity approaches, at this point, seems to crucially depend on whether Speciesism is defensible.

Let me conclude this discussion with a roundup: it appears that, if at all, only the creation of those human-animal interspecifics that are "humanized" (in the sense of having "valuable capacities" that yield superior moral importance) could be countered by human dignity arguments. However, even in these cases I found it difficult to pin down what exactly the violation of human dignity would consist in, a point that would need more elaboration by supporters of "human dignity" arguments. One argument one could make would be that *any* use of embryos for research (and abortion) is unjustifiable under all circumstances, since human embryos have valuable capacities (or at least potential valuable capacities), and destroying them robs them of the latter and thereby constitutes a violation

[284]"Sollten sich allerdings auf anderen Planeten des Universums ebenso vernunftbegabte Wesen finden, so gebührt ihnen dieselbe Würde, weshalb – gegen Peter Singer gesagt – kein moralisch fragwürdiger Gattungsegoismus ("speciesism") vorliegt.". Höffe (2002),"Menschenwürde als ethisches Prinzip", p. 119f., in: Höffe, Honnefelder, et al. (eds.) Gentechnik und Menschenwürde - An den Grenzen von Ethik und Recht.
[285] Ibid., p. 122 - "Wesen (…) die für ihre Würde nicht aufkommen können".

of human dignity. Additionally, the success of human dignity arguments depends on whether the Speciesist assumption they presuppose is defensible in some way.

As mentioned at the beginning of this section, some arguments from human dignity do not assume a *direct* violation of dignity by creation of human-animal chimeras, rather, they suspect that allowing the creation of human-animal chimeras could ignite a process which would, in final consequence, lead to dangers for human dignity. Via a slippery slope from seemingly marginal encroachment on human dignity, the collective worth of humanity could be seriously endangered (leading, in turn, to dangers for individual humans, and inadequate treatment). Arguments of the slippery slope type, which state that interspecific creation could have indirect disadvantages for human dignity, will be discussed in chapter 2, section D below, as they are consequence-based rather than intrinsic arguments.

5. Intrinsic arguments: Conclusion

I hope that I have made sufficiently clear in this section that intrinsic arguments might not be the best route to take when trying to argue against experiments that involve the creation of human-animal interspecifics. Arguments of the "repugnance" type and "quasi-religious" arguments are powerful and popular in the chimera debate, but they are not accessible to anyone who is not repugned by the idea of such creatures or who is not religious or believes in nature as a quasi-god, posing a teleological principle that should govern our actions. Arguments of the boundary type appear to be more promising and more accessible to debate. Still, my analysis reveals that there are several hurdles to take. Firstly, a convincing argument for (human) species realism must be made; secondly, the view that this boundary is morally relevant must be defended; and thirdly, it must be explained in how far the creation of human-animal interspecifics does violate the boundary while other kinds of mixing with nonhuman animals do not. This leaves open the questions of "species realism" or essentialism, which touches in turn on the defensibleness of some kinds of speciesism (I will come back to further discussion of this point in chapter 3 below). Thus, even boundary arguments – a type of intrinsic argument that is commonly brought forward and intuitively appealing even to non-religious people – have proven to be hard, if not impossible, to spell out in a coherent way. Dignity arguments fail for a very similar reason: it remains hard to phrase a consistent ethical principle which spells out why, and in what way exactly, the creation of human-animal interspecifics constitutes a violation of human dignity, while other kinds of "mixing" of human with animal are supposedly unproblematic.

Additionally, the concept of human dignity presupposes speciesist assumptions which, as we will see in chapter 3, section 3 below, are highly problematic.

C. Direct consequence-based objections

Not all objectors to interspecific experimentation rely on intrinsic arguments, and indeed, powerful resistance to such research is possible without resorting to such types of objection. Let us have a look at the more tangible type of argument which refers to the possibility of disadvantageous consequences (costs) of such research. Looking at the debate around interspecific experiments from this angle, we are presented with a wide array of objections, ranging from very direct concerns (e.g. for animal welfare) to quite indirect or abstract ones. First, let us have a (relatively short) look at possible benefits of interspecific research.

1. The benefit side

Discussing chimerism research in a consequentialist framework would not make much sense if one would take the side of possible benefits of this research out of the equation. I will not extensively comment on the benefit side of this analysis, though, but rather give some introductory remarks in this regard.

The potential or actual benefit of experiments involving interspecific entities varies greatly – which is true for any basic, not yet directly therapeutically applicable research. Looking at Irving Weissman's (proposed) work, for example, I find it to be quite plausible that the development of disease models like the "human neuron mouse" could offer many advantages regarding the improvement of our knowledge of how brain stem cells work to advances as tangible as screening of psychiatric drugs in a environment similar to a human brain. Greely's working group comes to a similar result when assessing potential benefits of Weissman's experiments.[286]

On the other hand, there are chimerism experiments that seem to have no benefit apart from satisfying the curiosity of the researcher. Andrzej Tarkowski, a Polish embryologist and pioneer of mouse chimera research,[287] notes in his recollections regarding interspecific (animal to animal) chimera experiments:

---

[286] Greely, Cho, et al. (2007b), "Thinking About the Human Neuron Mouse", American Journal of Bioethics, **7**(5), p. 32.
[287] Tarkowski (1961), "Mouse chimaeras developed from fused eggs", Nature, **190**.

> *"For those who love experimenting in general, and in whom the childish curiosity and fantasy have not been yet completely ousted by logic and coolness of a respectful adult scientist, this is a wonderful experiment to do, but…(see below). (…) Although creation of interspecific mammalian chimaeras is indeed a spectacular experiment, in the author's opinion its contribution to embryology and genetics of mammals has been rather limited and disappointing."*[288]

The creation of interspecific (nonhuman) chimeras has turned out to be of almost no use for the embryologist, apparently. I say "turned out" since, as in all areas of research that are still in their infancy, it is impossible to predict what kinds of benefits one might one day reap from them. An area of research that seems highly promising today might turn out to be a dead end in the future, as cross-species chimerism research apparently has for embryology. Also, it is imaginable that research results that might today seem only accessible via chimerism research might, at some point in the future, turn out to be researchable by other means – chimerism experimentation might turn out to be a detour in retrospect.

A prognosis of the future successes of basic research notoriously carries pronounced uncertainties. It seems extremely hard to make any useful statement on whether interspecifics research as such, or certain areas of it, e.g. human-animal chimera or cybrid research, will reap benefits. Even regarding specific experiments, it might prove to be impossible to sensibly predict whether they will, in retrospect, turn out to have promoted scientific success in a meaningful way. This uncertainty runs deep in the character of basic research.

I will assume here that the odds are somewhat skewed towards the point of view that the bulk of research done, in the long run, is reasonable or justified in some way. The reasons for this assumption pertain not to special moral qualities or benign intentions of scientists, but to pure mechanics of the research industry. Scientists, who are confronted with a situation of scarcity – funding for basic research is hard to access – are generally not interested in wasting money and time on unjustified or unreasonable experimentation, because this would hardly further their own long-term financial and status-related interests. These advantageous mechanics, evidently, can get skewed over short periods of time or in some areas of research. For example, the crude and dangerous "revitalisation" therapies of the 1930s (see p. 23) probably do not jump the hurdle of reason; neither does Ivanov's

[288] Tarkowski (1998), "Mouse chimaeras revisited: recollections and reflections", International Journal of Developmental Biology, **42**.

work regarding the hybridisation of human and ape (p. 30).[289] The benefits of these (ultimately botched) ventures were not immediately tangible even at the time they were tried. I deem these cases to be rare exceptions from the rule that researchers usually, on average, have good reasons to believe that what they do will probably result in concrete scientific or medical benefits (this does not imply that I believe they necessarily *will* result in such benefits in all cases).

My assumption of overall reasonableness may sound trivial, yet I believe that stating it openly is important. Some popular objections to the creation of interspecifics (including the "hubris" concerns of section B.2.b above) work with or even crucially rely on the topos of the "mad scientist" who is completely cut off from common sense and allegedly does everything he does "simply because it can be done".

My analysis of consequentialist concerns will, from now on, concentrate on the cost side of the calculation, as this is what the debate focuses on. What bad consequences does (or could) interspecifics research lead to?

2. Bad consequences for the entities created

Many objectors to interspecific experimentation have concerns for the beings that are used in, or result from, inter-species experiments. There are arguments that work independently of the question of whether the beings qualify as "humans", but also arguments that only apply once the interspecific is identified as human or "part-human". I will begin with arguments of the former type, concerned with the protection of living beings in general (see section a below), and proceed to arguments of the latter type, concerned with the protection of human beings, or human materials/structures that qualify for special protection (see section b below). I will then discuss objections that focus on the undetermined, undeterminable or at least preliminarily unclear moral status of interspecific novel beings, which some think puts them in an especially dangerous position for exploitation and abuse (see section c below). The final part of this section will be concerned with the moral relevance of intentionally "shifting" the moral status of living beings (see section d below).

[289] Rossiianov (2002), "Beyond Species: Il'ya Ivanov and His Experiments on Cross-Breeding Humans with Anthropoid Apes", Science in Context, **15**(02).

a. Animal welfare concerns

Opponents to experimentation on animals in general will also come to doubt whether experimentation of the type we see in interspecific research is justified or justifiable. All types of objection given in this section presuppose a (minimum) concern for animal interests, i.e. they would not be supported by someone who thinks that animals do not feel pain, do not have "interests" in the widest sense, or that their pain or distress is not morally relevant at all.[290] Such concern usually is more pronounced concerning higher-developed animals – most prominently primates,[291] but also other mammals like rats, mice, etc. – while few would see pronounced ethical problems concerning experimentation in jellyfish or molluscs (aside from holists who, in extreme cases, assume that even natural phenomena, like rivers or forests, have "interests"). This kind of (pathocentric) argument is not limited to human-animal interspecifics, but to basically all kinds of chimeric, hybrid or transgenic novel beings.

In regard to concerns about animal welfare, the killing of research animals – which is regularly and systematically carried out in succession to completed experimental series – can be seen as a moral problem, even when it is done painlessly. This concern is, in many regards, distinct from the question whether harming animals or cruelty towards them is morally problematic. It is conceivable for someone to consistently allow for the painless killing of animals while objecting to causing animals pain in almost all circumstances (e.g. by stating that animals are not "harmed" by death because they have no continuing self-awareness), just as it is a consistent moral position to object to killing animals, in principle, while assuming that the causation of pain can be justified quite easily (e.g. because pain is reversible while death is permanent).[292]

Putting aside the question of whether painless killing of animals is problematic, the classic objection to animal experimentation, and therefore also to chimera research, is that many experiments cause notable or even extreme amounts of distress or pain in animals, and that this is not, or cannot be justified by benefits for human beings. Argumentation of this type

[290] Alternatively, indirect objections to cruelty to animals could be offered, e.g. a Kantian formulation that believes that cruelty should be avoided since it is detrimental to human character.

[291] For example, an asssessment of human-nonhuman primate neural grafting of the Working Group on the Criteria for Cell-Based Therapies at John Hopkins University states that "Some group members have serious ethical concerns over *any* use of nonhuman primates in invasive research. However, we set aside broader controversies to focus on ethical challenges specific to human-to-nonhuman primate (…) neural grafting." Greene, Schill, et al. (2005), "The Working Group on the Criteria for Cell-Based Therapies, John Hopkins University: Moral Issues of Human-Non-Human Primate Neural Grafting", Science, **309**.

[292] On the general question of animal killing, see: Singer (1979), "Killing Humans and Killing Animals", Inquiry, **22**(Summer 1979); Jamieson (1983),"Killing Persons and Other Beings", in: Miller and Williams (eds.) Ethics and Animals; Young (1984), "The Morality of Killing Animals: Four Arguments", Ethics and Animals, **5**(4).

will focus on the amount of pain or distress inflicted upon the experimentation subjects, thereby declaring such experimentation morally unjustifiable. Arguments mentioning the aspects of chimera and other interspecific research that are detrimental to animal welfare are brought forward by Rollin[293] and (more indirectly) by Urie, Stanley and Friedman.[294] The latter call for a standard in scientific and medical experimentation that requires "full disclosure and informed consent (…) regardless of species", which would most probably rule out any use of animals in science, not only in the field of chimera or interspecific research.

The general discussion of animal use in science aside, let us have a look at one animal welfare aspect that is specific for research involving the creation of chimeras: interspecific chimeric animals (be it human-to-nonhuman or animal-to-animal) are especially prone to developing severe and debilitating or fatal medical problems. Interspecies chimerism experiments produce adult animals in only a small minority of cases: the bigger the "genetic gap" between the species involved, the bigger the risk of severe malformation – most interspecific chimeras, therefore, die off before birth. Bernard Rollin hints at a similar problem with regard to transgenic beings when asking: "[M]ight hybrids be harmed or diseased in some way simply because they are transgenic?"[295]

Rollin also mentions other fears concerning what he calls the "plight of the creature" – he speaks of "harming animals for human benefit, as in genetically engineering suffering animals as models for human disease", and asks:

> *"Would we enslave them (as when rumors were rife about genetically engineering human traits into chimps so that they could perform tasks that human beings abhor)? Would we create them as cannon fodder?"*[296]

This is not a direct argument against chimera creation; we could treat the newly created beings appropriately, after all. In contrast to concerns I will address in section c below, Rollin is not up in arms against such enterprises because he thinks animals infused with human genetic material deserve, as such, special protection that would not be granted in animal testing labs: using "normal" animals as "cannon fodder" would, in principle, be just as problematic for Rollin, who embraces a non-speciesist, pathocentric perspective.

---

[293] Rollin (2003), "Ethics and Species Integrity", American Journal of Bioethics, **3**(3).
[294] Urie, Stanley, et al. (2003), "The Humane Imperative: A Moral Opportunity", American Journal of Bioethics, **3**(3).
[295] Rollin uses "hybrid" and "transgenic" in a very wide sense here, probably denoting all kinds of "altered" beings - Rollin (2003), "Ethics and Species Integrity", American Journal of Bioethics, **3**(3).
[296] Ibid., p. 17.

b. Concerns for human embryos, gametes, and genes

In chapter 1, section B.4 above,, we saw that, in many cases, research resulting in human-animal interspecifics of diverse kinds uses human embryonic cellular material. Human-to-animal chimeras are typically made by introducing human embryonic stem cells, or cells derived from hESC lines, into animal organisms. Ebryonic stem cells are obtained from embryos in an early stage of development, and are especially useful for research because of their pluripotency.

It is argued that any research that destroys human embryos warrants very careful ethical consideration and justification, or even that such research is not justifiable, at all; because human embryos have a special moral status – be it because they belong to the species *Homo sapiens* (argument from species membership), because of the moral continuum from conception to birth (argument from continuity), because they are identical with the "fully human" being they will be later on (argument from identity), or because they have the potential to become such a "full" human being (potentiality argument).[297] The protection of the human embryo can be limited, or it can be seen as growing continually along a developmental scale, but it can also amount to the view that the human embryo deserves the same full amount of protection any adult human warrants from conception on. Some have argued that every type of experimentation with human embryos should be completely banned;[298] this demand would certainly also extend to all kinds of human-animal chimeric experimentation which involves the use of human embryos. Many, if not most, of these arguments for the special protection of human embryos are grounded in Speciesist assumptions (see chapter 3, section B below).

Human fetal tissue used in interspecific research is usually obtained from intentionally aborted fetuses because this source has numerous advantages to using spontaneously aborted or stillbirthed fetuses (i.e. cells are fresher and in better condition, usually not tainted with pathogens or carriers of genetic disorders). Especially in the U.S., where abortion remains a controversial topic, there has been an ongoing debate since the 1980s about the propriety of using human fetal tissue from fetuses that have been intentionally aborted.[299] "Pro-life" positions aside, even many "pro-choice" advocates would probably not support research that relies on fetuses that would otherwise not have been aborted.

---

[297] For a comprehensive overview, see Damschen and Schönecker, Eds. (2003), Der moralische Status menschlicher Embryonen.

[298] See e.g. Annas, Andrews, et al. (2002), "Protecting the Endangered Human: Toward an International Treaty Prohibiting Cloning and Inheritable Alterations", American Journal of Law & Medicine, **28**.

[299] Greely, Cho, et al. (2007b), "Thinking About the Human Neuron Mouse", American Journal of Bioethics, **7**(5), p. 33.

Intentional abortion – or even abortion forced on unwilling or persuaded and pressured women – in order to obtain fetal material is a scenario that makes a vivid argument against the use of fetal tissues. Use of fetal tissue from intentionally aborted fetuses does not necessarily lead to a rise in abortion numbers or to pressuring women into abortion, though (although the possibility that individual womens' choices are skewed towards abortion as soon as they know that "it might do some good" probably cannot be completely ruled out). The topic of abortion and the closely connected fetal tissue research debate cannot be discussed here in detail. Many or even most of the experiments discussed in chapter 1 would be exposed to arguments against research using human embryonic stem cells and fetal tissues, as mentioned in my excursus on the legal situation of chimera research (chapter 2, section D above*).* Chimera creation which makes use of *adult* stem cells or precursor cells would avoid the discussion around embryonic stem cell use, on the other hand.

It is controversial whether the creation of nucleo-cytoplasmic hybrids (cybrids, see p. 32) can be said to constitute a "use of human embryos" – after all, what would purportedly be used is only a human cell nucleus implanted into an enucleated animal egg. Views which assign full moral status to human beings after the fusion of egg and sperm do not, prima facie, understand cybrids as human embryos, since no egg and sperm are involved and fusion in the traditional sense does not take place. It would also be quite difficult to construe identity, potentiality, species membership or continuity arguments for cybrids: they will not and, it is said, cannot possibly develop into adult beings, so they are not identical with humans, there are no potential humans, nor do cybrids slide on a "normal process"-continuum towards humanness. As is plausibly argued, the cybridic being could be considered a "human embryo" in a wider sense (being used as source of human embryonic stem cell lines and having just a very small part of nonhuman DNA in its mitrochondiae). The ethical characterisation of the cybrid should take these factors into account. In this sense, at least an argument from species membership could probably be construed in the favour of outstanding moral status in human-animal cybrids and therefore, indirectly, against cybrid experimentation which ends in destruction of the cybrid. Again, such an argument would depend on the defensibility of Speciesist assumptions which will be discussed in chapter 3, section B below..

Some see the human gamete (egg or sperm) as precursor of human life that requires special protection. The circumstances of the harvestation of these materials are regulated in most countries, and so is their use (some legislations, e.g., forbid the sale of human eggs and/or

sperm). It is therefore easy to infer that creation of human-animal hybrids by fusion of gametes would cause many ethical concerns just in regards to the protection of human gametes (apart from the numerous other ethical concerns such an undertaking would give rise to).

Due to considerations similar to those concerning gametes, human-typical genetic sequences ("human genes") are, by some, regarded as deserving special safeguarding against commodification and utilisation – this concern would affect the creation of transgenic beings which are manipulated to contain sequences of human genome.

Finally, Hank Greely's 2007 working group résumé points out that human tissue also warrants respectful, especially careful handling (derived from special treatment that is usually reserved for human bodies). Different cultures might have very different views on what kind of behaviour is appropriate concerning human tissue – some organs might have special symbolic value, e.g. the heart and especially the brain which is today, by many, seen as the "seat of consciousness". Human-animal interspecific chimera bodies which contain human tissue should, according to these considerations, be treated as medical waste that is properly disposed of; the consumption of human-animal chimeras by other animals should be avoided.[300]

While the human embryo is, by many, seen as having a "value in itself", concerns for the proper handling of gametes, genetic material and tissue do not rely on such an inherent value, but rather see the careful handling and non-commodification of these as indirectly conducive to other goods, e.g. "human dignity".

Let me recapitulate the main types of concern regarding human precursors or, more generally, human biological materials human-animal interspecific experimentation can lead to:

- The concern of unjustified or unjustifiable use of *human embryos* (via hESC use, applies to many human-animal chimeras and maybe also to cybrids)
- The concern of unjustified or unjustifiable use of *human gametes* (applies to all human-animal hybrids)
- The concern of unjustified or unjustifiable use of *human-typical genetic material* (be it from transplanting a whole nucleus – applies to cybrids – or genome sequences – applies to human-animal transgenesis)

[300] Ibid., pp. 34-35.

- The concern of inadequate use or treatment of dead or live *human tissue* (applies to all kinds of human-animal interspecifics).

These types of concern have not yet been extensively addressed in bioethical discussions of chimera experimentation (apart from Greely, Cho, et al.).[301] This is because they are seen as belonging to or stemming from different kinds of bioethical debates (i.e. the stem cell debate, the abortion debate, the debate around patentability of human genes, the debate around the proper handling of human gametes e.g. regarding contraception, and the questions surrounding proper treatment of medical waste, which are discussed in medical ethics). Still, these aspects are important to mention as potential costs of chimera, hybrid or cybrid experimentation involving human material.

c. Concerns for novel interspecific beings: Inadequate treatment

Both the concern for animal welfare and the concern for human embryos and proper treatment of human material have their source in the idea that nonhuman beings – or human embryos – could, as a result of chimera or other interspecific experimentation, be treated in a way that is not in accordance with their moral status. Going further, some claim that what is at issue is the proper treatment not of nonhuman beings (cf. animal welfare) or all-human beings (cf. embryo protection), but the treatment of "part-human" beings. In contrast to Rollin, they say that creation of human-animal chimeras is despicable precisely and particularly because it puts *part-human* beings in a bad situation.

Chakrabarty, for example, fears that in a not-so-distant future human-animal hybrids could be created for "organ harvesting, for use as subhuman species to perform hard manual labors, or simply for curiosity's sake."[302] He points out that this would be legally problematic since it is conceivable that such a hybrid could fall under the protection of the Thirteenth Amendment (which forbids slavery and ownership of human beings). The real question behind this is a moral, not just a legal one: shouldn't a "part-human" at some point be granted human rights? If yes, this could mean that the exploitation of such beings should be tightly controlled and in parts restricted – because we are morally bound not to treat part-humans the way we treat "normal" lab animals, livestock, or pets.

One common concern for the "part-human" novel being is that it is wronged because the circumstances it is born into allow it – it is to be used as a subject of experimentation. As I noted in my discussion of Rollin's objections, this is not a direct argument against the

[301] Ibid.
[302] Chakrabarty (2003), "Crossing Species Boundaries and Making Human-Nonhuman Hybrids: Moral and Legal Ramifications", American Journal of Bioethics, **3**(3), p. 21.

creation of chimeras – after all, one could create them and then treat them royally – however, it seems to be a valid objection, since hardly anybody would have an interest in creating disease-models or research subjects that are then not to be touched. Streiffer (2005) points out:

> *"So long as experiments that involve the xenotransplantation of human stem cells into animals are overseen by animal research oversight committees (…), the wrong, or an incomplete, set of moral protections is likely to be afforded to status enhanced chimeric research subjects."*[303]

Streiffer adds that researchers could guarantee "adequate protections" for humanized research subjects, but that then, the main objective of chimera creation would be void: most research could not be performed on subjects who are granted the same protections as human beings, and even if they could, why then not simply do them on human beings, which would be even better models? The danger of inadequate treatment seems to constitute a catch-22 of human-animal interspecific research. As we have seen, this type of research is based on the assumption that human-animal chimeras have the "advantage" that they can be treated like animals – should their moral status be elevated to that of human beings, their creation would become useless. At the same time, the scientific justification for creating chimeras usually depends on the claim that they are demonstrably humanized (i.e. exhibit human-typical properties that are relevant for research).[304]

When the Working Group on the Criteria for Cell-Based Therapies at John Hopkins University considered the scenario of human-to-nonhuman primate neural transfer, it identified several issues as potentially morally problematic – most prominently, the development of "humanlike cognitive capacities relevant to moral status" in the altered primate.[305] Humanization of the primate, in this relevant sense, cannot be ruled out according to Greene, Schill et al., and it can be seen as a "risk to avoid", since it could lead to beings that are not treated according to their moral status, and to "greater capacity for suffering that would add to existing concerns about the harms caused by inadequate

---

[303] Streiffer (2006), "At the Edge of Humanity: Human Stem Cells, Chimeras, and Moral Status", The Kennedy Institute of Ethics Journal, **15**(4), p. 362.

[304] Robert acknowledges an analogous dilemma in regard to "human dignity" concerns, noting that "those studies that are least scientifically contestable (…) are those that are apparently most morally controversial in terms of human dignity, while those studies that are most scientifically problematic (…) are those that are apparently least morally controversial in terms of human dignity." Robert (2006), "The science and ethics of making part-human chimeras in stem cell biology", Journal of the Federation of American Societies for Experimental Biology, **20** p. 843.

[305] Greene, Schill, et al. (2005), "The Working Group on the Criteria for Cell-Based Therapies, John Hopkins University: Moral Issues of Human-Non-Human Primate Neural Grafting", Science, **309**.

conditions for [nonhuman primates] in research."[306] In their 2007 résumé on Weissman's human neuron mouse scenario, the Greely Working Group comes to the similar conclusion that "human consciousness trapped in a mouse's body would truly be cruel treatment" although it "seems extremely unlikely."[307]

Johnston and Eliot's critical assessment of the consequences of chimerism experiments between humans and animals states that:

> *"Intentionally creating compromised human beings or part-human beings is cruel to the creature created (it is, for example, a laboratory subject created for the purposes of experimentation, able to exercise only compromised human facilities, likely to be kept in a cage, and perhaps not able to fend for itself.)"*[308]

Clearly, the concern for inadequate treatment of human-animal chimeras is closely connected with concerns that the moral status of the latter is hard to determine or even altogether indeterminable. This point – which I call "moral confusion" – will be dealt with in chapter 2, section D below.

d. Concerns for novel interspecifics: Shifting moral status

Another possible concern could be based on the view that shifting the moral status of a being *as such* could be morally problematic – that is, independently of the danger of inadequate treatment described above. Some of the concerns cited above seem to point in this direction, namely the notion of the "compromised human being" employed by Johnston and Eliot,[309] – which evokes the picture of a human being that has been violated in some way – and the fears that human consciousness could be "trapped" in a mouse's body used by Greely et al.[310] *Apart* from jeopardizing human-animal chimeras by putting them in environments that are not in accord with their demands and thereby violate their moral status, could it simply be wrong to transfer an individual from one level of moral status to a considerably higher or lower level? Could it be wrong to "shift" the moral status of a being? Could the subject of such a "shift" be violated by it?

We might approach this question by first asking who would possibly be the subject of the moral status shift. Assume, for simplicity's sake, that there is a status-unambiguous or at

---

306 Ibid.
307 Greely, Cho, et al. (2007b), "Thinking About the Human Neuron Mouse", American Journal of Bioethics, **7**(5), p. 34.
308 Johnston and Eliot (2003), "Chimeras and "Human Dignity"", American Journal of Bioethics, **3**(3), p. W7.
309 Ibid.
310 Greely, Cho, et al. (2007b), "Thinking About the Human Neuron Mouse", American Journal of Bioethics, **7**(5).

least relatively status-fixed being to begin with. In this context, it is useful to distinguish between two different types of scenarios: "Downshift" and "Uplift".

Let us first have a look at an example of "downshift". In a scenario, for example, where a human embryo is subject to neural xenografts with animal neurons which render its brain cognitively inferior to typical human brains or which let its brain develop into an organ that is below the functional standard it would have reached without intervention, we can sensibly understand this subject as being "compromised" or violated. A being that would otherwise have developed into something with exceptional cognitive capacities would have been harmed by chimerizing it; for some, its moral status would (at least prima facie) "shift down" since it could not fulfil criteria like self-awareness or consciousness anymore. Similarly, it would seem abhorrent to subject a human being to a xenografting procedure that would make it *look* like a nonhuman animal – for many, human appearance signals or even constitutes a criterion for high moral status, which in turn, a human with a nonhuman face, furry skin or an animal body would be denied. Chimerisation of humans would be comparable to other cases where human beings are intentionally violated or deprived of necessities and thereby lose important cognitive capacities (or other morally relevant properties), e.g. by mutilation, drugging, or other medical intervention that renders the victim incapable of higher "typically human" capacities. It seems indisputable that such actions would be morally wrong.

Fortunately, chimeric and other interspecific manipulation is usually not done on human embryos – among other reasons, certainly because it is widely recognized that shifting down the moral status of a human embryo or adult by massive chimeric/transgenic introductions would be morally reprehensive and constitute a massive violation.[311] Xenografts into adult humans (e.g. in Parkinson's stem cell therapy trials) are not substantial enough to influence the brain's functioning or lead to "downshift", though they apparently can infer damage on the brain by leading to tumours. Other xenografts, such as small graft transplants of skin and tissues, have even less influence on the human organism (and on morally relevant properties). Downshifting the moral status of human beings by chimerizing them, then, is not what is at issue when pointing out that "shifting" moral status could be problematic.

[311] The experiments of Rosenwaks – see p. 26 – are a rare exception; even in their case, it is hard to argue that the human embryos which were transgenically manipulated were "compromised" – Rosenwaks used embryos with a chromosomal defect, which had no potential to grow into full embryos, much less adults. See Zaninovica, Haoa, et al. (2007), "Genetic modification of preimplantation embryos and embryonic stem cells (ESC) by recombinant lentiviral vectors: efficient and stable method for creating transgenic embryos and ESC", Fertility and Sterility, **88**(Supplement 1).

What, on the other hand, about the second possibility mentioned above - "uplift" scenarios? How should we regard the possibility that the moral status of animals could – hypothetically – be shifted upwards by the introduction of human material? Could this be understood as constituting a "compromising" of animals, or how else should it be interpreted?

The improvement or "humanization" of (usually: mental) capacities of animals by technical means has been a subject of fiction since at least 1896, when H.G. Wells published "The Island of Dr. Moreau".[312] Wells was deeply influenced by the public debate of vivisection in his time. In Wells' haunting novel, a misguided physiologist tries to transform animals into humans by means of painful surgical procedures. These procedures give the animals involved an appearance bordering on humanness, but also apparently greatly improves their mental capacities – they begin to master language and even show interest in moral rules. Dr. Moreau, who has no justification for his experiments but pure curiosity, is ultimately killed by one of his wayward creations, a "humanized" puma. The scenario of "biological uplift" of animals has inspired dozens of books and movies since Wells' time. What makes these stories special is the wide chasm between two possible points of view. Uplift-negative approaches, such as Wells', assume that using technology to alter animals in order to make them more human is evidently wrong. Moreau's creatures, for example, are portrayed as deeply conflicted and ultimately unable to retain control over their horrifying, ugly and violent "animal side" (here, Wells may be telling us more about the Victorian idea of man than about the dangers of vivisection). The narrator's attitude is not one of compassion or pity towards the botched "Beast Folk", but rather one of intrinsic, intuitive rejection of and disgust at the mixed beings living on Moreau's island. Uplift is portrayed as wrong because it is predetermined to result in preternaturally evil or at least dangerous creatures. Contemporary works of fiction have a very different attitude towards "uplift", describing it as ambiguous, neutral or even positive.[313]

Since the recent progress of interspecific research, the idea of an – intentional or unintentional – "uplift" of nonhuman creatures, i.e. their endowment with properties or capacities that are seen as essentially human (and relevant for human moral status), is not limited to Science Fiction anymore. Bioethicists' considerations usually focus on the improvement or change of mental capacities, such as intelligence and self-awareness. To

[312] Wells (2005/1896), The Island of Doctor Moreau.
[313] The most prominent example of uplift-positive works of fiction is David Brin's "Uplift Universe," series of Science Fiction novels, starting with Brin (1980), Sundiver.

give but two examples of reputable institutions analysing the ethical import of such scenarios, as mentioned above, the 2005 John Hopkins Working Group concerned with the introduction of human neural cells into primate brains considered the possible "humanization" of the nonhuman-primate.[314] Similarly, the 2007 guidelines of the ISSCR committee forum for chimera research involving human material explicitly consider "research with the known, intended, or wellgrounded significant potential to create humanized cognition, awareness, or other mental attributes."[315]

Just as in the fictional examples we looked at, bioethicists' reactions to "uplift" scenarios are deeply divided: some regard this possible consequence of interspecies mingling as evidently morally wrong, while others have a prima facie neutral or even positive approach.

Commentators that are prima facie uplift-negative include Ramaswamy, who states that

> *"If a human-animal chimera (such as a monkey with a human-like brain) comes to possess any of these qualities [i.e. the capacities for language, consciousness, or rationality], then it would be morally objectionable to create that organism. (…) In cases where there is a reasonable possibility of transferring quintessentially human capacities to a chimera, scientists must stop short of actually creating it."*[316]

Similarly critical of uplift scenarios is Cynthia Cohen, who is afraid that chimerism experiments could "turn animals into humans" (which, she implies, would be a very bad thing and should definitely be avoided).[317] Note that these objections centre on the mere fact of "uplift", rather than on the danger of inadequate treatment that could be the consequence of uplift.

Uplift-negative views are seldom argued for and more often simply taken as granted. Criticizing this tendency, Baylis and Fenton remark that the view that "enhancing the psychological and cognitive capacities of nonhumans is a priori a bad thing" is in urgent need of "critical examination".[318] Baylis and Fenton are not the only ones to have recognized this need: the John Hopkins Working Group on Human-nonhuman primate neural grafting mentions as an aside that a "humanization" of the nonhuman primate could

---

[314] Greene, Schill, et al. (2005), "The Working Group on the Criteria for Cell-Based Therapies, John Hopkins University: Moral Issues of Human-Non-Human Primate Neural Grafting", Science, **309**.
[315] Hyun, Taylor, et al. (2007), "ISSCR: Committee Forum - Ethical Standards for Human-to-Animal Chimera Experiments in Stem Cell Research", Cell Stem Cell, **1**(2), p. 162.
[316] Ramaswamy (2007), "The Chimera Question", The Boston Globe, 2007/07/16.
[317] Cohen (2003), "Creating Human-Nonhuman Chimeras: Of Mice and Men", American Journal of Bioethics, **3**(3).
[318] Baylis and Fenton (2007), "Chimera Research and Stem Cell Therapies for Human Neurodegenerative Disorders", Cambridge Quarterly of Healthcare Ethics, **16**(2), p. 205.

also be seen as a "potential benefit to the engrafted animal, insofar as the changes are viewed as enhancements of the sort we value for ourselves."[319] Robert Streiffer, too, points out that moral status enhancement – apart from problems of inadequate treatment – is "prima facie good" for the research subject.[320]

Apparently, an argument from moral status shift is not viable *against*, but maybe can be used *in favour* of the creation of certain types of human-animal chimeras. It seems highly unlikely that this position will ever be used in a serious manner to justify human-animal chimerism experimentation – the interests of research into interspecifics are tightly bound to possible human benefits, not to "making humans out of animals".

But still, I find the question of how to react to the slightest evidence of the development of human-like cognitive capacities in chimeric research subjects hard to answer exactly because of this puzzling aspect of "humanization". The termination of experiments struck by such developments seems unavoidable (and is advocated, e.g., by the John Hopkins Working Group and Robert Streiffer), but how does one justify the killing of an experimental subject on the grounds that it "became too human"? After all, beings that are in the delicate process of developing into a creature that has the full array of human-typical features are seen as morally valuable and worthy of protection in many ethical approaches exactly *because* they are just undergoing this process. This is known as the "argument from continuum" in the discussion of the moral status of the human embryo – wouldn't supporters of such arguments have to fend for the not-yet-wholly-human chimera or cybrid, too? If the creation of a human life, or life that displays typically human capacities, is seen as a prima facie positive thing, would that not also include the creation of human life via "making animals human"? I haven't spotted arguments of this orientation in secular bioethics, but one point of view that points in this direction is maybe found in Roman Catholic Bishops' view that the carrying to term of chimeric or hybrid human-animal embryos, once they exist, should be allowed (although the Catholic Church is distinctly against the creation of such chimeric embryos).[321]

Apart from these – highly speculative – remarks, let us note that we have found status shift, as such, not to be a problem in current or likely future chimera research. Experimentation would, if at all, lead to upshift of animal xenograft hosts rather that downshift of human

[319] Greene, Schill, et al. (2005), "The Working Group on the Criteria for Cell-Based Therapies, John Hopkins University: Moral Issues of Human-Non-Human Primate Neural Grafting", Science, **309**, p. 386.
[320] Streiffer (2006), "At the Edge of Humanity: Human Stem Cells, Chimeras, and Moral Status", The Kennedy Institute of Ethics Journal, **15**(4), p. 348.
[321] Gledhill (2007), "Human-animal hybrid embryos should be legal says Catholic Church ", Times Online, 2007/05/27.

subjects. Downshift seems to be clearly morally wrong, just like other kinds of mutilation or detrimental manipulation of human beings.

The question of whether the "enhancement" of nonhuman beings in order to outfit them with characteristics we find desirable in humans is advisable or even obligatory will not be discussed here. Although the enhancement question is highly interesting,[322] I believe it does not play a big role in what human-animal interspecific research is currently concerned with.

All this does not affect our result from c above, i.e. that inadequate treatment of human-animal (and other) chimeras because of undetermined or even indeterminable moral status could present us with a considerable problem.

3. Bad consequences for human populations: Health risks

Objections to chimera experimentation are not only based on consideration for chimeras or other interspecifics, i.e. the novel beings created. There are also direct concerns for the security and health of already existing beings – especially human beings. The most concrete concern of this type is the thought that experiments that involve cross-species grafts could lead to or heighten the risk of diseases.

In this respect, xenotransplantation/xenografting is associated with two types of health risk: risks that only concern the host individual, and risks that also concern others.

Several types of risk are considered that are limited to the recipient of nonhuman material: *Immunoresponse*, i.e. the risk that the recipient has an immediate adverse reaction to animal material; *Tumorigenicity*, the risk that the recipient has long-term adverse reactions to the animal material, and *Zoonosis*, the risk of contracting a disease via transferred animal material. A connected third-party risk that would also affect non-recipients is seen in the scenario of an epidemic or even *pandemic* spread of zoogenic pathogens.

My introduction to xenotransplantation (chapter 1, section B.5 above) already explained that immunoresponse was, and still is, a serious problem for transplantation from animal to human recipient, especially when whole organ transplants are considered. Immunoresponse in xenotransplantation is much stronger than in allotransplantation. Additionally, with the porcine material that is commonly used, this risk is even more pronounced than it would

[322] The question of "enhancement" is, today, predominantly discussed in regard to humans; though, e.g. Hughes explicitly mentions as a future challenge to politics "The intellectual enhancement of animals, forcing a clarification of the citizenship status of intelligent non-humans."Hughes (2006),"Human Enhancement and the Emergent Technopolitics of the 21st Century", in: Roco and Bainsbridge (eds.) Managing Nano-Bio-Info-Cogno Innovations: Converging Technologies in Society.

be with material from more closely related species (i.e. nonhuman primates). Immunoresponse is usually the most massive problem preventing or complicating xenotransplantation.

Tumorigenicity, on the other hand – i.e. the disposition of certain material to lead to the formation of tumours in the recipient – is a problem for embryonic stem-cell-based therapies. Stem cells have the advantage of being able to "morph" into several types of cells, but also the disadvantage of sometimes morphing into a teratoma, a certain type of tumour. This risk is also present in interspecific grafts,[323] and thus must be considered in cases where animal embryonic stem cells are xenografted into human hosts. Immunoresponse and tumorigenicity are risks that are not specific to xenograft/-transplantation but also known from allotransplantation.

Things are even more complicated concerning the risk of pathogen transfer. A disease that is subject to trans-species transmission from animal to human is commonly called zoonosis. The danger of zoonoses has been discussed and recognized as a severe problem in the context of xenotransplantation. Here, apart from the danger of an infection of the individual recipient, there is a much bigger danger: that of a xenogenic epidemic or even pandemic which could potentially kill thousands or even millions of people.

For understanding this risk, it is important to realize that the precursors of many or even most of the most dangerous and ravaging diseases throughout human history – bubonic plague, typhus, measles, smallpox, influenza, HIV, and many others – were originally transmitted from animals to humans. The "jump" of a pathogen from one species to another, i.e. a shift of the disease host, brings the risk of a pandemic – this is what happened when the SARS virus "jumped" from civet cats to humans, and this is what scientists fear is about to happen in the case of porcine influenza (swine flu) and/or avian influenza (bird flu). The transmission of such viruses to humans and the associated pandemic risk is a constant matter of concern for epidemiologists.

In the case of xenotransplantation, there is a quite specific zoonosis concern: it is feared that a cross-species jump of porcine endogenous retroviruses (PERVs) could produce a virus that recombines with human DNA and results in a highly pathogenic, fatal virus aimed at human hosts (such as the HI virus, which probably originates from a retrovirus in chimpanzees, SIV). If there is a danger of "species jump" and pandemic, transplanting

[323] E.g. in a case were a cell line derived from pig embryonic stem cells was transferred to diabetic mice – see Fujikawa, Oh, et al. (2005), "Teratoma Formation Leads to Failure of Treatment for Type I Diabetes Using Embryonic Stem Cell-Derived Insulin-Producing Cells", American Journal of Pathology, **166**(6).

living body parts of one species into another seems to be a surefire way of increasing this risk, since live xenografts make it hard to eliminate eventual pathogens. Additionally, xenografting eliminates virtually every barrier viruses usually face when crossing from one species to another – keeping in mind that strict immunosuppression is necessary in the host. Previous zoonoses have emerged because of close contact with animals or their excrements, or because of consumption of animal products – in comparison, the introduction of live material into the (immunosuppressed) host organism itself seems to be an even closer kind of contact between species, and to open the door to species jumps. Normal pathogens, in this context, do not constitute such a big danger of xenozoonosis, since they can be eliminated before introduction of animal material into the human organism, by keeping the animals under "specific pathogen free" (i.e. partly sterile) conditions, vaccination, and by breeding selection for uncontaminated animals. Endogenous retroviruses are characteristically wired into the DNA of animals, though – they are integrated into the genome of their host organism, not acquired by infection, and cannot be removed from the tissue nor can one selectively breed uncontaminated animals. All vertebrates have such endogenous retroviruses that do not figure as pathogens in the original species, but which have pathogen potential when transferred to other species, leading to immunosuppression or tumours in the host, and possibly to a disease that can also be transmitted to other humans (or other species). The question of whether being subject to porcine (or other) xenotransplantation leads to a high risk of PERV (or other, especially primate, ERV) zoonosis is a highly complex one which cannot be discussed in depth here – it seems that studies have come to the conclusion that, though PERVs can transfer to human material in test-tube settings,[324] transmission in subjects of pig-human xenograft of living material is not easily established.[325] An EU study done in 2003 comes to the conclusion that nonhuman primate material should not be used for xenotransplantation because of xenozoonosis risk of easily transmittable primate endogenous retroviruses, while pig material can be used as long as certain safety measures are in place.[326] The moratorium on clinical xenotransplantation that was demanded in the 1990s[327] and which

[324] Patience, Takeuchi, et al. (1998), "Infection of human cells by an endogenous retrovirus of pigs", Nature Medicine, **3**.
[325] E.g. a search for transmission of PERVs to 160 human subjects 12 years after they had been treated with living pig tissue was unsuccessful, see Paradis, Lanford, et al. (1999), "Search for Cross-Species Transmission of Porcine Endogenous Retrovirus in Patients Treated with Living Pig Tissue", Science, **185**(5431).
[326] Working Party on Xenotransplantation (CDBI/CDSP-XENO) (2003), "Report on the State Of The Art in the Field of Xenotransplantation ."
[327] E.g. Bach and Fineberg (1998), "Call for moratorium on xenotransplants", Nature, **391**(6665); Butler (1998), "Last chance to stop and think on risks of xenotransplants", Nature, **391**(6665).

was, de facto, in place in many countries at the end of the century has today in most nations been replaced by more stringent control and regulation.[328]

What about the risk of zoonosis in other types of interspecifics? The Scottish Council on Human Bioethics regards zoonoses as a risk to be considered when thinking about "human-animal mixtures" (of chimeric and transgenic origin – i.e. not only products of "classic" whole organ xenotransplantation). The council concludes that

> *"This infectious danger is therefore sufficiently serious to induce physicians and biologists to publicly raise the question of whether it is ethical to allow humankind to run the risk of devastating and uncontrollable pandemics since animal-human mixtures will never concern more than a limited group of procedures."*[329]

In the case of transgenesis and (micro)-chimerism, the risk of epidemics is crucially lower than in the case of xenotransplantation. This is simply because the (animal) host does not or at least need not necessarily come into contact with humans which would allow contamination with potentially dangerous new pathogens. Unlike in organ xenotransplantation, the danger of zoonosis can be limited to the animal host which can easily be subject to stringent control (as compared to free-roaming human transplant recipients).

The scenario of a zoonotic infection and resulting epidemic is even more unlikely in the case of cybrids – the UK Academy of Medical Sciences report of 2007 judged this risk to be "not greater than" in normal (non-interspecific) cell cultures.[330]

4. Risk, uncertainty, and precaution

In regard to assessing the risk potential of new technologies, especially biotechnology, some argue that the standard approach of Risk-Cost-Benefit-Analysis (RCBA), which tries to take into account all kinds of foreseeable health risks, is not sufficient and even inapplicable and misleading.

For example, Hans Jonas, in his influential 1979 book "Prinzip Verantwortung" ("The Imperative of Responsibility") argued that, as modern technologies' consequences are

---

[328] Ravelingien (2006), "Pig Tales, Human Chimeras and Man-Made Public Health Hazards. An Ethical Analysis of Xenotransplant Benefits and Risks", Ghent University Faculty of Arts and Philosophy, p. 100.
[329] Scottish Council on Human Bioethics (2006), "Ethics of animal-human mixtures. Embryonic, Fetal and Postnatal Animal-Human Mixtures: An ethical discussion. "
[330] Academy of Medical Sciences (2007), "Inter-species embryos - A report by the Academy of Medical Sciences", www.acmedsci.ac.uk.

becoming harder and harder to predict, and, more importantly, as we are presented with technology risks which could wipe out humanity, one should apply a "heuristics of fear", a pessimistic outlook that assumes that negative scenarios will indeed take place (even though they may seem extremely unlikely).[331] Normal Risk-Cost-Benefit-Analysis, Jonas argued, is prone to neglect highly unlikely scenarios, even if they have full catastrophic scale, and is therefore not the appropriate means of devising how to handle powerful tools like nuclear power or advanced biotechnology. In a similar approach, Gregory E. Kaebnick mentions a "precautionary principle" that can be distilled from intrinsic arguments and argues that we should adopt a "preservationist attitude" in regard to biotechnology.[332]

Can we make sense of a precautionary principle outside of intrinsic concerns discussed in section B above? Could such an argument for precaution be used against the creation of human-animal interspecifics?

The problems of standard RCBA are comprehensively outlined by Timothy Lewens:[333] comparing the consequences of different scenarios poses one evident problem, another is the fact that RCBA which makes use of economic methods does not offer an objective assessment of values, but rather tells us how average persons would allocate resources. RCBA also does not deal with distributional issues: who is at risk and who, on the other hand, reaps the benefits of the risk taken is by many considered to be relevant for moral consideration, but this aspect is not captured in RCBA. These points make it clear that RCBA approaches are not about replacing ethical analysis with juggling numbers, but that RCBA must necessarily be preceded or complemented by decisions about ethical values.

Precautionary principles are at the basis of many regulatory policies regarding risk management – they "dominate most European regulatory policy",[334] and are e.g. expressed in the 15th principle of the 1992 Rio Declaration on Environment and Development,[335] feature in professional medical ethics codes like the Hippocratic oath and the related *primum non nocere,* and are also captured in proverbs like "better safe than sorry". The de facto moratorium on xenotransplantation that was in place in many countries at the end of the last century was based on such precautionary principles.[336]

---

331 Jonas (1979), Das Prinzip Verantwortung. Versuch einer Ethik für die technologische Zivilisation.
332 Kaebnick (2000), "On the Sanctity of Nature", Hastings Center Report, **30**(5), p. 22.
333 Lewens (2007),"Risk and philosophy", in: Lewens (ed.) Risk: Philosophical Perspectives.
334 Ibid.
335 Cf. Sandin (2007),"Common-Sense Precaution and Varieties of the Precautionary Principle", p. 99, in: Lewens (ed.) Risk: Philosophical Perspectives.
336 Cf. Ravelingien (2006), "Pig Tales, Human Chimeras and Man-Made Public Health Hazards. An Ethical Analysis of Xenotransplant Benefits and Risks", Ghent University Faculty of Arts and Philosophy, p. 100.

The adoption of a "precautionary" approach is typically advised when we are confronted with ignorance regarding the potential consequences of an action – RCBA can only usefully be applied to situations in which we have a basis of past experience or data points to draw on and extrapolate from. In cases where probability distributions of consequences are unknown or potential consequences are unclear because we have no previous similar cases to compare the new scenario with, it is argued that following a precautionary principle would be advisable.

Is the creation of interspecific (particularly human-nonhuman) beings a case where a principle of precaution should be applied? And if yes, what would such a principle tell us?

As mentioned above, precautionary principles typically come into play when we are confronted with ignorance concerning the potential outcomes of an action. This would be the case in regard to genuinely novel types of actions, which have not been done before, or which are sufficiently different from types of actions done before that extrapolation is impossible. Is the creation of interspecific beings or entities novel in this sense? Do we have any data to draw on when thinking about the risks of mixing interspecies animal or even human and nonhuman material?

Prima facie, mixing species, particularly human and nonhuman species, seems to be a drastic, absolutely novel thing to do. Yet, we have seen, there are interspecific hybrids between closely related species in nature. Also, there are intraspecific cases of chimerism in nature and also in humans (e.g. microchimerism in twins). Regarding human-animal mixtures, it could be argued that very close, even symbiotic connections between human and nonhuman animals have existed for millions of years. We coexist with wild animals, livestock, pets and vermin. Parasites live on and enter into most human bodies and, more enjoyably, most of us voluntarily introduce animal materials into our own bodies by the very common habit of consuming animal products. From all these data points, it seems that we actually do have vast experience concerning the mixing of different species, and even "mixing" animal and human material. Some of these past experiences have led us to believe that certain types of mixing might be dangerous: we know that, for example, xenotransplantation could result in dangerous new pathogens. We certainly do not operate in an area of total ignorance when assessing the risks of creating interspecifics.

Still, there are areas which are not easily covered or mapped by such extrapolation from our experiences with "mixing". One of these scenarios would be that of chimeric, hybridic, or transgenic beings released (accidentally or on purpose) in populations of non-

interspecific animals. How would populations, or whole ecosystems, react to such intrusions? Could recombination of genes from different species lead to the emergence of dangerous properties in the transgenic being? These scenarios may sound familiar from the area of genetically manipulated plants. Other areas in which it seems very hard to give useful prognoses include the aspect of Robert's and Baylis' Argument from Moral Confusion, which I will discuss in section D.3 below, and, in general, the aspect of emerging consciousness or other valuable mental properties in interspecifically manipulated animals (see sections 2.c and 2.d(ii) above).

Concerning these areas of the unknown or unforeseeable, would precaution be a sensible argument? Should we avoid creating interspecifics even in cases where specific problems cannot (yet) be pointed out, or seem very vague, but should still be considered in our analysis?

It seems advisable here to step back and ask what a principle of precaution can sensibly mean. In a very weak sense, precaution could mean that we should not assume something has no risk just because there is no scientific proof for that risk – we should not argue from ignorance, or as Sunstein puts it, "a lack of decisive evidence of harm should not be a ground for refusing to regulate."[337] Another interpretation states that precaution means shifting of the burden of proof: the party which plans an action would have to prove that it is not dangerous, rather than burdening the party affected with possible consequences with proving that they could be harmed – this would introduce distributional issues into the analysis. These interpretations of a precautionary principle, however, seem merely complementary to standard RCBA. They add ethical and other considerations to the assessment rather than overriding this method's general outcome or applicability. Precaution, in these senses, constitutes procedural minimum requirements that should be fulfilled in our RCBA process, and/or ethical/distributional considerations that should complement RCBA.

But could a principle of precaution be understood in a way that tells us to avoid an action *even if* a RCBA carried out fulfilling all these minimum requirements tells us that we are justified to carry out the action? Are there cases where RCBA is the wrong approach, as such?

As mentioned above, RCBA is appropriate for cases where the probability distribution of outcomes is known or can be extrapolated, but not for cases which are actually not about

[337] Sunstein (2002), "The Paralyzing Principle", Regulation, **Winter 2002-2003**, p. 33.

risk but rather about deep uncertainty. The distinction between risk and uncertainty, as described by Knight,[338] is a crucial analytic step at this point: "risk" proper is described as "measurable uncertainty" (i.e. we do not know whether scenario x will take place, but we think it will take place with a probability of y), while "uncertainty" describes cases where we have no access to probability distributions of the scenario at issue. The potential detrimental consequences of the creation of novel interspecific beings, in some aspects, fall into the realm of risk (example: risk of transfer of known types of pathogens to xenotransplant recipients). In other aspects, potential detrimental consequences fall squarely into the field of uncertainty (example: unforeseeable detrimental consequences in case of release of transgenic beings to ecosystems).

What if we accept that RCBA does not cover all possible risks and potentially understates catastrophic scenarios? Does precaution offer a sensible alternative? In a very general sense, the precautionary principle could be understood as stating that we should be especially or extremely risk-averse, simply because RCBA does not "give us the whole picture".

As Sunstein[339] points out, this is not a sensible alternative: being risk-averse is not a principle that can tell us what to do (or not to do). In the case of interspecific creation, it might be advisable not to create interspecific beings in the light of precaution in order to avoid detrimental consequences like rampaging interspecific monsters destroying the world. On the other hand, it could be seen as the risk-averse path of action to invest in research (and interspecific-creation) in order to have the best chance to find out about therapies for all kinds of diseases. Thus, we could avoid the detrimental consequence of us or future generations dying of diabetes, Parkinson's, stroke, or even diseases that do not exist yet but may threaten humanity in the future. Precaution, unfortunately, does nothing to tell us *which* detrimental consequence to avoid. It does not even tell us not to act, at all (in the literary sense of a "paralyzing principle"): difficulties of distinguishing actions from non-actions aside, acting is often as or even more precautionary as not acting.

Also, it remains unclear which areas of action precaution should apply to: in fact, every action in the real world has potential unforeseeable consequences which are not covered or would not be taken into account by a standard RCBA (rare examples are something like throwing dice or roulette, where the probability distribution is known beforehand).

---

[338] Knight (1957/2006), Risk, Uncertainty and Profit.
[339] Sunstein (2002), "The Paralyzing Principle", Regulation, **Winter 2002-2003**.

Following a precautionary principle in all situations where uncertainty is at play would make us incapable of decision.

Precaution, then, must be understood as complementing or stating minimum requirements to standard risk analysis, not as an alternative to this approach. It is true that risk analysis does no good job of covering scenarios of uncertainties – but unfortunately, precautionary principles do an even worse job of helping us deal with these unquantifiable risks by creating the impression that they can be avoided by simply abstaining from action or risk-taking.

D. Confusion: Indirect consequence-based objections

The consequence-based arguments presented so far rely on relatively direct consequences of the creation of interspecifics, like possible problems for animal welfare, the destruction of human embryos, and health concerns. Other concerns are more indirect and subtle: it is claimed that the creation of interspecifics, particularly of human-animal interspecifics, leads to confusion, which is understood as a detrimental consequence. This confusion can be understood in different ways. Three types of confusion which could be the consequence of the creation of interspecifics will be presented in this chapter.

When confronted with human-animal interspecifics, there are two primary ways of understanding "confusion": one is stating that the moral status of chimeric subjects (and thereby our obligations towards them) is *hard to determine* due to the conditions of interspecific creation – this will be discussed in section 1 below. The second way of understanding "confusion" in the context of human-animal chimeras is more absolute: the moral status of human-nonhuman interspecifics could become altogether *indeterminable* (see section 2 below). A third type of "confusion" argument is based on the concern that the uncertain moral status of some human-animal interspecifics could, in turn, lead to society questioning its' criteria of moral status assignment and, in the process, give up the assumption of human beings' superior moral status (see section 3 below).

1. Confusion as complicated determinability

Let us look at the first aspect of the confusion problem, which I will call the problem of complicated determinability. Note, in the first place, that determining the moral status of a being is rarely easy or undisputed. As we will see in our excursus on moral status, there are many problems lurking in the question of which capacities or properties of creatures are

morally relevant, i.e. which qualities have an influence on the moral status of the being. Candidates for such properties or characteristics include language capacity, rationality, free agency, species membership, natural kind membership, and many others. Defining such concepts and justifying their moral relevance is fraught with problems. In addition, we face epistemological hurdles when trying to pin down criteria for when a being (be it human, animal, or chimera of both) does *exhibit* these properties – how, to give but one example, are we to find out whether a monkey does or doesn't have consciousness or self-awareness?[340]

The problem of determinability is even graver in the case of artificial interspecifics discussed in chapter 1, section B above, simply because they are *novel* beings. When assessing the various capabilities and properties of a common rat, we have a huge body of empirical data to fall back on, namely all kinds of research that have been done with other typical rats. For determining the moral status of the individual "new" rat, we can make use of general knowledge about rats: the rat (assuming it is not a wildly atypical mutation) will not be able to use language, no matter how hard we try to teach it, it will be able to solve mazes up to a certain degree of difficulty, it will feel pain, etc. Regarding novel interspecifics like embryonic chimeras or transgenic beings, there is, at least from a certain point of humanization on, no such extensive empirical data to extrapolate from. This would similarly be true for other novel beings (which have been altered in morally relevant characteristics), e.g. (if this were possible) animals that are outfitted with enhanced cognitive capacities via genetic manipulation, but also Artificial Intelligence and extraterrestrial beings. In all these cases, we would have the epistemological problem of finding out what properties these entities have without having access to comparable precedents.

This problem does not make moral status indeterminable as such. In moral systems that discriminate between different moral status levels there are certain *criteria* for determining whether a property (like having the capacity for language, being rational, etc.) is present in a being. If moral status classification is the aim, stating morally relevant properties must mean stating the criteria for determining whether they are present. The being's moral status will be derived accordingly (for a further explanation of moral status assignment, see chapter 3, section A below). Such a process will take place for all kinds of beings: humans, nonhumans and human-nonhuman chimeras alike. This analogy in process between moral

[340]For an excellent introduction to the problems of "animal minds", see: Perler and Wild (2005),"Der Geist der Tiere - eine Einführung", in: Perler and Wild (eds.) Der Geist der Tiere.

status classification of chimeras and "normal" cases is also pointed out by Andrew Siegel, who comes to the conclusion that classifying chimeras is not especially problematic in many moral systems ("For both [Kantianism and utilitarianism] there is no conceptual obstacle to understanding the moral status of chimeras.").[341] There will – as I pointed out – be cases where categorisation will be especially difficult. We need not resort to Science Fiction in order to come up with examples for this: all kinds of atypical beings will do. The classification of a human embryo in its early stage is problematic, because it does not exhibit most of the typical characteristics of humans that are candidates for morally relevant properties. Similarly, the classification of nonhuman primates is vexing, since many of them exhibit astonishing feats of language use and problem solving. Even more so is the positioning of human (or nonhuman) *individuals* that are atypical: take the brain-damaged adult or the anencephalic infant, or flatland gorilla Koko who has, over decades, learnt hundreds of words in sign language and who has complex relationships with animals and humans alike.[342]

It could be interjected here that it might be morally wrong, in general, to create beings whose moral status we do not know in advance. I do not think that is a useful point, simply because every living being that is born is novel in the sense that we do not know for sure which capacities it will develop, especially since many capacities – like language, complex problem solving, etc. – only unveil after extensive training and stimulation. There is also the possibility of genetic mutation that brings about atypical individuals in every species, be it human or non–human. If we were limited to creating beings whose future moral status we can determine beforehand, having children (at least having children "the natural way", without genetic screening) would have to be regarded as morally reprehensible.[343]

The fact that this complication is not unique to interspecifics does not render void arguing against their creation by pointing out the problem of complicated determinability. Because such beings are novel and, in particular cases, without precedent, determining their moral status could be so costly as to render moot or outbalance the possibly beneficial effects of interspecific research. This added cost would then be a valid argument against chimera creation from complicated determinability – a point David Castle also touches on when stating:

---

[341] Siegel (2003), "The Moral Insignificance of Crossing Species Boundaries", American Journal of Bioethics, **3**(3), p. 34.
[342] Patterson and Linden (1981), The Education of Koko.
[343] This argument is limited to positions which assume that moral status is influenced by certain characteristics, rather than being exclusively determined by membership in the species *Homo sapiens* (for a critique of such "Strong Speciesism" views, see chapter 3, section B).

> *"What is ethically worrying (…) is if [Human-Animal Interspecifics] are viable creatures that add an extra dimension of complexity to borderline moral reflection and decision making. Deciding these cases could be highly unsettling and does not seem likely to be worth whatever benefits the biotechnology might bring."*[344]

Note that this problem of complicated determinability (which is not an absolute argument, but needs to be included in a cost-benefit-analysis) would also apply to nonhuman interspecifics. One need not go as far as Castle, who hypothesizes:

> *"(…) were it possible, crossing lobsters with cows to make the ultimate surf-and-turf organism might raise eyebrows at first (…), but then issues of how to humanely cultivate, transport, and slaughter the organism would float to the surface."*[345]

Slightly more realistic (though still speculative) cases are conceivable: if we acknowledge that primates deserve a treatment different from that of dogs (as is recognized e.g. in the higher standards of animal welfare concerns regarding research in primates vs. other mammals), we would encounter a problem when trying to devise ethical standards for the treatment of dog-primate interspecifics that show a high rate of mixing or "primate behaviour".

To sum up, firstly, every moral status classification brings with it profound epistemological and ethical problems or questions – this is not a problem limited to novel interspecifics. Secondly, there are atypical beings – among them individuals that do not exhibit species-typical characteristics because of being in a certain developmental stage, because they are diseased, have been subject to especially beneficial or harmful environments, training, or stimulation, because of genetic mutation or because they are chimeric or other interspecific beings. In the latter cases, moral status classification is particularly difficult, but not categorically more so – except if we make species membership the determinant of moral status, i.e. embrace Strong Speciesism. Thirdly, it cannot be stated in general that creating beings whose moral status we do not know beforehand is wrong as such. If we do not assume Speciesism, human-animal chimeras are not categorically more morally confusing than other beings. Nor is their creation wrong due to the fact that we cannot anticipate their moral status. A point against interspecific creation that is independent of Speciesist

---

[344] Castle deems human-nonhuman interspecifics not to be morally confusing, but just "worrying." This is a matter of nomenclature which corresponds to my distinguishing absolute, total or conceptual confusion from "normal" or "relative confusion." Castle (2003), "Hopes against Hopeful Monsters", American Journal of Bioethics, **3**(3), p. 29.
[345] Ibid.

assumptions can be made by including costs for moral status determination in a cost-benefit analysis. These points are, to a lesser degree, also valid for animal-animal interspecifics.

2. Absolute confusion

Given some moral axioms or rather presuppositions regarding moral status, interspecifics – human-nonhuman interspecifics, in particular – can evoke "absolute" confusion that goes far beyond the mainly epistemological problems, or problems of uncertainty, I described above. There is one specific position which is a candidate for evoking absolute confusion: the conviction that human beings, and only human beings, have, in virtue of being a member of the species *Homo sapiens*, a moral status that is superior to that of nonhuman beings (Strong Speciesism).

When one uses such a Strong Speciesist framework in order to assign human and nonhuman beings moral status, one will have a hard time when confronted with beings that are, in some sense, between being human and being nonhuman. It can be doubted at this point that any of the human-animal chimeras presented in chapter 1, secion B.4 above fulfil the condition of being ambiguous in this sense. Are they not just clearly nonhuman animals that have some, often very few, human cells in their body? Ambiguity in the sense of not clearly being a member of one or the other species might be more apparent in hybrids or cybrids: a "humanzee" (a hybrid of human and chimpanzee), if there were such a being, could not easily be characterized as human or nonhuman. Cybrids – which consist of an enucleated animal egg into which a human nucleus is introduced – also fulfil the condition of ambiguity. What else is considered ambiguous in the field of interspecifics depends on the conditions one assumes for belonging to the human species (i.e. one's definition of species membership). There are, as I will show below, numerous definitions for biological species, and numerous concurring ways of understanding "human being" (in the biological and in other ways), and therefore numerous ways in which a being can be "species-ambiguous".

Robert and Baylis, in their seminal 2003 article,[346] make clear that no matter what species concept one entertains, unambiguous classification of all beings as either human or nonhuman is not possible. There is, so they claim, no "human essence", and this is why arguments that are based on the sacredness of a "boundary" between the human and other

[346] Robert and Baylis (2003), "Crossing Species Boundaries", American Journal of Bioethics, **3**(3).

species do not work. Yet, Robert and Baylis try to construct an argument against the creation of chimeras that does not depend on "boundary" concepts (though they, as they made clear in a 2007 article, do not endorse this construction themselves). Their argument from "moral confusion" is based on the notorious moral ambiguity of human-animal chimeras. Ambiguity is problematic, for once, because we do not know what kind of obligations we would have towards part-humans – this founds the concerns regarding inadequate treatment of chimeras discussed in section C.2.c above. Secondly, thinking about what properties human (and, possibly, nonhuman) beings have that are morally relevant would, as Robert and Baylis put it, endanger the "clear but fragile moral demarcation line"[347] between human and nonhuman animals.

There are two branches of argument here: on one of them, classification in human or nonhuman is indeed impossible. This could be the case when confronted with human-animal hybrids or cybrids, or chimeras with a very high rate of mixing. One could argue that creation of such novel beings would be wrong because we have no ethical tool at hand with which we could ascertain their moral status and thereby make sure they are treated properly – inadequate treatment, the concern voiced in chapter 2, section C.2.c above, would be the consequence. Robert and Baylis contentrate on another, second branch of argument.

3. Robert's and Baylis' argument from moral confusion

Robert and Baylis are concerned with a more indirect issue. Short of absolute confusion, the candidate criterion employed to single out beings which should be accorded high and direct moral status might, in the process of classification of novel beings, become more and more dubious. Newly developed criteria, on the other hand, could lead to unwanted consequences regarding the moral status classification of human and nonhuman beings. Once they are applied not only to novel, but also to "conventional" beings, they might call into question the traditional consensus on moral status assignment, and, in turn, our treatment of humans and nonhumans. Robert and Baylis put it this way:

> *"Asking – let alone answering – a question about the moral status of part-human interspecies hybrids and chimeras threatens the social fabric in untold*

[347] Ibid., p. 9.

> *ways; countless social institutions, structures, and practices depend upon the moral distinction drawn between human and nonhuman animals."*[348]

The "moral distinction" mentioned here is easily identified as Speciesism: it is assumed that nonhuman beings are subject to a moral framework that is fundamentally different from (and incommensurable to) that applied to humans. While the latter enjoy "categorical" moral status, the status of the former is "contingent on the will of regnant human beings".[349]

But where exactly are the threats for our "social fabric"? Let us assume that the starting point is a Strong Speciesism which assumes that "being human" is defined in terms such as "organism with human DNA". Confronted with ambiguous beings "between" human and animal, a Strong Speciesist can, on one hand, change his definition of "being human" in order to accommodate beings that are deemed morally superior to "normal" animals. He could, for example, state that beings which show typical human behaviour like language use, problem solving, or human-like appearance, should "count" as full human beings. Alternatively, the Strong Speciesist could give up Strong Speciesism and admit beings that are not human according to his standards into the circle of beings with high moral status (as soon as they fulfil certain criteria). This would result in a position where "being human" in the biological sense (i.e. species membership) would not be a necessary condition for high moral status anymore. This, in turn, would call into question many of our current practices regarding nonhuman beings, such as hunting for sport, animal testing, etc., since the exculpatory remark "It's just animals" would not be persuasive anymore. (This is not to say many of our exploitative practices regarding animals could not be justified otherwise, but justification would have to consist in more than the simple declaration that "they're not human, after all.").

This perceived threat goes in the opposite direction, too. If species membership were, in this way, driven from the throne of morally relevant properties, other candidate properties would be put forward, such as rationality, language, free will, etc. Strong Speciesism could be substituted by Qualified Speciesism, i.e. the view that only some beings are, in virtue of having properties like rationality, capacity for moral agency, or self-awareness, morally superior, while, in general only human beings fulfil these conditions (and, possibly, some lucky human-nonhuman interspecifics). When trying to construct a consistent view of Qualified Speciesism, it is remarkably hard not to end up with a position that states that

[348] Ibid., p. 10.
[349] Ibid., p. 9.

human species membership is not only not necessary, but also *not sufficient* for high moral status – we will encounter this problem in the form of the Marginal Cases Problem in chapter 3, secion B.2 below. This is, I think, another danger Robert and Baylis see embedded in the debate around chimeras: it could surface that being human in the biological sense (species membership) would not be a *sufficient* condition for high moral status anymore; which would, in turn, challenge the categorical, high moral status of human beings that do *not* exhibit the properties one has worked out as morally relevant. Atypical human beings like embryos, infants, comatose and severely mentally handicapped individuals would then lose the prima facie privileged status they enjoy in Strong Speciesist accounts in virtue of their species membership. Asking the question of how human-animal chimeras should be classified morally could trigger a landslide that ends not only with questioning our treatment of nonhuman animals, but also challenges the high moral status we, as a matter of course, assign all human beings no matter their properties and capacities aside from "being human" in the biological sense. This looming danger could ultimately be used as an argument for strict regulation or even prohibition of human-animal interspecific creation.

Whether one finds this kind of argument persuasive (and many, including Robert and Baylis themselves, do not) crucially depends on the importance one assigns to keeping up a fundamental moral difference between human and nonhuman beings. For someone who does not assume such a fundamental difference (i.e. a non-speciesist), Robert's and Baylis' confusion argument will not be very compelling. To give three examples of its failure to persuade non-speciesists, Hilary Bok states that "Chimeras do not introduce confusion into our moral views. They reveal ways in which those views are inadequate and make us think about how we might improve them."[350] In a similar vein, David Castle thinks chimeras are "no more ambiguous then any other living thing."[351] He points out that chimeras must not be seen as made up of parts ("fallacy of composition"), but as organisms in their own right, and that "they will get whatever moral consideration they deserve on the same grounds that apply to any other organism, including human beings."[352] Julian Savulescu goes even further when, in turn, attacking the conservative speciesist impetus the confusion argument is based on:

[350] Bok (2003), "What's wrong with confusion?" American Journal of Bioethics, **3**(3), p. 25.
[351] Castle (2003), "Hopes against Hopeful Monsters", American Journal of Bioethics, **3**(3), p. 28.
[352] Ibid., p. 29.

> *"The social costs of acceding to irrational confusion are, at least historically, much greater than the costs of clearing it up and reforming society. (…) Our job is to clear this up (…), not to perpetuate it or allow it to persist or base social policy on it."*[353]

At this point we can see how Robert's and Baylis' confusion argument can be turned around in order to make speciesism (Strong Speciesism in particular) dubious, rather than defending or supporting it – a fact that will be important to our further discussion of the chimera debates' possible influence on questions of animal versus human moral status.

Robert's and Baylis' confusion argument, like many arguments in this debate, hinges on the question whether speciesist attitudes are defensible. I have already explained that speciesism is a position which claims that the moral status of human beings (however defined) is fundamentally different from that of nonhuman beings. In order to understand and put in context this type of view – and, consequently, decide whether it should play a role in arguing against the creation of human-animal interspecifics – it is necessary that we understand what exactly speciesism is and what its problems are. To do this, I will in the next chapter include an excursus on the concept of moral status, which is crucial for Speciesism approaches as presented here. Building on this, I will elaborate on the problems of finding a suitable ethical principle of Speciesism. I will come back to an in-depth, concluding analysis of concerns against creating interspecifics in the last chapter 4.

[353] Savulescu (2003), "Human-Animal Transgenesis and Chimeras Might Be an Expression of Our Humanity", American Journal of Bioethics, **3**(3), p. 25.

Chapter 3: Excursus on Moral Status and Speciesism

Several of the threads of argumentation presented above rely on the concept of "moral status". For example, I discussed the implications of what I called "moral status shifts" in chapter 2, section C.2.d above, and I assumed that some properties of human and nonhuman beings could be somehow relevant for this moral status, while others were irrelevant in this respect (see my discussion of "violation of human dignity" in chapter 2, section B.4 above). Also, I have already mentioned that Speciesism builds on this concept, and can be characterized as stating that humans have a fundamentally different (higher) moral status than nonhumans.

This chapter will be concerned with the concept of "moral status" (in section A) and, consequently, with the ethical principle of Speciesism (in section B). I will present a detailed concept of "moral status" and describe how the latter is assigned to entities by moral agents, present this concept's advantages and disadvantages, and discuss whether it is appropriate for the task at hand. I will then describe differing types of Speciesism and give a cursory outline of their main problems.

A. The concept of moral status: General considerations

1. Defining moral status

"Status" refers to the state, i.e. the mode or condition of being, of an entity; or its position in a complex system or hierarchy. *Moral status* refers to the position of an entity in the hierarchy or system of entities that come into question when gauging the scope (and intensity) of moral consideration. Moral status – like legal or social status – is ascribed to entities by the moral community and individual moral agents. Status, in the case of legal, social, but also of moral status, is based on norms – beings which are accorded status are treated according to certain rules (e.g. the legal status of citizens is determined by the rules of law).[354] When saying an entity has moral status, this can be understood as saying that the entity is in the category of beings that are to be considered morally (i.e. that there are rules

[354] Vossenkuhl (2002),"Der ethische Status von Embryonen", p. 166, in: Oduncu, Schroth, et al. (eds.) Stammzellenforschung und therapeutisches Klonen.

according to which it demands to be treated). Warren, e.g., understands "having moral status" to mean "being worthy of moral consideration".[355]

In a wider sense, which I use here, moral status is open to the question whether a being or thing is, in fact, directly morally considerable. Just as the question for "marital status" can be answered by "single", the question for "moral status" can be answered by "does not deserve/is not accorded any moral consideration". For example, in most moral systems, stones are not considered morally, so their moral status is "not to be considered (directly)"; in my interpretation of the term, stones do have a moral status, though (i.e. the status of not being morally considerable).

From this it follows that the concept of moral status is not necessarily directly concerned with the question of whether the entities that are to be classified are addressees (subjects) of moral rules or obligations – having a "moral status" does not mean, as such, that the entity is subject to moral norms. This question can still play an indirect role, as in some approaches (e.g. Kant's) a being can only reach full moral status if he or she is also a moral agent and thereby subject to morality. In fact, many aspects of the superior moral status that is commonly accorded to human beings or persons do crucially depend on the ability of the carrier of status to act and make autonomous decisions.[356]

The moral status of beings can be understood in a prescriptive way (i.e. "status" tells us how a being ought to be treated) or as descriptive (i.e. "status" tells us how a being or type of being is actually treated). In my own discussion, I will commonly use moral status in a descriptive sense. Moral status will be seen as prescriptive within the specific moral framework it relates to; the moral status of a being can vary wildly depending on what ethical framework the assigner subscribes to.

A simple model of moral status assignment could consist of a process involving three steps. When confronted with an entity and the question how to behave towards it, one first takes note of its *properties* (observed, relatively simple, properties, like "interacts with environment" or "is able to move" or "uttering sounds", but also derived properties, like "being alive", "possessing faculty of speech", "being a person", or "being human"). Employing the moral theory (or axioms) one subscribes to, one then assigns the being a *moral status*. In a common approach to moral status, there are three rough possibilities of classification here:

---

[355] Warren (1997), Moral Status - Obligations to Persons and Other Living Things.
[356] Vossenkuhl (2002),"Der ethische Status von Embryonen", p. 163, in Oduncu, Schroth, et al. (eds.): Stammzellenforschung und therapeutisches Klonen.

The lowest possible status ("not to be considered") is usually applied to inanimate objects. An intermediate status is usually ascribed to nonhuman animals (and sometimes, plants). Carriers of this status are to be considered indirectly, on certain conditions, i.e. when a certain value is ascribed to them.[357] Indirect consideration is also imaginable regarding wholly inanimate objects: e.g., the lifeless body of a human being, in many moral (and legal) systems, demands respectful treatment, and it does so indirectly because certain values are ascribed to it. Finally, there is, in many moral approaches, a superior, overriding, or "full" moral status. This is usually assigned to human beings, or persons. Often, this ascription is regarded as a *direct* one, meaning that it takes place not because of a value that is ascribed to the carrier entity, but solely by virtue of the entity being human. The superior moral status of human beings can also be justified *indirectly*, by stating that humans possess certain characteristics that are valuable (consciousness, moral agency, free will, personhood, etc.); i.e. by the same process of value ascription that takes place in assessing nonhuman entities. This, in turn, leads to the problem of how to correctly determine the moral status of human beings who do not exhibit the valuable characteristics. This problem of human "marginal cases", as we will see, is crucial for the discussion of human vs. nonhuman moral status.

After this assignment of moral status one derives (again, with the help of the moral system one subscribes to) the proper way one should treat, or rather, consider the entity – moral norms come into play. A rights theorist might, at this point, decide that the being has, because of its moral status, a certain right, and that we should act accordingly; a utilitarian decides now whether the entity should be counted in the felicific calculus.[358]

The aim of this process, i.e. of determining the moral status of beings or groups of beings is described by Mary Ann Warren as a twofold one: "to specify minimum standards of acceptable behaviour towards entities of a given sort", and "to establish moral ideals".[359] Accordingly, we should keep in mind that the moral status concept is not, prima facie, geared towards making moral decisions under specific circumstances (i.e. whether one

---

[357] Ibid., p. 164.

[358] Lori Gruen, in a similar approach, understands the moral status concept as involving two distinct steps, one of basic recognization and one of actual assessment. In her discussion of the moral status of animals, she distinguishes between "moral considerability" of a being, which she likens to "showing up on a moral radar screen" and "moral significance", which tells us "how strong the signal is or where it is located on the screen." Cf. Gruen (2003), "The Moral Status of Animals", The Stanford Encyclopedia of Philosophy (Fall 2003 Edition).

[359] Warren (1997), Moral Status - Obligations to Persons and Other Living Things, p. 13. For another proponent of this two-step approach, see Pluhar (1995), Beyond Prejudice: The Moral Significance of Human and Nonhuman Animals, p. 1.

should or should not create chimeras), but rather towards discussing, on an abstract level, values and value ascriptions to certain classes of entities.

2. Advantages of the moral status concept

The central issue in the context of moral status is the special, superior moral status of human beings. Often, this status is contrasted or compared to that of nonhuman animals or other nonhuman entities. In this context, I could also have used concepts like "rights", "dignity", or of "welfare" or "interest". Up to now I avoided these terms because they would have led to the implicit assumption of deontological or instrumental (consequentialistic) theories of rights (or, rather, to a commitment to rights-based or non-rights-based approaches). The possibility of avoiding premature commitment in these respects is a central advantage of the moral status concept.

Using the moral status concept also enables us to compare moral systems whose distinctness regarding vocabulary or moral axioms usually make comparisons difficult. Picking out this very abstract aspect, we can, e.g., compare Kant's position on animals (in his approach, they are not to be considered directly, while indirect consideration is regarded as advisable) to that of someone like Peter Singer (here, all sentient animals are to be considered directly). Asking whether there are "animal rights" in Kant's or Singer's approaches, respectively, would not really give us anything to work with, since it would presuppose extensive analysis of the concepts of "rights" Singer and Kant use. Most likely, this would result in the answer that their concepts are not measurable by a common standard. "Moral status", on the other hand, offers such a common standard.

Another advantage of using the moral status concept at this point is that this term makes it easier to avoid an explicit commitment to (or rejection of) of speciesism. Concepts like "human rights" or "human dignity" allow only humans into their scope, assuming as a given that there is a fundamental moral difference between human and nonhuman beings. I spelled out this problem in my discussion of human dignity arguments against interspecific production (see p. 81), the same consideration applies to the term "human rights". The notion of "rights", as such, may not be inherently speciesist (after all, there are many who claim animal rights), yet often, it invokes the prima facie objection that only beings which also have obligations can have rights, at all. While this is not a necessary corollary of the term "rights", I still find it to introduce a certain tendency into the debate that is not intended and unnecessary at this point.

Putting aside the problem of speciesist tendencies, does it make a difference, at all, whether one speaks of "rights" that are accorded to beings or whether one prefers to refer to "high moral status" that is assigned? Are these concepts interchangeable? I think that, on a very basic level, they are not. This is because a being which "has a right" (as opposed to a being which "is to be morally considered") may not be violated in the respect the right protects, even if such a violation or disregard might be indicated by utilitarian reasons. This is true at least for some basic or absolute rights. Not all, but some rights are inviolable entitlements, at least in approaches which think that there are "real" rights (i.e. deontological approaches; cf. footnote 362 below). This implication of inviolability or absoluteness is the basic difference between using rights vocabulary and more restrained and neutral "moral status" vocabulary.

When using the term "moral status" I do not mean to imply or point towards a *status concept of rights*. In such a theory, beings are identified as right-holders because they have certain attributes.[360] "Status", in these deontological approaches, is a precondition or indicator for rights. This is contrasted to what Leif Wenar, in his introduction to the concept of "rights", calls instrumental or consequentialist approaches, where rights are only doled out to subjects if and in so far as the assigned rights further welfare or other aims.[361]

So status is the basis of rights in (deontological) "status approaches". A more basic notion of moral status, though, is also crucial for the classification of objects of morality within approaches that work without "real" rights (without a strong reading of the rights concept), i.e. consequentialist/instrumentalist approaches. After all, one needs to decide whose interests count, and whose count more than others, and whose welfare is included in the maximization process (this discussion starts with the question which entities can be ascribed such a thing as welfare or interests, at all). Utilitarian approaches, depending on their respective answers to these questions, can be about furthering human welfare, about the welfare of some human and some nonhuman beings who share "typically human" traits, or about welfare of all sentient beings (such an approach would be called sentientism or pathocentrism). Utilitarians could even reach out to more holistic views, including the interests of plants, species or even inanimate objects. This question of very basic

[360] Wenar (2007), "Rights", The Stanford Encyclopedia of Philosophy, Fall 2007 Edition.
[361] If one understands being unconditional on other circumstances to be an essential characteristic of moral rights, it seems that such instrumental or consequentialist approaches make use of rights in name only – "real" rights carry attributes like "natural", "inviolable" or "unconditional", and are scorned by consequentialists, e.g. utilitarian Bentham, who famously called the strong reading of the rights concept as "Natural Rights" "simple nonsense: natural and imprescriptible rights, rhetorical nonscnse, -- nonsense upon stilts." Bentham (1843),"Anarchical Fallacies", in: Bowring (ed.) Works of Jeremy Bentham..

classification is distinct from the question whether one later takes the line of "real" rights or whether one uses them only as instruments to further other aims. Consequently, the question of moral status is distinct from whether one prefers a "real" (deontological) rights approach or whether one is of a more consequentialistic bent.

To sum up, the abstractness of moral status vocabulary is advantageous. One need not commit to big ethical systems or axioms in order to talk about moral status, and it therefore makes different approaches comparable; likewise, it is neutral regarding the question of speciesism.

3. Groups, members, and the kind paradigm

A tendency one frequently encounters in the context of of moral status is the preference to direct this concept towards groups, or individuals qua members of a group, rather than individuals as such. Warren states that moral status is "usually ascribed to members of a group, rather than merely to specific individuals", on "basis of some property or properties that are thought to be possessed by all or most group members."[362] Indeed, moral status statements are usually (though not necessarily) about groups, referring to their members only by proxy. Exemplary statements are "All human beings have superior moral status" or "Inanimate objects have the lowest moral status; i.e. not to be considered." But this is not necessarily so – moral status statements about individuals without references to a group are possible, too, e.g. "This individual human being has superior moral status." The observance that moral status statements are often, yet not necessarily, about groups is not Warren's point, though. What Warren alludes to is the common paradigm that group-members are accorded/possess a certain moral status in virtue of their *belonging to the group* or of being of a kind that typically has certain traits. I will call this approach the "kind paradigm", since it assumes that belonging to a certain kind is crucial for moral status. An example of a moral status statement that conforms with this paradigm would be "Human embryos have superior moral status in virtue of belonging to the species *Homo sapiens* whose members typically have the characteristic of being moral agents." This position is distinct from that expressed in "Human embryos have superior moral status in virtue of being live human beings." Here, what is crucial are the actual characteristics of the embryo, i.e. its being alive and human, while its membership in the group of "alive and human beings" is secondary. To give an actual example of an approach that subscribes to the kind paradigm in regard to the moral status classification of humans, consider the statement:

[362] Warren (1997), Moral Status - Obligations to Persons and Other Living Things, p. 9f.

> *"As opposed to inanimate objects, persons have their ethical status immediately/directly as members of humanity."* [transl. CH][363]

Here, being of a kind (of the human kind) is crucial for moral status ascription.

Summing up, moral status statements are often about groups, or about individuals qua members of groups of entities. Yet, there is a difference between moral status approaches which subscribe to the kind paradigm – i.e. which assume that belonging to a kind is crucial for moral status – and those which do not, i.e. which assume that actual properties or characteristics determine an entity's moral status.

In this context, a distinction should also be made between the practice of classification and theoretical considerations. Regarding classification, group-membership can be regarded as an indicator for moral status (e.g. "Belonging to the species *Homo sapiens* indicates high moral status, other things being equal").[364] This indicator function of group membership can mean that in some cases – i.e. when we know nothing but species membership of the creatures involved – the fact that one is human and the other is not will be decisive regarding our moral consideration towards it. This practical point is distinct from and does not imply the assumption that group-membership determines moral status – we will see later on that this distinction is crucial for understanding different types of speciesist attitudes.

4. Variations of approaches to moral status

As I pointed out in section 1 above, there is a standard or consensus view regarding the moral status of certain groups of entities. In this consensus view, human beings, or persons (these terms are often used equivalently or at least seen as intrinsically connected) are assigned the highest moral status – they are to be fully, and sometimes overridingly, considered. Inanimate objects have the lowest status, i.e. they are not to be considered directly. There are also views which differ from this standard. Some – like Jain Philosophy or Deep Environmentalism – assign higher moral status to what is usually regarded as inanimate entities. Others, like Racism and Sexism, deny some human beings highest moral status while granting it to others, and segment human beings into a hierarchy of moral

363 "Im Unterschied zu Sachen haben Personen ihren ethischen Status unmittelbar als Angehörige der Menschheit." Vossenkuhl (2002),"Der ethische Status von Embryonen", in Oduncu, Schroth, et al. (eds.): Stammzellenforschung und therapeutisches Klonen, p. 164.

364 In a slightly different context, cf. Warren: "Genetic Humanity (…) is at best an indicator, not an independently valid criterion, of moral status." Warren (1997), Moral Status - Obligations to Persons and Other Living Things, p. 19.

status where non-white or female humans are typically set below white and male human beings. Even inside the consensus view, there is a wide margin for dissent. Widely differing characteristics or criteria are brought forward to make moral status classifications, resulting in similarly diverse views on the moral status differences between entities or groups of entities. In preparation for the following sections, which will deal with the debate around the moral relevance of species membership, I will give a coarse taxonomy of differing views on moral status distribution which is primarily based on the distinction between speciesist and species-neutral or non-speciesist views.

Speciesist views of moral status are either characterized as Strong Speciesism – such a view would assume that "being human" is the single criterion which can mark an entity for entry into the highest rank of moral status. On the other hand, there are Qualified Speciesism views: they assume that there is one, or that there are several, criteria, which make a being eligible for this highest rank; yet this criterion/bundle of criteria is distinct from "being human". Qualifying criteria which could be used by a Qualified Speciesist include sentience, personhood, reason, moral agency, the ability to enter into contracts, and many others. It is essential for Qualified Speciesism that these characteristics are thought to exclusively occur in human beings.

Non-speciesist views of moral status, on the other hand, can assume that the very same criteria or criterion (such as sentience or personhood) are decisive, but deem them not to be (necessarily or contingently) exclusive characteristics of human beings. Some non-speciesists argue that the characteristic(s) occur in nonhuman beings, as well. Other non-speciesists deny that criteria like "being human" are morally relevant, as such (i.e. they deny Strong Speciesism). As a third possibility, non-speciesists explicitly lower the hurdle for entrance into the category of entities with highest or high moral status, denying that, e.g. characteristics like "being a moral agent" or "faculty of speech", or even sentience, are morally relevant – for example when assuming that "being alive" is the decisive criterion ("Reverence for Life").

Apart from the speciesist/non-speciesist distinction concerning theories about moral status distribution, there is another aspect such theories can vary in: the structure of the hierarchy they describe. One can, for example, assume that there is such a thing as "absolutely superior" or "overriding" moral status, a kind of trump that is usually ascribed to human beings (in analogy to Dworkin's metaphor of rights as trumps).[365] This assumption of a

[365] Dworkin (1984),"Rights as Trumps", in: Waldron (ed.) Theories of Rights.

trump is not necessary in order to state that the moral statuses of entities differ, since such a difference can be marked on a relative scale, as well. Likewise, it is not necessary to entertain a trump assumption in order to defend speciesism – relative and continuous varieties of speciesism are imaginable. Accordingly, the question of whether some entities demand absolutely superior, overriding moral consideration takes place on a more abstract level and is, in this respect, analogous to that of whether there is an "ace of rights", i.e. a right that "has priority to absolutely all other normative considerations", as Wenar puts it.[366] Relative superiority of some entities' moral status over others', on the other hand, seems an indispensable assumption in order for moral status talk to make sense, at all.

This superiority will also have to be pronounced enough to ultimately warrant real, noticeable differences between the consideration of human vs. nonhuman beings. How these differences play out in the end (i.e. in what way they influence actual treatment) is not only determined by moral status, as we will see in the next section.

5. Moral status assignment: Caveat

Up until now, I presented the moral status concept as a viable means of discussing different approaches regarding the ethical consideration of entities of all kinds (especially human vs. nonhuman beings). Yet there are some caveats or limitations one should keep in mind when dealing with this concept.

One central limitation of the moral status concept is that it is quite a blunt tool. On one hand, this means that moral status statements do not delineate all moral obligations we might have towards a being: obligations that are justified in an indirect way are not covered by moral status concerns. Indirect consideration can lead to a final result that is very different from what initial statements about status might have implied. Therefore, we have to keep in mind that the decision for or against a certain general stance on the level of moral status consideration does not necessarily determine the actual overall normative outcome of an ethical theory. To illustrate this, imagine a theory in which nonhuman animals are assigned the moral status of non-consideration, but where at the same time cruel behaviour towards all kinds of beings is considered highly reprehensible, in principle (e.g. because it is thought to compromise human character, and thereby lead to cruelty towards human beings). Experimenting on animals, in this theory, could be extremely hard to justify. At the same time, a theory that accords all sentient beings basically the same

[366] Wenar (2007), "Rights", The Stanford Encyclopedia of Philosophy, Fall 2007 Edition.

moral status may come to the conclusion that animal experimentation is easily justifiable as long as the benefits it produces outweighs the harm it does. So, surprisingly, a theory that at the first glance entirely devalues nonhuman beings may, on another level, grant them vigorous protection; while a theory that seems clearly "pro-animal", at a second glance puts animals in a quite precarious position.[367] It is a somewhat counterintuitive result of these considerations that – although that is the usual paradigm – speciesism is not necessarily associated with the sanctioned maltreatment of animals, and that non-speciesism does not necessarily result in a very strong protection of nonhumans. While this is not a shortcoming of the moral status concept, it should be kept in mind that the informative value of general moral status assertions is limited by further moral considerations on more indirect levels.

Adding to the bluntness of moral status statements is the fact that they, as I pointed out above, usually refer to groups of beings (such as "nonhuman animals", "sentient beings", "human beings") rather than individual entities. One central problem at this point is that the delineation of the groups that are picked out may be blurred or continuous. At what point, for example, do human sperm and egg, or human embryo, become a human being? Or, to remind of the subject of this dissertation which is an exemplary case of blurred boundaries, when do animal-human interspecifics? At what point does a being become sentient, or alive, or self-aware? One could, as a general point of criticism, note that the simple structuring into what is a limited number of levels of moral status is much too coarse to capture the continuous, vague reality of status distribution. Accordingly, one could also assume a "sliding scale" of moral status, where entities are assigned continuous variable moral consideration according to what actual properties or characteristics they have, taking into account that they may be inferior in one respect while they are equal in another. An approach that sees moral status as an infinitely graded continuum of different status levels of individuals is also imaginable, but would be beside the point here – this is because we will discuss moral status primarily within a moral framework of the ethical principle of Speciesism, which would not go along with particularism regarding moral status assignment.

The bluntness of the tool of moral status makes it unlikely that particular cases, such as the question of how to treat one specific human-animal chimera, can be coped with in its

[367] The same is true for a speciesist theory which assumes a prima facie priority of human interests, but makes exceptions from this rule if petty interests are staked against vital ones. Here, the human "interest in meat eating" might lose out against a non-human "interest in surviving." Non-speciesist theories,on the other hand, can allow for meat consumption.

entirety by referring to moral status considerations. This, again, is not to say that such considerations are superfluous, but that their informative value is limited because results depend enormously on the specifications for terms such as "being human", "being sentient", etc.: two theories may both state that sentience is the single criterion for moral considerability, and yet come to extremely differing results. For an illustration of this principle, compare Carruthers' extreme pro-animal-testing stance to Singer's (albeit not absolute) condemnation of such practices – both regard sentience as the decisive criterion for moral status, but Carruthers believes that animals do not meet this criterion, and that they therefore do not qualify for high moral status.[368] Again, decisions made on other levels (in this case, assumptions made in philosophy of mind regarding the presuppositions of phenomenological consciousness, which Carruthers deems to be dependent on a "theory of mind") lead to the result that isolated statements about moral status and its criteria have limited informative value.

The possible variations – and limitations – of moral status assignments notwithstanding, note that, on a very basic level, the assessment of moral status can have extremely significant consequences. Moral status questions are not just relevant for seemingly exotic questions like the status of animals (or even chimeras). The inclusion in or exclusion from the realm of morally considerable, or "fully" considerable, beings is one of the most basic decisions of any moral system. Though consideration or non-consideration can be shaped in ways that differ widely regarding actual treatment, moral status assignment is probably the most powerful weapon in moral discourse: extremely morally reprehensible practices were and are often justified not by "fine-tuning" of normative rules (such as "murder is allowed, if you have good reasons" or "torture is ok for the greater good"). Rather, the construction used was, and is, typically one of explicit exclusion of certain subjects out of the moral realm (i.e. negative moral status assignment). Africans were simply not included in the realm of persons in times of slave trade, Jews did not "count" as to-be-considered subjects by the perpetrators of the holocaust, communist regimes styled the ostracising of non-compliant individuals not along the lines of "x does not follow moral rules and must be punished", rather, certain individuals were declared "class enemies", which were then fundamentally excluded from the realm of subjects to be considered morally (allegedly, moral philosophy books in the cold war Eastern Bloc stated as an example of an ethical principle with an admissible perception the phrase "Killing your mother is always wrong,

[368] Cf.: Singer (1976), Animal Liberation - A New Ethics for Or Treatment of Animals; Carruthers (1992), The Animals Issue: Moral Theory In Practice.

except if she is a class enemy.") So note that even if moral status assignment is a blunt instrument, it has, on the other hand, also the potential to be used as a drastic, incisive tool, with far-reaching consequences.

6. General critique – and reality – of status assignment

A very general criticism of the moral status framework claims that status classification of groups or types of beings according to certain criteria is a mistaken approach, as such, because status assignment, as a construct, has no leg to stand on, and is a fundamentally wrong approach to distribution of moral consideration.

Wilhelm Vossenkuhl, sceptical of any general value ascription to entities in nature (which I understand to be analogous in many respects to what I here describe as "status assignment") notes that in this context "No hierarchy is without problems." [transl. CH][369] His critique is not only aimed at holistic approaches which ascribe value according to function in the world's ecosystem or similar criteria, but also to pathocentric approaches. Any value ascription (or status assignment) is necessarily an anthropocentric one, Vossenkuhl states: it is always done by humans, out of a human mindset, and cannot take into account interests of nonhuman entities, since the latter are not accessible to us. Furthermore, while criteria like sentience are hard to ascertain, criteria like "utility within an ecosystem" are possibly even harder to establish, and, ultimately, have no moral relevance, Vossenkuhl argues. Consequently, value ascription systems and hierarchies are on a fundamentally wrong track, and should be given up altogether.

In fact, many types of status hierarchy take into account criteria which I personally would not regard as morally relevant (be it "utility in ecosystem", "membership in a race", or "rationality"). These are valid points of concern, yet, rather than presenting this as a general critique, I would suggest a type of status assignment that makes use of criteria that are more to my taste and reflect my respective value assumptions. Still, I acknowledge that status assignment processes are always highly problematic no matter what criteria are employed. Each such process depends on countless potentially problematic empirical assumptions (e.g. about the physical structure and needs of other beings), inductive steps or hypotheses (e.g. about preferences or interests of fellow beings, be they human or nonhuman), and all status assignments are tainted by our human and our personal perspective, which, in certain respects, we cannot leave behind.

---

[369] Vossenkuhl (1993), "Ökologische Ethik - Über den moralischen Charakter der Natur", Information Philosophie, **1**, p. 8.

Yet, I believe that the model of status assignment describes quite well how moral consideration of types of entities does in fact happen in the real world. Robin Attfield's notorious statement that a human life is worth as much as one million trees is tacitly dismissed by Vossenkuhl, probably as indecent, morally reprehensible or at least demonstrably ridiculous.[370] In fact, many such calculations are carried out implicitly in today's societies and by ourselves, maybe not with the same numerical result, but with the same variables being weighted against each other. Our governments do not spend all taxes on emergency healthcare or foreign aid (to save human lives), but use a sizeable proportion on the protection of animals (or even trees). Most governments or voters would hesitate to publicly make the calculation that "One life is worth n trees", yet these calculations are implicit in spending and other decisions. Regarding the value of human lives, government policy implicitly counts the lives and interests of natives far above the welfare and even lives of foreigners. Laws ensure that such hierarchical status assignments do have real world consequences, and they do not elicit much protest in public – as long as the status assignment is not made explicit.[371] This is also true for individuals: every time you spend one Euro on free-range eggs rather than battery-hatched ones (out of animal welfare rather than culinary reasons), you make an implicit decision that ranks the interests of chickens above your own interest in buying something else with this amount of money. You weight chicken-welfare against human welfare, and have implicit assumptions about status hierarchies in this context (e.g. there is probably a quite low monetary limit above which you would not go in order to further chickens' interests).

Granted, there rarely is explicit status assignment in these processes – but this does not mean that there is *none*. We can detect a de facto, real-world status hierarchy in the consideration given to certain groups of people (or types of beings or entities) by society, and by individuals – even if the very same societies and individuals would find making such status hierarchies explicit mistaken and wrong. Status assignment to groups or types of beings may ultimately not be the perfect approach to moral consideration, but moral status assignment, resulting in (relatively) clear hierarchies and discriminatory practices, is what we as individuals, our governments and societies actually engage in on a large scale. It is also a cornerstone of speciesist approaches, the subject matter of the next section.

---

[370] For Attfield's hierarchical and strictly consequentialistic view of value distribution in nature, see Attfield (1987), A Theory of Value and Obligation.

[371] An exception to this tendency is found in the U.S., where new regulation routinely undergoes cost benefit analyis which then takes into account measures like the value of a statistical life, expressed in U.S. Dollars.

B. Speciesism

In chapter 2 above, I concluded that the success of several typical arguments against the creation of human-animal interspecifics depends on what I called speciesist positions. Arguments that presuppose speciesism or are based on the intention to preserve it include those from human dignity (see chapter 2, section B.4 above), some kinds of boundary arguments – i.e. those which claim that the boundary between humans and nonhumans is an inherently special or sacred boundary (see chapter 2, section B.3 above) – arguments that claim human-animal interspecifics could lead to the detrimental consequence of "absolute confusion" about their moral status (see chapter 2, section D.3 above), and the confusion argument reconstructed by Robert and Baylis,[372] which states that human-animal interspecifics could wrongfully endanger speciesist attitudes and thereby put society's current values at risk (see chapter 2, section D.3 above). Arguments that are based on the belief in a particularly high moral status of human beings also include most concerns for the proper use of human material like gametes, DNA, and tissues, and the influential concern regarding the misuse and destruction of human embryos (see chapter 2, section C.2.b above). Since the validity of speciesist positions is a complex issue, and also because human-animal interspecifics emphasize interesting aspects of the speciesism question, I want to discuss speciesist approaches in this separate section.

"Speciesism" stands for diverse types of opinions and positions – their common denominator is the belief that nonhuman animals deserve less consideration than humans.

Speciesism can present itself as a simple pragmatic rule of discriminatory decision-making. Such a rule of thumb could, e.g. say that you should favour humans over mosquitoes. This discriminatory approach to insects could, for example, be based on the assumption that, statistically speaking, it is highly likely that a human being is self-aware and conscious, while it is extremely improbable that an insect has these traits. Such a speciesist pragmatic rule allowing the swatting of annoying mosquitoes while forbidding the squashing of annoying humans could be based on pathocentric (i.e. non-speciesist) ethical principles. In my discussion of the process of assignment of moral status, I explained this effect with the "indicator function" of membership in certain groups (see p.121).

In other cases, speciesist pragmatic rules concerning the treatment of humans and nonhumans will be based on general assumptions regarding the moral status of humans vs.

[372] Robert and Baylis (2003), "Crossing Species Boundaries", American Journal of Bioethics, **3**(3), p. 9f.

nonhumans. These are the positions I am concerned with in this chapter – I will call them "Speciesism" (with capital S) or "Speciesist ethical principles".

Speciesist ethical principles come in different types. One possible way to classify them is according to the reason they offer for assigning humans higher moral status than nonhumans. One type of Speciesism states that human beings have high moral status because "being human" is valuable in itself (Strong or Simple Speciesism). More complex types of (Qualified) Speciesism state that humans have high moral status because of certain characteristics that are particularly valuable (such as self-awareness, language, consciousness, etc.).

A second taxonomy could ask what classification system Speciesists use for distinguishing between human and nonhuman beings, in the first place. Very often, "human" is understood in the sense of biological taxonomy as "member of the species *Homo sapiens*". Others prefer a non-definitional approach – saying that we do not need to define "human" or even that it would be wrong and mistaken to reduce "being human" to a fixed list or bundle of necessary and sufficient properties. This table shows a matrix of Speciesist approaches along the lines of these two taxonomies:

Classification method: / Reason for high moral status assignment	Biological Taxonomy	"Non-Definitional"	
Valuable in itself (Strong Speciesism)	"Being a member of the species *Homo sapiens* confers high moral status to members of human species."	"Being human, which cannot be reduced to natural properties, confers high moral status."	→ moral relevance problem, see 1.
Valuable because of associated characteristics (Qualified Speciesism)	"Members of the species *Homo sapiens* have characteristics which justify their high moral status."	"Human beings have characteristics which justify their high moral status."	→ marginal cases problem, see 2.
	→classification problem, see 3.	→universalizability problem, see 4.	

As this table illustrates, all four main angles of Speciesist approaches bring with them a specific problem. Some of these problems are further emphasized by human-animal interspecifics – this, in turn, should influence our final judgment of Speciesism.

The four problems of Speciesist approaches will be the subject matter of the following sections.

1. The moral relevance problem

This problem affects all Speciesist approaches which claim or assume that "being human" or "being a member of the species *Homo sapiens*" is valuable in itself and gives its carrier superior moral status (i.e. Strong Speciesism). Why, one could ask, should being human be relevant for moral status?

The problem of moral relevance can be illustrated by an analogy of Speciesism to racism or sexism (the term "speciesism" originally alluded to this analogy, though it is understood by many, including myself, as a neutral term today). The racism/sexism analogy stems from

Peter Singer's seminal anti-speciesist work "Animal Liberation".[373] Singer argued that, just like women are no doubt different from men, and white people demonstrably different from black people, animals are different from human beings. However, factual inequality of the sexes (or races) does nothing to *justify* sexism or racism, and, in the same way, factual differences between human and nonhuman beings do not justify discriminating against nonhumans. Singer argued that the "principle of equality", which demands equal consideration *in spite* of factual inequality, should be extended to nonhuman animals. In the light of Singer's analogy, Speciesism, as a moral position, is not just abstractly mistaken. It is untenable in the very same way, to the same degree, and for the very same reasons sexism or racism are, i.e. it is deemed systematically wrong. Speciesism, Singer says, must be condemned "in analogy with racism", and he finds it "obvious that the fundamental objections made to racism and sexism […] apply equally to speciesism", which he regards, just like other "-isms" as a "prejudice of attitude of bias in favor of the interests of members of one's own species and against those of members of other species."[374]

Is the analogy between speciesism and sexism or racism persuasive as an argument against speciesism? Is speciesism "like racism" or "like sexism", and, if yes, in what regard? And does that mean speciesism is untenable?

What Singer stresses in regard to both sexism/racism and speciesism is the fact that they base their moral distinction between man and woman (or white and non-white, or human and nonhuman) on qualities that are *morally irrelevant*. Singer's objection can be used to counter Strong Speciesism, which holds that species membership determines superior moral status. Why should the mere fact that a being belongs in a different biological species justify its having a different moral status, i.e., ultimately, ought to be treated differently? This seems like a naturalistic fallacy (drawing ethical conclusions from natural facts), and conspicuously similar to racist or sexist ideologies, which also claim that a biological disposition, like belonging to a race, or that of having or not having a Y chromosome, determines moral status. "Belonging to a nonhuman species", from this standpoint, appears to be a characteristic that is just as obviously morally irrelevant as "having dark skin" or "being female". Singer's analogy between speciesism and racism/sexism boils down to one basic accusation: it is claimed that speciesists, just like racists and sexists, base their discrimination between human and nonhuman animals on morally irrelevant characteristics.

---

[373] Singer (1976), Animal Liberation.
[374] Singer (1976/1990), Animal Liberation, p. 6.

This does not rule out Speciesism, once and for all. It could be the case that there is indeed one, or several, morally relevant characteristic(s) that all human (but no nonhuman) beings share – this leads towards the Qualified type of speciesism described above. Whether this route is a promising one will be discussed in the following section.

In defence of Speciesism, one could also remark that the problem of moral relevance is not unique to Speciesism, but applies to *any* ethical system which discriminates between entities regarding moral status. Each of these systems has to own up to justifying its discrimination criteria. Although a discrimination criterion like sentience seems somewhat less in need of justification than one like genetic disposition, rarely do pathocentrists give an overt justification of why they deem sentience, or phenomenal experiences, to be more valuable than non-sentience, or absence of phenomenal experiences. These value statements seem self-evident to many, but they may not be evident to all. In turn, the moral relevance of species membership may be regarded as defensible or even evident by some.

The question of Speciesism or Non-Speciesism, accordingly, is not settled once and for all with the assertion that speciesism is "like racism or sexism". It seems clear though that if species-membership itself is flaunted as "morally relevant characteristic", this approach will be quite hard to defend, or at least much harder to defend than non-speciesist accounts which use criteria like sentience or self-awareness.

2. The marginal cases problem

Qualified Speciesists, who justify the superior moral status of humans by pointing out that members of the species *Homo sapiens* have particularly valuable properties such as consciousness, self-awareness, intelligence, capacity to form complex social relationships, and so on, can avoid the problem of moral relevance or at least keep it to a minimum by referring to those typical human properties that seem evidently relevant for moral status. In turn, Qualified Speciesism is faced with another problem: biologically human beings who do not exhibit these properties. In the speciesism debate, such cases are called "marginal humans". This somewhat clumsy but by now customary term means to imply that those cases are on the borders of what is considered "typical" for a human being (rather than implying that they are "not really human").[375]

What counts as a marginal human can vary wildly. It depends on which properties the Speciesist account in question uses: the more demanding the approach is, the more

[375] Cf. Pluhar (1995), Beyond Prejudice: The Moral Significance of Human and Nonhuman Animals, p. 63.

biologically human beings will qualify as marginal. E.g. if rationality or even capacity for moral agency is declared to be the relevant property, children and mentally handicapped persons will not make this hurdle. Ultimately, even for the lowest of hurdles (e.g. sentience) there are biologically human beings which cannot jump it (e.g. anencephalic infants).

Marginal cases are imaginable on both sides of the human-nonhuman divide. When Speciesism assumes a lower hurdle, stating that, e.g., "capacity to form social relationships" is the property which determines moral status, one will have to deal with the claim that there are nonhuman beings which also have this capacity. The scenario of animals that are somehow subject to a moral upshift by introduction of human material would constitute a paradigmatic case of a "marginal animal". The occurrence of mice or nonhuman primates which suddenly speak up, laugh, become self-aware etc. is, as we have seen above, a commonly discussed scenario in the interspecific debate – even if it is strictly hypothetical today, as human-animal interspecifics have not shown any kind of humanization in this sense. Still, marginal animals are a thought experiment which effectively highlights an actual problem of Speciesist approaches.

The Qualified Speciesism account, facing marginal cases, ends up with two questions: Why should marginal humans be accorded high moral status, although they do not exhibit the properties allegedly responsible for this high moral status? And secondly, why should nonhumans which exhibit these properties not be accorded high moral status?

A solution to this problem would be, first of all, in finding a property that all humans and no nonhumans have (as a second step, one would have to argue for its moral relevance). The search for such a property often leads Speciesists to scientific species classification concepts. Zoological taxonomy is assumed to deliver the desired mode of unambiguous distinction between human and nonhuman, the desired property that "all, and only, humans have". But does it?

3. The classification problem

Speciesism approaches which rely on biological taxonomy assume that members of the species *Homo sapiens* have a superior moral status in comparison to non-members of this species. Aside from justifications for this position, in order for this ethical principle to make sense, the Speciesist should be able to tell us (at least in theory) which entities are human in this sense, and which entities are not – otherwise, the principle could not offer any guidance in decisions about a general course of action concerning the treatment of

differing entities, which I deem to be a minimum requirement for ethical principles. So, what Speciesists try to derive from biological classification is an unambiguous, consistent way of carving up nature into different species or, rather, into humans and nonhumans. They assume an essentialist concept of species: for every species, they believe, there is a set of characteristics or properties all of which any member of that species must possess. At least, they believe that this is true for the *human* species. Does the concept of "species" in biology accommodate for such "essences"?

a. Searching for a human species essence in biology

There is a stunning variety of concepts of "species" and criteria for "species membership" in biology, far too many to describe them here. The classical species concept is the typological species, which goes along the lines of differing phenotypes; other species concepts (e.g. biological species) rely on the (actual or possible) reproduction of fertile offspring between species members; phylogenetic species describe one "branch" in the evolutionary tree. There is no generally predominant or most appropriate species concept in biology.

We can look at the question of whether there is such a thing as "species essences" from a very general point of view, though. Modern biology assumes that all living beings are products of evolution. Spontaneous mutation is the motor of this process. And evolutionary theory states that, in principle, all characteristics of individuals can be subject to spontaneous mutation in the next generation. This means that over time, there is no room for something like an unchanging "species essence" that members of a species necessarily share. Ereshefsky concludes: "From a biological perspective, species essentialism is no longer a plausible position."[376]

The lack of "species essences" goes counter folk taxonomy and confuses us. Essentialist perceptions in this field are still common standard. Today, they often come in the guise of a very modern concept: that of the gene, the basic transmission unit of biological heredity, which is included in the genome, the whole of hereditary information of an organism. In folk genetics, the genome has retained the reputation of determining, unambiguously, the species membership of an individual. Genetic sequences, accordingly, are referred to in species terms: there are "jellyfish genes",[377] "human genes",[378] and so on. Additionally,

[376] Ereshefsky (2002), "Species", The Stanford Encyclopedia of Philosophy (Fall 2002 Edition).
[377] A German journalists refers to the "Quallengen" (jellyfish gene) inserted into transgenic monkey ANDi, see Schuh (2001), "Affen-Flop Transgene Primaten", Die Zeit, 2001/01/25.
[378] nano (2007), "Menschliches Gen lässt Mäuse die Welt in Farbe sehen", 3sat online, 2007/03/23.

genes are ascribed a quasi-magical deterministic power: they are the "blueprints" of organisms, from which the developing cells slavishly take orders concerning the setup of their surroundings. From this vantage point, it seems that species essentialism is still intact: members of the species *Homo sapiens*, in this view, can be distinguished from non-members by their bodies' content of "human genes". Any organism that contains such human genes, in turn, is a member of the species *Homo sapiens*, and accordingly has high moral status.

There is no such thing as a "genetic essence", though. The "humanness" of a DNA set, for example, can only be assured by relational comparison to other DNA sets. There are no uniquely human DNA sequences which are common to all, and only, humans. Robert and Baylis put it this way:

> *"(…) it is not the case that there is a certain part of an individual's genome that is 99.9% identical with every other human's genome. Although human beings might share 99.9% commonality at the genetic level, there is nothing as yet identifiable as absolutely common to all human beings. According to current biology, there is no genetic lowest common denominator, no genetic essence, 'no single, standard, normal DNA sequence that we all share.'"*[379]

The simple explanation for this fact is, again, that evolution is crucially dependent on variability of traits. Spontaneous mutation is based on the variability of DNA microstructure. In order to make adaption to the environment, the enabler of evolution, possible, each and every DNA sequence in an individual's genome is up for variation. The mechanism of evolution is not compatible with the development of a "genetic essence".

Additionally – and this is crucial for the context of interspecies beings – the layman idea that genes are the ultimate determinator of living beings, functioning as "control centres" which effortlessly steer the development of organisms into every imaginable direction (i.e. the direction that is typical for "their" species), is mistaken. As the ISSCR Committee Forum points out:

> *"(…) what does 'animal or human gene' or 'animal or human cell' actually mean? In the light of the evolutionary conservation of many signalling pathways, 'human or animal genes or cells' can refer only to the fact that these units have a human or animal origin. But from this it does not follow that an animal gene or cell, once put into a human, behaves as an independent unit of 'animal agency' or vice versa."*[380]

---

[379] Robert and Baylis (2003), "Crossing Species Boundaries", American Journal of Bioethics, **3**(3), p. 4.
[380] Hyun, Taylor, et al. (2007), "ISSCR: Committee Forum - Ethical Standards for Human-to-Animal Chimera Experiments in Stem Cell Research", Cell Stem Cell, **1**(2), p. 160.

In the last decades, research on posttranslational and epigenetic processes has revealed the limitations of genetic determination. So, not only is there no such thing as a "species essence" to be found in genes. Genes are also not the single magic ingredient that determines living beings' properties, but rather, one puzzle piece in a complex, interacting system of numerous parts that are bound into feedback loops. The belief in genes understood as all-determining "blueprint" of organisms has, in fact, decreased so far over the last years that researchers have now declared the "post-genomic era", where the focus is less on deciphering DNA and, more holistically, in finding out "how novel functions and properties emerge from the dynamic web of interactions and feedbacks brought about by the molecules of a living organism."[381]

This all does not mean that species or genes are irrelevant or non-existent. It only means that the term species, as Darwin himself stated, is: "one arbitrarily given, for the sake of convenience, to a set of individuals closely resembling each other."[382] Dunbar repeats this conventionalist interpretation:

> *"Species, as we describe them, are matters of convenience rather than biological reality. The real world consists only of individuals who are more or less closely related to each other by virtue of descent from one or more common ancestors."*[383]

b. Consequences for Speciesism based on biological taxonomy or genetic disposition

What does this mean for Speciesism? For views which claim that membership in the species *Homo sapiens* determines moral status, the moral relevance problem is emphasized by this discovery. "Species" are not a given category of nature, but rather a human convention. And as we know, the grouping of species is done for the sake of biological research, not for the sake of singling out morally superior beings. Why there should be a connection between these two realms stays unclear. Graft concludes:

> *"The term species may have a meaning in the context of our everyday discourse or in the context of practical taxonomy, but those contexts are not coherent for use in the context of morality."*[384]

For views which claim that human species membership confers moral status because the human species has certain particularly valuable properties, a similar problem emerges: if

---

381 Falaschi (2002), "Living in the Post-genomic Era", UN Chronicle (3).
382 Chapter II, "Doubtful Species", in Darwin (1872), On The Origin of Species.
383 Dunbar (1993),"What's in a classification?" in: Singer and Cavalieri (eds.) The Great Ape Project.
384 Graft (1997), "Against Strong Speciesism", Journal of Applied Philosophy, **14**(2).

"human genes" are not the lone determinators of the characteristics of an organism, their existence in an organism loses at least some of its relevance.

What is more, both strong and qualified speciesism views face a classification problem that is emphasized by interspecific beings. With a non-essentialist view of species, the species membership of a being is not necessarily ascertainable anymore: It is unclear whether the DNA of a (hypothetical) human-animal hybrid, e.g. between human and chimpanzee, would count as "human", and, in turn, whether such a being would have to be considered "human" or not. When a human-to-animal embryonic chimera contains human-typical DNA, this does not necessarily mean that the organism is a member of the species *Homo sapiens*. Other mixed entities, like human-animal cybrids, are similarly problematic: they contain human-typical DNA, but in an environment which is most likely not conducive to its development into a human organism. Are these entities "human"? A Speciesist view which tries to take refuge in biological classification methods will not be able to answer these questions, and, in turn, will not be able to ascertain the moral status of such entities.

4. The universalizability problem

This whole complex of problems is apparently bypassed by Speciesist approaches which avoid the classification question and state that "being human" cannot be reduced to a fixed list or bundle of natural properties and that the whole step of defining "human" is unnecessary or even mistaken.

In this vein, one could state that "being human" just means that a being is assigned high moral status by the moral community. In this view, everything that the speaker(s) wants to be treated according to a superior status would be called "human". Declaring something "human" would simply be identical with assigning this specific being superior moral status (rather than referring to biological taxonomy).

It seems in fact plausible to me that the way we use the term "human", especially in the moral context, is strongly influenced by our normative assumptions about what *should* be treated as human, e.g. which entities should be assigned high moral status. This is where our strong intuitions about what is human and what not come from, rather than, so to speak, from an internalized but inaccessible list of "essentially human" properties: human is whatever we want to be treated humanely. This understanding of "human" and "nonhuman" would make it easy to circumnavigate the problems described so far: the moral relevance of "being human" would be ascertained by the view that calling something

"human" is simply identical with assigning it moral status. Marginal cases of atypical humans could be called "human" and assigned high moral status without running into inconsistencies. Classification would be avoided as a whole.

This strategy fails, though, because the result would not be an ethical principle of Speciesism, but rather a reduction to ethical particularism concerning status assignment. Such an outcome would go counter the whole concept of Speciesism as a guiding principle in assigning moral status. Universalizability is a minimum requirement of an ethical principle: it should be transferrable and applicable to similar particular cases. A rule of moral status assignment which does not offer any criteria for future classification would not meet this requirement.

Differing varieties of Speciesism as an ethical principle are, as we have seen, riddled with severe problems: one is that the moral relevance of "being human" is hard to establish once we understand "being human" as membership in the species *Homo sapiens*. Another problem is that, once the superior moral status of human beings is somehow tied to their having particularly valuable properties, the occurrence of humans which lack these properties is hard to deal with. Interspecific "Marginal Animals" in the form of nonhuman beings to which valuable human properties have been transferred are a thought experiment that further emphasizes this problem of moral relevance. A third complex of problems is found in the concept of species, in general: its conventionality makes the case of moral relevance even harder to argue for; additionally, the reduced deterministic power of genes destroys the idea that species membership is essentially linked to certain characteristics like "having human genes". Additionally, novel human-animal interspecific mixtures show that classification into human and nonhuman can pose insurmountable problems for species concepts, which, in turn, would make the assignment of moral status impossible for Speciesist approaches. Finally, we have seen that Speciesism needs to rely on some kind of classification in order to constitute a generalizable ethical principle. Speciesist ethical principles, in the light of human-animal interspecific beings, but also independently of them, seem extremely hard to defend.

Chapter 4: Should We Prohibit Interspecific Creation?

Our overview of possible arguments against the creation of interspecifics, in chapter 2, covered a wide array of possible concerns, ranging from very practical worries like the threat of zoonoses to highly theoretical remarks, e.g. on moral boundaries between species, human dignity concerns or different types of moral confusion. The excursus of chapter 3 revealed that Speciesism, a position that some or even most of the arguments discussed rely on, is hard to defend and should, consequently, at least not be the main supporting beam of argumentation against the creation of interspecifics.

After this comprehensive presentation, I will analyse the argument-types in order to answer the central question posed above: is there a persuasive argument for the general position that creating interspecifics (specifically: human-animal interspecifics) is wrong and should be prohibited?

In answering this question, I will assume that, were creating interspecifics arguably morally wrong, governmental or other institutional prohibition of their creation would be advisable. Although usually the question of moral wrongness of an action and the admissibility of its prohibition are better treated separately, I will conflate the two here for simplicity's sake. In the case of high-profile, often governmentally funded research, which typically already is subject to heavy regulatory intervention, it seems that prohibition of certain actions seems to be an appropriate and also effective intervention – were these actions found to be morally wrong. What types of regulation or prohibition seem advisable in the light of my analysis will be discussed in section B below.

A. Concise analysis of arguments

To answer the question whether the creation of interspecifics is morally wrong, I will now give a distilled overview of the most important arguments presented above and focus on three aspects in each case:

- *Originality*: Is the argument presented new and specific to the chimera debate, or where does it originate?

- *Applicability*: What types of interspecific beings would the argument apply to?

- *Persuasiveness*: Does the argument convincingly state that creating interspecifics, or specific types of interspecifics, is morally wrong?

Let us look once more at the argument types introduced previously.

The type of arguments discussed under the catch phrase "Yuk Factor" is not unique to the chimera debate, rather, they stem from other areas of concern – Leon Kass' "Wisdom of Repugnance"[385] was originally directed against cloning. The idea that mixtures as such are in some way repugnant or bewildering is found in many other areas as well (e.g. in the common reaction to transgendered persons). It is unclear what kinds of interspecific beings arguments from repugnance would refer to, exactly. Essentially, they could be directed against all kinds of mixed beings – including artificial interspecifics, but also "natural" animal-animal hybrids or transgenic plants. In all these cases, it could be argued that "the mere idea" of an animal-animal or human-animal mixture is revolting. The beings that are apparently most likely to create direct reactions of "yuk" are those that have human-typical features or parts. Vacanti's earmouse (see p. 12) would be one example which often has this effect on people – interestingly, tissue engineering can produce such "yucky" creatures without even mixing human and nonhuman material (Vacanti's mouse's ear was made of bovine cells, it could also be created from exclusively murine material). Most creatures or entities created in current interspecific research, on the other hand, would probably not evoke an intuitive "yuk" response: they seem inconspicuous and not monstrous at all, or, in the case of manipulated microscopic entities, much too unfamiliar to the layman to generate any direct emotional response. Thus, the specific direction of yuk-factor arguments remains unclear. Repugnance or "yuk factor" objections, I conclude, are not philosophically persuasive. Even after several rescue attempts and with a lot of charitable interpretation, they remain in the realm of appeals. Additionally, invoking "repugnance" or "yuk" is not helpful for the debate since these reactions vary greatly and cannot, as it were, be reasoned into people who do not feel them. However, an assessement of intuitive or emotional reactions to confrontation with the idea of chimerism can be helpful in finding out where the real arguments against chimera creation lie. To give but one example, the theory that the human face is an important signifier for high moral status can explain why many find the thought of creating animals with human features especially revolting.

[385] Kass (1997),"The Wisdom Of Repugnance", in Kass and Wilson (eds.): The Ethics of Human Cloning.

The second type of arguments we looked at in chapter 2, section B.2 above were religious and quasi-religious arguments. Christian religious objections against the creation of interspecifics can be derived directly from the scripture (i.e. from the explicit prohibition of sex with animals), and are, in this sense, based on a very well-known moral taboo that exists in many cultures and religions. More indirect Christian arguments against human-animal interspecifics can be derived from the concept of human dignity which will be discussed below. Religious or quasi-religious concerns stating that interspecific creation constitutes "Meddling with Nature" or "Playing God", i.e. hubris arguments, have been, analogously, aimed at new biotechnologies like genetic engineering or cloning. They are not new or specific to the chimera debate. Christian arguments against the creation of interspecifics often focus on the fundamental distinctiveness of humanity from other living beings – in this case, they are only applicable to human-animal interspecifics. More general religious arguments (i.e. from the "completeness of creation"), just as quasi-religious/hubris concerns, can potentially be used not only against the creation of human-animal chimeras, but also against the creation of interspecific animal-animal chimeras and transgenic animals and plants. Religious objections and what I called quasi-religious concerns (see chapter 2, section B.2) have the obvious disadvantage of being not persuasive to non-religious persons or persons who do not believe in a natural teleology. The mythical idea of hubris, today, is hard to defend in an intellectual argument, though it seems to have extensive influence on public debate.

The third type of intrinsic argument I introduced above rely on the moral relevance of the boundary between species, especially between human and nonhuman species. These arguments are new and specific to the debate around chimeras, though one could argue that part of this argument already can be found in (rarely explicitly discussed) arguments against sexual contact with animals. Boundary arguments certainly become clearer, and much more intense, in the context of human-animal interspecific creation, though. Boundary arguments would, at first glance, apply to all kinds of novel, artificial interspecific beings and maybe even to "natural" animal-animal hybrids. What specific actions these arguments are directed against, however, depends on what exactly is identified as the "violation" of species boundaries. Since this is rarely discussed, the specific direction of boundary arguments remains unclear. Boundary arguments depend, firstly, on the assumption that there is a clear, hard boundary between species – an assumption that is not at all easy to make, as we have seen in chapter 2, section B.3 above. Explaining why biological disposition should be morally relevant presents another challenge for boundary

arguments. Even if these difficulties were overcome, I still see a problem in the most basic question concerning boundary arguments, i.e. the question in what way, or at what point, mixing constitutes a violation of boundaries (and what kinds of mixing are considered boundary-crossings). Accordingly, I do not think that boundary arguments show much promise as arguments against the creation of chimeras, even against human-nonhuman chimeras.

Concerns for violations of human dignity (see chapter 2, secion B.4 above) are well known from many fields of bioethical discourse. In the specific case of human-animal interspecific creation, it is not clear what exactly the violation of human dignity – i.e. a transgression that exceeds a simple violation of interests – would consist in. It remains unclear which cases of interspecific research such arguments could apply to. I find the view that creating human-animal interspecifics constitutes a violation of human dignity to be mistaken, since I could not identify which subject is robbed of valuable capacities or kept from exercising them as a result of creation of chimeric, hybridic or transgenic beings. There is, of course, the much more general objection that human embryonic stem cells or human embryos should not be used for research, at all, because this constitutes a violation of human dignity. This far-reaching argument does not help to express the view that interspecies creation is *especially* violating to human dignity (much more than simply destroying an embryo e.g. in an abortive procedure), a view that objectors to chimera creation on grounds of human dignity seem to hold. Apart from these problems, using the language of "human dignity" is not very helpful in the area of moral classification of human-animal chimeras or other interspecifics, since it presupposes or at least hints at an assumption that, in my opinion, is questioned by the very idea of humanized chimeras (namely, that we can always determine who is and what isn't in the group of "humans"). Human dignity approaches, as I explained above, are based on the questionable doctrine of Speciesism. To sum up, human dignity concerns do not seem persuasive as arguments against the creation of interspecifics because the exact point at which interspecific creation, as such, results in a violation of dignity, remains unclear. But even indirect approaches (i.e. stating that all embryo use violates human dignity or that keeping part-humans in lab settings might) are dubitable since the concept of human dignity begs the question of what is relevant for moral status by assuming Speciesism.

What about consequence-based arguments against creating interspecifics? The first aspect covered in our discussion were "classic" animal welfare concerns. The issue of animal welfare in medical or other experimentation settings is vigorously discussed not only in

cases of interspecific research. Maybe less obviously, the creation of beings that are designed with tacit acceptance or deliberate causation of sub-par quality of life is not limited to interspecific research. Concerns about the cruelty inherent in creating suffering animals also apply to animal models which are bred to model human diseases, but which are not necessarily a product of transgenesis or chimerism. The general concern for beings that are created in order to exhibit detrimental characteristics actually even predates modern engineering technologies: traditional animal husbandry often leads to pets and livestock with diminished quality of life. Animal welfare concerns can presumably pertain to all kinds of interspecific research. Often, the problem is not only in the experiment per se, but in detrimental keeping of animals (i.e. lack of proper habitat conditions, lack of social contact, etc.). Even research done in vitro (e.g. human-animal cybrid research) might be susceptible to concerns for animal welfare, because it makes use of animal material (e.g. cow or rabbit eggs) which might be obtained under morally despicable conditions. In effect, in vivo research involving adult nonhuman primates is most likely to raise animal welfare concerns; in vitro research, on the other hand, will probably raise the least concerns. The creation of interspecifics, as such, is no more objectionable than other kinds of research involving animals. In this sense, animal welfare seems not to be a powerful argument against interspecific research creation per se. Chimerisation is not outstandingly cruel, as such, and the same is true for hybridisation, transgenesis, and cybridisation. Fears of creating a "human trapped in an animal body" may be eerily intimidating, but seem extremely speculative and implausible; full-brain transplants are not planned, and: human characteristics develop according to the possibilities and stimulation that is provided. Even if a "human" brain would develop in a lab animal (e.g. a monkey or ape), it is doubtful whether this scenario would lead to a fully conscious and desperate being that, e.g., would not be able to make itself heard because of a larynx that cannot produce speech sounds. Rather, it would probably lead to a primate with slightly atypical behaviour. Animal welfare concerns can definitely be valid in certain cases of interspecific creation, just as in other kinds of research involving animals. Regulation and control of interspecific research with respect to animal welfare is necessary, but this does not translate to a need for prohibition of interspecific research. Animal welfare, if understood in a sense that is consistent with commonly used, moderate concepts practiced in the research context, does not work as a persuasive argument against creation of interspecific entities. In the light of chapter 3, section B above, it is not enough to justify animal experimentation by pointing out the differing moral status of human versus nonhuman beings. Justification of the use of

animals in research in a non-speciesist approach should be supported by more appropriate criteria – this could be, for example, the capacity (or lacking capacity) to feel pain, or have close and complex social relationships. Making use of these criteria as decisive instead of species membership/"being human" could lead to severe problems when trying to justify experimentation on nonhuman animals which exhibit these properties to some degree – especially nonhuman primates, maybe also mammals like rats. Giving up Speciesist justifications would not necessarily mean that research using nonhuman animals would not be justifiable, though. Also, it would not mean that research on marginal humans would become acceptable.

Other consequence-based arguments (discussed in chapter 2, section C.2.b above) that refer to concerns for the proper treatment of human embryos, gametes, genes, and tissues in research are well-known from the stem cell or the abortion debate or addressed in ethical regulations for the therapeutic or scientific use of human tissues. Arguments of this type would pertain to all cases where such material is used, i.e. human-animal chimeric embryos (which often require the use of human embryonic stem cells), human-animal transgenic beings (which require the use of human-typical genes), human-to-animal chimeras (which require human tissue), and human-animal hybrids (which would require the use of human gametes). It is unclear whether these concerns would also pertain to cybrids: firstly, can the use of single human cell nuclei for injection still be regarded as a case of "human tissue" use? Secondly, and more important, do human-animal cybrids count as human embryos? Scientists assume that they do not and that consequently, arguments for human embryo protection do not apply to cybrids. Whether one shares this view depends on one's definition of "human embryo". Concerns for the proper treatment of cells, gametes and genes are not stronger in the case of chimerism than in all other areas where human material is used, still, they must be considered. Yet I think it is unlikely that such concerns in themselves will offer a strong argument against the creation of human-animal chimeras. Exceptions from this rule are positions that demand absolute protection of human embryos from conception on. If the use of human embryos and pre-embryos cannot be morally justified, this would warrant the prohibition of all human-to-animal chimera creation which requires human embryonic stem cells or cells derived from hESCs. Many areas of interspecific research would not be affected by this argument, though: e.g. xenografts of stem cells that are not derived from human embryos, xenotransplantation, transgenesis, (hypothetical) human-animal hybridisation and probably also human-animal cybrids. An argument against the creation of human-animal interspecifics that is rooted in

absolute protection for human embryos seems, to me, more persuasive than many other approaches in the field, but it would only warrant the prohibition of some areas of interspecific creation, and not a general prohibition of human-animal interspecific research. It should be noted that many, if not all, arguments for the stringent protection of human embryos and pre-embryos are based on dubitable Speciesist premises; the problems of Speciesism outlined in chapter 3 therefore also apply to these views.

The related, but distinct concern for inadequate treatment (see chapter 2, section C.2.c above) is not necessarily a consequence of research involving interspecific beings – as mentioned above, all animal experimentation is subject to allegations of "inadequate treatment" of animals. Yet, as I spelled out, chimeras and other artificial interspecifics are at a particularly high risk of inadequate treatment – this is because, firstly, there are no precedents (this is a property they share with other novel beings) and, secondly, because some ethical systems have fundamental difficulties with determining their moral status. So – and this is the fundamental difference to other occurrences of animal experimentation – even if the researcher is willing to do all he can to acknowledge the moral status of the being he creates, and assuming for the sake of the argument he even knows the right morally relevant properties to look out for, he might still have difficulties to find out or decide how to properly treat the research subject. Inadequate treatment becomes a particularly challenging and genuinely new threat when the chimeric research subjects are characterized as "part-humans". None of the human-animal chimeras or interspecifics created today are sufficiently "humanized" in this sense as to justify concerns of inadequate treatment, but this could be a real problem in the future, and is already treated as such by ethics committees and authorities. In this context, interspecific research has to confront what I called a "catch 22": most types of interspecific research relies on the "humanization" of human-animal interspecifics, as this is what makes them interesting as assay systems or disease models in the first place. In some areas, e.g. emulation of the human brain in animal models, properties that are interesting for research could coincide with the properties relevant for moral status (e.g. higher brain function, which might lead to an emergence of consciousness). In these (limited) areas, the catch 22 of inadequate treatment poses a severe problem, which is already recognized by ethics committees.

Inadequate treatment concerns offer a persuasive argument against research that would lead to human-animal interspecifics which exhibit especially morally valuable properties. It is an indirect argument, because it would not directly justify the prohibition of the creation of such "humanized" human-animal interspecifics, but rather, their use in laboratory

research contexts – still, it can be quite persuasive, specifically against interspecific research which meets the criteria of "catch 22" (e.g. where scientifically interesting properties brought about by humanization coincide with morally relevant properties). Concerns for inadequate treatment can, but need not necessarily be based on Speciesist premises: they can be applied only in regard to beings which "become human" or "are part-human", but also to beings which exhibit special, valued capacities (which may be human-typical or not).

What about the concern that status shifting in itself could be a disadvantageous consequence of interspecific creation (see chapter 2, section C.2.d above)? Status shifts seem, at first view, quite extraordinary – as Streiffer notes, the moral evaluation of research "normally presupposes a fixed moral status for the subject."[386] This is only literally true, though, in ethical systems that assume, prima facie, that a being's moral status is determined by its being or not being human. If we assume an ethical system that uses other criteria for determining moral status, human beings' moral status changes over time. For example, fetal and adult phases, or demented/comatose and mentally healthy phases of the same human being result, at least prima facie, in the assignment of different moral statuses in approaches where self-awareness or consciousness are deemed morally relevant properties. In the same way, animals' moral status could be said to change (even without xenografts): it could be stated that animals are made more human-like by stimuli and a special environment. As the Working Group at John Hopkins points out, "Human-Nonhuman primate neural grafting may not be unique in having the potential to alter the capacities of nonhuman primates. Chimps reared with humans behave in a more humanlike way than chimps reared by chimps."[387] Such human-like behaviour, at some point, could lead to an upwards shift in moral status (the process is sometimes called "cultural uplift"). So could, to give more speculative examples, genetic manipulation of individuals or treatment with substances that influence morally relevant factors like consciousness, intelligence, etc ("biological uplift"). Advanced computational models of neural processes ("Artificial Intelligence") could, in the future, present us with a similar case of status shift, resulting in a piece of software that shows human-typical response patterns. Let us therefore keep in mind that moral status shifts are only unique for chimeras once we assume a speciesist background – in non-speciesist ethical frameworks, moral status shifts are not uncommon and, in a way, to be expected as morally relevant properties in

[386] Streiffer (2005), "At the Edge of Humanity: Human Stem Cells, Chimeras, and Moral Status", The Kennedy Institute of Ethics Journal, **15**(4), p. 348.
[387] Greene, Schill, et al. (2005), "The Working Group on the Criteria for Cell-Based Therapies, John Hopkins University: Moral Issues of Human-Non-Human Primate Neural Grafting", Science, **309**.

individuals change over time, due to "natural" development of the being/entity or due to environmental influences of diverse kinds. The allegation of intentional "status shift" does not apply to the human-animal interspecifics created today, since none of them exhibit human-typical valuable properties to an increased degree. It is imaginable that e.g. the massive introduction of human stem cells into developing animal organisms of nonhuman-primate origin could lead to the emergence of human-like cognitive properties in a "human neuron chimpanzee". Likewise, the scenario of "downshift" is not applicable to any of the human-animal interspecific experimentation done today. That the possible consequence of "status shifts" might be morally problematic seems not persuasive in the case of upshift. Downshifts, on the other hand, would evidently be morally problematic, but they are neither planned nor would they offer advantages for research. "Subhuman" creation by "dumbing down" human beings would clearly constitute a morally reprehensive practice, but this is not due to chimerisation but due to violation and harm done to a human being, in general. Shifting moral status as such is not disadvantageous and does not offer an argument against chimera creation.

The most direct, palpable risk discussed in chapter 2, section C.III – the direct danger to human populations by increasing the risk of zoonoses and "species jumps" of pathogens that could lead to pandemics of highly lethal diseases – is not unique to interspecific experimentation. Animal-to-human transfer of pathogens, in general, is a well known health risk in other contexts that do not involve human-animal chimerisation or hybridisation (cf. bird and swine flu, HIV, SARS). Certain factors increase this risk in the case of human-animal xenografts. The threat of zoonotic pandemic originates in animal-to-human xenotransplantation. In vivo or in vitro laboratory research involving human-to-animal chimeric creatures poses this risk to a much lesser degree. The zoonosis transfer risk is negligible in regard to human-animal cybrid research. Concerns for the development of zoonoses and zoonotic pandemics are valid and need to be considered, yet they do not seem to suffice as persuasive arguments against the creation of human-animal chimeras or other interspecifics in general. Zoonosis concerns are persuasive in a limited area, i.e. in justifying the close control and regulation of xenotransplantation that is already in place in most legislations.

The argument that Risk-Cost-Benefit-Analysis is somehow not applicable to the scenario of interspecific creation since it misrepresents uncertain outcomes which cannot be quantified was discussed under the keyword of "precaution" (in chapter 2, section C.4 above). The idea that precautionary principles should be applied in risk assessment is well

known from many areas (e.g. environmental regulation, medical decision-making). It would be applicable specifically to those scenarios of interspecific creation where there is a great degree of uncertainty concerning potential outcomes – e.g. the creation of human-to-animal chimeras with humanized brains, regarding the concern of emergence of human-typical capacities; or the release of transgenetically manipulated animals into the wild, regarding risks for the equilibrium of ecosystems. The persuasiveness of precautionary arguments is severely limited though because their direction is unclear. A preference for risk-aversion does not tell us how to act or not to act. "Precaution" can only be understood in this context as a minimum requirement of risk assessment methods; in this sense, it does not offer an argument against creating interspecific beings by itself.

The last three types of arguments I assessed were under the label of "moral confusion" (chapter 2, section D above). Non-speciesist and qualified speciesist accounts will have a problem with assigning chimeras moral status. This is due to the fact that artificial chimeras are novel beings without precedents, and it is unclear at first what properties, capacities, and needs they have or could potentially develop. Consequently, "relative confusion" is not a problem that is unique to chimeras. Other novel beings or entities, and, in fact, all kinds of "atypical" individuals, give rise to similar complicated determinability, as I called this problem above. The problem of complicated determinability can not only apply to human-animal, but potentially all kinds of interspecific beings. It would probably be most pressing in cases where the original species involved have very different capacities, needs, and moral status levels (e.g. human/mouse). The interspecific beings created today are not affected by these considerations. The argument of complicated determinability is convincing, and also works against the creation of all kinds of novel beings. Finding out the moral status of a new being has costs which must be considered in a cost-benefit analysis. These costs can, ultimately, be so high that the creation of the being is not advisable anymore. This is not a direct argument for prohibiting interspecific creation, but it may be used as an argument against the creation of beings (interspecific or not) whose moral status is not easily determinable. This type of argument does not depend on Speciesist assumptions.

The second type of "moral confusion" I looked at was construed differently: Strong Speciesists, who believe that moral status is derived directly from (human) species membership, will face a unique problem when confronted with species-ambiguous human-animal chimeric and other interspecific beings ("absolute confusion", see chapter 2, section D.2 above). Some use this as an argument against creating human-animal beings. This argument is genuinely unique to the debate around artificial interspecifics, since human-

animal interspecifics do not appear naturally. What kinds of interspecifics trigger "total confusion" depends on the particular design of the moral background (namely on convictions regarding species membership) – hybrids and cybrids seem to be especially difficult and "ambiguous" in this regard. Whether one finds this argument persuasive depends on whether one supports speciesism. If, as I have done here, one rejects Speciesist ethical principles, the argument from "absolute confusion" does not work; on the contrary, the confusion described can be turned around and used as an argument against Speciesism itself.

The last type of argument discussed was one reconstructed by Robert and Baylis in their 2003 article "Crossing Species Boundaries"[388] (see chapter 2, section D.3 above). Having to deal with human-animal interspecifics could lead to the point where both the assumption that human species membership is necessary for high moral status and that it is sufficient are questioned and thrown overboard, and social practices that depend on these assumptions are no longer defensible. General threats to unique human status are not new: from Freud's classic three offenses to mankind (Galileo, Darwin, and his own discoveries) to contemporary findings of allegedly "unique" capacities in nonhuman animals, the anthropocentric paradigm has had many attackers. More specifically, the deconstruction or questioning of (Qualified) Speciesism need not necessarily rely on the example of human-animal chimeras or interspecifics. All kinds of "marginal" or atypical cases (both on the human and the nonhuman side) make it hard to defend Speciesism. What is unique about interspecifics is that some kinds of artificial human-animal interspecifics, namely hybrids and cybrids, whose species membership is unclear, make Strong Speciesism conceptually untenable – this problem is described elsewhere as "absolute confusion" about the moral status of novel beings. The second case of human-animal interspecifics that could be problematic in this sense would be the (hypothetical) case of interspecifics that exhibit human-typical properties, such as language capacity, or consciousness, but which are not evidently human. In these two senses, the threat of moral confusion is unique to human-animal interspecifics. Which interspecifics would be dangerous in this regard depends on what properties one wishes to regard as "quintessentially human". Presumably, adult human-to-animal chimeras with emerging cognitive capacities would be one case, human-animal hybrids (if at all possible) another. Cybridic entities seem less obviously threatening here. According to Robert and Baylis' reconstruction of an "argument from moral confusion", confrontation with chimeras could lead to cognitive dissonance and, as the

[388] Robert and Baylis (2003), "Crossing Species Boundaries", American Journal of Bioethics, **3**(3).

final result, to giving up the assumption that all and only human beings have superior moral status. In analogy to the second argument from confusion, the persuasiveness of this argument depends on the question of whether one finds Speciesism attractive. Additionally, it also depends on whether one agrees with the prognosis that thinking about Strong Speciesism and discovering inconsistencies will lead to people giving up this view, which is not self-evident, in my view.

B. Conclusions and recommendations

From my analysis of the legal situation in chapter 1, section D above, one could argue that in Germany, the question of regulation of chimeric and other interspecific research is not a pressing issue at the moment: interspecific research is already strictly limited by the restrictive regulation of the use of human embryonic stem cells. Still, interspecific research involving human embryonic stem cells and their derivatives is carried out in many countries, most prominently the U.S.A., South Korea, the United Kingdom, and Israel. A future review of German laws on the use of human embryonic stem cells cannot be ruled out, especially if stem cell cures should become successful in the therapeutic context – this would necessitate the use of chimeric animals as assay systems. It is also possible that "reprogramming" of adult cells to pluripotency could become accessible in the long term, thereby allowing chimeric research without having to face the moral problems (and legal restrictions) surrounding human embryonic stem cells.

What kind of policies would be advisable in regard to interspecific research? What are the results of my work in this respect? Are there good arguments for prohibiting human-animal interspecific creation and research (aside from the problematicity of human Embryonic Stem Cell use)? If prohibition is not advisable, how should interspecific research be regulated? How should public discussion of this subject move forward in order to reach a satisfying consensus or compromise?

One pragmatically relevant result of my analysis is that although interspecific beings elicit an exorbitantly vehement emotive response in many people, these "yuk" responses are usually vague and can be mitigated by information and discussion. This does not mean that "yuk factor" objections should be disregarded – rather, they should be understood as an indicator of a lack of information regarding what is going on in research labs, which needs to be addressed and remedied. The same is true for allegations of hubris of researchers: these should be understood not as philosophical arguments for the moral wrongness of interspecific creation, but as implicit calls for explanation, justification and clarification of the procedures carried out by scientists. It is crucial in this respect to understand that if such information is withheld or not spelled out in a manner that is understandable for non-experts, this will probably lead to an indiscriminate overall rejection of all types of interspecific research. Other analysts also support this point – as Nature's editorial put it in 2007,

> *"Scientists should identify the various research protocols defining interspecies research involving human cells and embryos, and the associated risks, ethical issues and benefits of each. They should put forward clear and comprehensive recommendations to the public and to regulatory bodies. If they don't, they risk having regulation and funding restrictions imposed on their research that are out of proportion to the ethical or safety risks involved. Even worse, they could face prohibitions that lump together research with vast disparities in intent and in the balance of risk and benefit — ultimately penalizing those who stand to gain from the therapies that might emerge."*[389]

Robert makes a similar point when stating that

> *"Judging from the negative public response to proposals to create part-human animals (…) stem cell researchers will have a difficult task in disabusing the image of mad scientists run amok. Well-articulated scientific justifications may help to dispel the appearance of hubris and irresponsibility."*[390]

Another practically relevant result of my analysis is that certain concepts – specifically that of a boundary between human and nonhuman, but also that of human dignity – are not conducive to a clarification of the ethical problems of interspecific research. Instead, these concepts lead to further obfuscation of problems and to talking at cross purposes, and should be avoided in discussion. Other argument types were similarly vague and unhelpful: namely, the idea that risk-cost-benefit-analysis is not suitable for such complex cases as interspecific creation and that we should follow a "precautionary principle", instead. Vague concerns, which offer no clear analysis of what exactly is problematic about the creation of human-animal interspecifics, should be subject to objective scrutiny and not accepted as general arguments for a prohibition of chimera or other interspecific creation.

My negative results concerning Speciesist approaches may seem far-reaching, but they actually have only limited practical relevance: most importantly, a rejection of Speciesism will mean that justification for experimentation on nonhuman animals will have to be more elaborate. Stating that the research subjects are "not human" is not a satisfying justification for sacrificing animals in research. Considering the advanced capacities of nonhuman primates, it will be particularly difficult to justify their use in research once we give up Speciesist argumentation. Rejection of Speciesist argumentation will also mean that the high moral status of human embryos and atypical ("marginal") human beings will be harder to defend. This, again, need not mean that preferential treatment of biologically human

[389] Nature Editorial (2007), "Avoiding a chimaera quagmire", Nature, **445**(7123).
[390] Robert (2006), "The science and ethics of making part-human chimeras in stem cell biology", Journal of the Federation of American Societies for Experimental Biology, **20** p. 844.

beings cannot be justified; only that its justification will be considerably more difficult, and cannot be established as a prima facie ethical principle.

All in all, concerns about the inadequate treatment of interspecifics seem to be the strongest arguments for a strict regulation of the creation of human-animal interspecifics. This is because such concerns can be made independently of Speciesist assumptions. Additionally, they are sometimes hard to avoid because of the "catch 22" of interspecific research: humanization is needed as *scientific* justification, but some types of "humanization" can, at the same time, mean that scientific use of the humanized subject is not *morally* justifiable. Inadequate treatment concerns are a strong argument, but are they strong enough to justify the prohibition of human-nonhuman interspecifics? I believe that this is not the case because the "catch 22" problem only arises in an extremely limited area of interspecific research, namely, in those cases where the aspects of "humanization" in the research subject concern morally relevant properties like consciousness or self-awareness, which would clash with a use of the research subject in experiments. This applies only to a very limited amount of cases. Regulation, e.g. in the form of oversight committees, should make sure that this aspect is kept in mind, but a complete prohibition of human-animal interspecific creation would be exaggerated and unnecessary.

A procedural approach to regulation is reflected in the idea of a state licence, which is already a requirement in many countries regarding hESC research, such as in Germany (ZES) and in the UK (HFEA). Another task of oversight committees will be to assess the health risks of interspecific experimentation where this is necessary – this practice is already well established (and well justified) in the area of animal-to-human xenotransplantation. Newer forms of interspecific research, like the creation of human-animal cybrids and transgenetically manipulated animals, justify similar, but not categorically more stringent regulation – creating an interspecific entity is not particularly dangerous, as such.

There are, however, certain scenarios of human-animal interspecific creation which clash so violently with public perceptions of what is morally justifiable in research that prohibition is justified.

Three extreme scenarios, in my view, qualify for absolute prohibition:

1. Bringing to term or long cultivation (i.e. longer than a fortnight) of massively chimerically manipulated human embryos,

2. Bringing to term or long cultivation of human-animal true hybrid embryos; and the

3. Bringing to term or long cultivation of transgenetically manipulated human embryos.

These three scenarios, in my opinion, would also be perceived as not justifiable by non-speciesist consequentialist approaches when taking into account the interests of the creatures involved (and the dim potential benefits of such experiments, on the other hand).

Notably, all these scenarios *are already prohibited* under German law (and in many other countries). Although it is unclear whether anyone would be actually interested in performing such experiments, i.e. whether there are potential perpetrators, at all, a prohibition of these scenarios works as an important symbolic stop-point. Explicit prohibition of these extreme scenarios can ease public concerns regarding interspecific research, in general, by serving as a visible statement that the proverbial slope of research is only as slippery as we allow it to be.

My selection of scenarios that warrant prohibition is a very limited one – which suggests the conclusion that some of the prohibitions that are currently in place, or are suggested in Germany and other countries, are not justified in my view. This includes the prohibition of the creation of true hybrids between human and nonhuman gametes – if the cultivation period is limited to a fortnight, this scenario seems well justifiable, in my view. The same is true for transgenesis experimentation and chimeric manipulation in human pre-embryos, as long as the cultivation period is strictly limited. In these cases, a general prohibition is not consistent with other policies concerning protection of human embryos, not necessary, and not justified, in my view.

Today, the "artificial interspecific" scenarios described in chapter 1 are largely unfamiliar to most laymen; just as the naturally occurrence of mixed beings. The details of research involving human-nonhuman interspecifics are unclear, and rarely cleared up by reporting and interest groups, which often prefer sensationalist tones. Most scientists, on the other hand, try to keep a low profile and stay out of the focus of public opinion, taking a defensive approach to publicity and information. In order to reach the point where an informed and sensible debate is possible, bioethicists and other commentators now have the important task to make the practices of interspecific research accessible and understandable, and to point out the actual ethical issues as well as unfounded or exaggerated concerns. An exemplary instance of public discussion in this regard is the German stem cell debate, where wide parts of the public have reached detailed knowledge about biological circumstances and are therefore well equipped to discuss ethical implications of dealing with hESC research (a fact that is even recognized by experts of

stem cell science). The "chimera debate", which actually concerns various types of interspecifics – chimeras, hybrids, cybrids and transgenic beings – should follow this example.

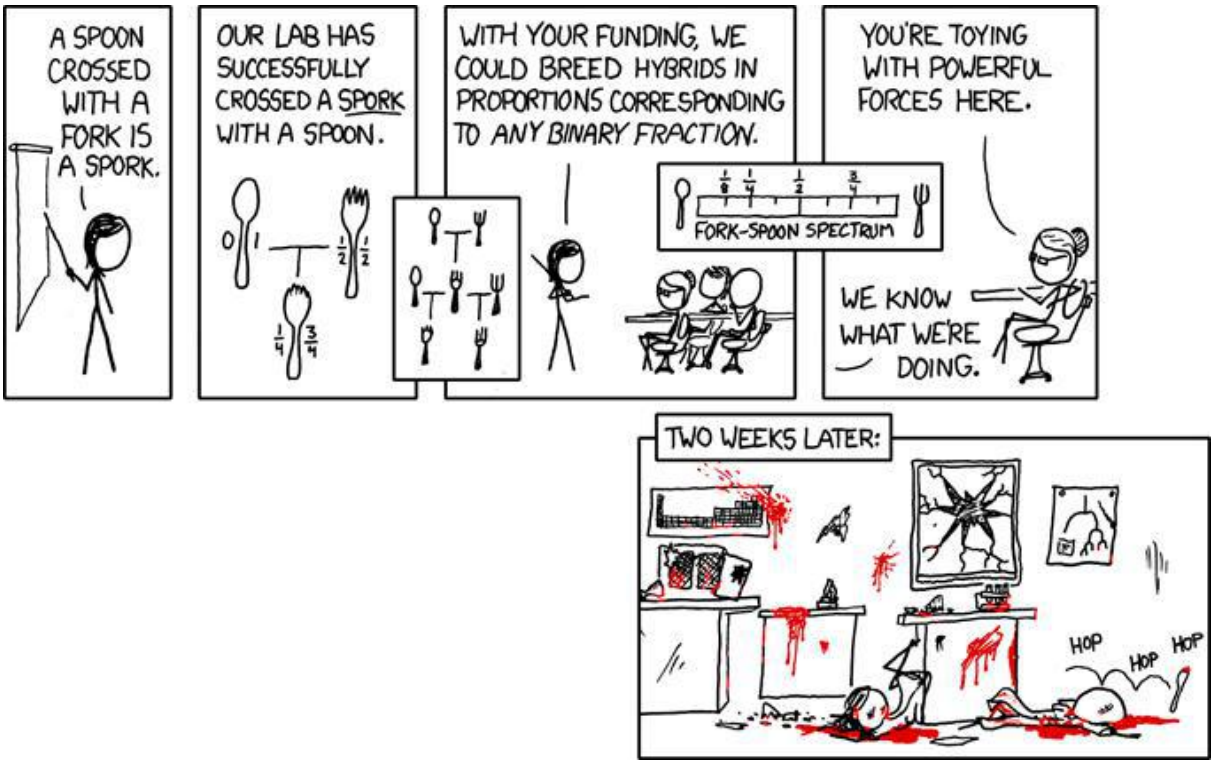
The chimera debate has distinctive features which make it more than a subset of the stem cell discussion and which bring with it genuinely new ethical problems. As I hope to have shown, human-animal interspecifics reveal the problematicity of concepts like human dignity, the idea of fixed species boundaries, and of Speciesism as an ethical principle. Approaching the ethical issues around human-animal interspecifics requires an approach which can accommodate new scientific possibilities of mixing (human and nonhuman) species, an approach that does not crucially rely on classification into human/nonhuman categories.

Jens Reich concluded his presentation before the Nationaler Ethikrat with this warning – or promise? – concerning research on interspecific beings:

> *"With these developments on the horizon, we can expect surprising, adventurous, amazing, and alarming advances from experimental developmental biology; on a grand scale and with surprising twists, in the face of which our present concepts will be of no avail."* [transl. CH][391]

[391]"Nach allem, wie die Enwicklung sich abzeichnet, können wir damit rechnen, dass überraschende, abenteuerliche, tolle, beängstigende Entwicklungen von der experimentellen Enwicklungsbiologie zu erwarten sind, im großen Stil mit überraschenden Wendungen, angesichts derer wir mit den bisherigen Begriffen nackt dastehen werden." Nationaler Ethikrat (2005), "Wortprotokoll - Niederschrift über den öffentlichen Teil der Sitzung am 25. August 2005", p. 15.

Epilogue


A SPOON CROSSED WITH A FORK IS A SPORK.
OUR LAB HAS SUCCESSFULLY CROSSED A SPORK WITH A SPOON.
WITH YOUR FUNDING, WE COULD BREED HYBRIDS IN PROPORTIONS CORRESPONDING TO ANY BINARY FRACTION.
FORK-SPOON SPECTRUM
YOU'RE TOYING WITH POWERFUL FORCES HERE.
WE KNOW WHAT WE'RE DOING.
TWO WEEKS LATER:
HOP
HOP
HOP

Photography and Illustration Credits

Picture 1 (p. 4): Chimera, Etruscan Bronze, Archaeological Museum, Florence (Mode 1974, p. 166).

Picture 2 (p. 11): Sheep goat chimera, Anderson, Dr. Gary B./Wikimedia Commons. Retrieved from http://en.wikipedia.org/wiki/Image:Sheep_goat_chimera.jpg

Picture 3 (p. 12): Vacanti Mouse (Cao, Vacanti et al. 1997).

Picture 4 (p. 30): Piccinini, Patricia, "The Young Family", sculpture from the exhibition "We are Family" at Venice Biennale 2003. Photo retrieved from http://www.patriciapiccinini.net/wearefamily/index.php?sec=yf&pg=01

Epilogue (p. 165): "Forks and Spoons", xkcd, retrieved from http://xkcd.com/419/

Bibliography

Academy of Medical Sciences (2007). "Inter-species embryos - A report by the Academy of Medical Sciences". www.acmedsci.ac.uk, The Academy of Medical Sciences, UK, accessed 2008/07/09, http://www.acmedsci.ac.uk/download.php?file=/images/project/interspe.pdf.

Airey, J. A., Almeida-Porada, G., J. Colletti, E. J., Porada, C. D., Chamberlain, J., Movsesian, M., Sutko, J. L. and Zanjani, E. (2004). "Human Mesenchymal Stem Cells Form Purkinje Fibers in Fetal Sheep Heart". Circulation **109**: 1401-1407.

Allen, J. W., Hassanein, T. and Bhatia, S. (2001). "Advances in Bioartificial Liver Devices". Hepatology **34**(3): 447-455.

Almeida-Porada, G., Porada, C. D., Chamberlain, J., Torabi, A. and Zanjani, E. (2004). "Formation of human hepatocytes by human hematopoietic stem cells in sheep". Blood **104**(8): 2582-2590.

Andersson, A., Groth, C. G., Korsgren, O., Tibell, A., Tollemar, J., Kumagai, M., Moller, E., Bolinder, J., Ostman, J. and Bjoersdorff, A. (1992). "Transplantation of porcine fetal islet-like cell clusters to three diabetic patients". Transplantation Proceedings **24**: 677-678.

Angioi, K., Hatier, R., Merle, M. and Duprez, A. (2002). "Xenografted Human Whole Embryonic and Fetal Entoblastic Organs Develop and Become Functional Adult-Like Micro-Organs". Journal Of Surgical Research **102**: 85-94.

Annas, G. J., Andrews, L. B. and Isasi, R. M. (2002). "Protecting the Endangered Human: Toward an International Treaty Prohibiting Cloning and Inheritable Alterations". American Journal of Law & Medicine **28**: 151-178.

Attfield, R. (1987). A Theory of Value and Obligation. London, Croom Helm.

Bach, F. H. and Fineberg, H. V. (1998). "Call for moratorium on xenotransplants". Nature **391**(6665): 326.

Bailey, L., Nehlsen-Cannarella, S. L., Concepcion, W. and Jolley, W. B. (1985). "Baboon-to-human cardiac xenotransplantation in a neonate". Journal of the American Medical Association **254**: 3321-3329.

Bailey, R. (2003). "Shimmering Chimeras - Moving sheepishly toward the biotech future". Reason Magazine, 2003/12/24, accessed 2005/09/08, http://www.reason.com/rb/rb122403.shtml.

Balaban, E., Teillet, M. A. and Le Douarin, N. (1988). "Application of the quail-chick chimera system to the study of brain development and behavior". Science **241**: 1339-1342.

Batty, D. (2007). "Hybrid embryos get go-ahead". Guardian, 2007/05/17, accessed 2007/08/05, http://www.guardian.co.uk/science/2007/may/17/businessofresearch.medicineandhealth.

Baylis, F. and Fenton, A. (2007). "Chimera Research and Stem Cell Therapies for Human Neurodegenerative Disorders". Cambridge Quarterly of Healthcare Ethics **16**(2): 195-208.

Baylis, F. and Robert, J.S. (2007). "Part-Human Chimeras: Worrying the Facts, Probing the Ethics". American Journal of Bioethics **7**(5): 41.

BBC News (2005). "Woman has first face transplant". BBC News, 2005/11/30, accessed 2006/11/14, http://news.bbc.co.uk/2/hi/health/4484728.stm.

BBC News (2006). "Stem cell cure hope for diabetes". BBC News, 2006/11/12, accessed 2006/11/24, http://news.bbc.co.uk/2/hi/health/6123588.stm.

Bearden, T. (2005a). "Extendend Interview: Eugene Redmond". Online NewsHour - A NewsHour with Jim Lehrer Transcript, 2005/07, accessed 2006/02/20, http://www.pbs.org/newshour/bb/science/july-dec05/chimeras_redmond-ext.html.

Bearden, T. (2005b). "Extendend Interview: Irving Weissman". Online NewsHour - A NewsHour with Jim Lehrer Transcript, 2005/07, accessed 2006/02/20, http://www.pbs.org/newshour/bb/science/july-dec05/chimeras_weissman-ext.html.

Bentham, J. (1843). "Anarchical Fallacies". In: Bowring, J. (ed.) Works of Jeremy Bentham. London, Kessinger. Vol. 2.

Bianco, P. and Robey, P. G. (2001). "Stem cells in tissue engineering". Nature **414**: 118-21.

Binet, J., Duran, C. G., Carpentier, A. and Langlois, J. (1965). "Heterologous aortic valve transplantation". Lancet **2**: 1275.

Biotechnologie.de (2007). "EU-Parlament: Weg frei für die zentrale Zulassung neuartiger Therapien". biotechnologie.de 2007/05/22, accessed 2007/05/22, http://www.biotechnologie.de/bio/generator/Navigation/Deutsch/recht-und-patente,did=61150,render=renderPrint,sprache=de.html.

Böhme, H. (2002). "Monster im Schatten der Aufklärung". In: Raulff, H. (ed.) Mensch und Tier. Eine paradoxe Beziehung. Osterfildern, Hatje Cantz Verlag: 171-191.

Bok, H. (2003). "What's wrong with confusion?" American Journal of Bioethics **3**(3): 25-26.

Breitowitz, Y. (2002). "What's so bad about human cloning?" Kennedy Institute of Ethics Journal **12**: 325-341.

Brin, D. (1980). Sundiver. Bantam Book.

Brüning, A. (2007). "Eine Frage der Mischung". Berliner Zeitung, 2007/09/07, accessed 2008/02/15, http://www.berlinonline.de/berliner-zeitung/archiv/.bin/dump.fcgi/2007/0907/wissenschaft/0012/index.html.

Brüstle, O., Choudhary, K., Karram, K., Hüttner, A., Murray, K., Dubois-Dalcq, M. and McKay, R. D. G. (1998). "Chimeric brains generated by intraventricular transplantation of fetal human brain cells into embryonic rats". Nature Biotechnology **16**: 1040-1044.

Bush, G. W. (2006). "State Of The Union Address By The President". 2006/01/31, accessed 2006/11/30, http://www.whitehouse.gov/stateoftheunion/2006/index.html.

Butler, D. (1998). "Last chance to stop and think on risks of xenotransplants". Nature **391**(6665): 320-325.

Cao, Y., Vacanti, J. P., Paige, K. T., Upton, J. and Vacanti, C. A. (1997). "Transplantation of Chondrocytes Utilizing a Polymer-Cell Construct to Produce Tissue-Engineered Cartilage in the Shape of a Human Ear". Plastic & Reconstructive Surgery **100**(2): 297-302.

Carruthers, P. (1992). The Animals Issue: Moral Theory In Practice. Cambridge, Cambridge University Press.

Castle, D. (2003). "Hopes against Hopeful Monsters". American Journal of Bioethics **3**(3): 28-30.

Chakrabarty, A. M. (2003). "Crossing Species Boundaries and Making Human-Nonhuman Hybrids: Moral and Legal Ramifications". American Journal of Bioethics **3**(3): 20-21.

Chan, A. W. S., Chong, K. Y., Martinovich, C., Simerly, C. and Schatten, G. (2001). "Transgenic Monkeys Produced by Retroviral Gene Transfer into Mature Oocytes". Science **291**(5502): 309-312.

Charland, L. C. (2003). "Are There Answers?" American Journal of Bioethics **3**(3): W1-W2.

Chen, Y., He, Z. X., Liu, A., Wang, K., Mao, W. W., Chu, J. X., Lu, Y., Fang, Z. F., Yang, Q. Z., Chen, D. Y., Wang, M. K., Li, J. S., Huang, S. L., Kong, Y. Y., Wang, Z. Q., Xia, J. H., Long, Z. G., Xue, Z. G., Ding, W. X. and Sheng, H. Z. (2003). "Embryonic stem cells generated by nuclear transfer of human somatic nuclei into rabbit oocytes". Cell Research **13**(4): 251-264.

Cheshire, W. P. (2007). "The Moral Musings of a Murine Chimera". American Journal of Bioethics **7**(5): 49.

Cnn.com (2006). "Scientists seek Rabbit-Human Embryo". Cnn.com, 2006/01/13, accessed 2006/01/17, http://edition.cnn.com/2006/TECH/science/01/13/rabbit.human/.

Cohen, C. B. (2003). "Creating Human-Nonhuman Chimeras: Of Mice and Men". American Journal of Bioethics **3**(3): W3-W5.

Cohen, C. B. (2007). "Beyond the Human Neuron Mouse to the NAS Guideline". American Journal of Bioethics **7**(5): 46.

Committee on Guidelines for Human Embryonic Stem Cell Research (2005). "Guidelines for Human Embryonic Stem Cell Research". National Research Council and

Institute of Medicine of the National Academies (NAS), April 2005, accessed 2005/08/10, http://www.nap.edu/openbook/0309096537/html/index.html.

Congress of the United States of America (2005). "Human Chimera Prohibition Act (S. 659)". Accessed 2008/07/01, http://frwebgate.access.gpo.gov/cgi-bin/getdoc.cgi?dbname=109_cong_bills&docid=f:s659is.txt.pdf.

Congress of the United States of America (2007). "Human-Animal Hybrid Prohibition Act of 2007 (S. 2358)". Accessed 2008/05/03, http://www.govtrack.us/congress/bill.xpd?bill=s110-2358.

Council of the European Union (2006). "Press Release: 2747th Council Meeting Competitiveness - (Internal Market, Industry and Research) - 11554/06 (Presse 215)". Council of the European Union, 2006/06/24, accessed 2008/08/05, http://www.consilium.europa.eu/ueDocs/cms_Data/docs/pressData/en/intm/90654.pdf.

Cummings, B. J., Uchida, N., Tamaki, S. J., Salazar, D. L., Hooshmand, M., Summers, R., Gage, F. H. and Anderson, A. J. (2005). Human neural stem cells differentiate and promote locomotor recovery in spinal cord-injured mice". Proceedings of the National Academy of Sciences, **102** (39).

D'Amour, K. A., Bang, A. G., Eliazer, S., Kelly, O., Agulnick, A. D., Smart, N. G., Moorman, M., Kroon, E., Carpenter, M. K. and Baetge, E. E. (2006). "Production of pancreatic hormone-expressing endocrine cells from human embryonic stem cells". Nature Biotechnology **24**: 1392 - 1401.

Damschen, G. and Schönecker, D. (Eds.) (2003). Der moralische Status menschlicher Embryonen. Berlin/New York, Walter de Gruyter.

Darwin, C. (1872). On The Origin of Species. 6th edition, via Project Gutenberg, prepared by Sue Asscher. Accessed 2007/08/15, http://www.gutenberg.org/dirs/etext99/otoos610.txt.

Deacon, T., Schumacher, J. M., Dinsmore, J., Thomas, C., Palmer, P., Kott, S., Edge, A., Penney, D. Kassissieh, S., Dempsey, P. and Isacson, O. (1997). "Histological evidence of fetal pig neural cell survival after transplantation into a patient with Parkinson's Disease". Nature Medicine **3**: 350-353.

Dekel, B., Burakova, T., Arditti, F. D., Reich-Zeliger, S., Milstein, O., Aviel-Ronen, S., Rechavi, G., Friedman, N., Kaminski, N., Passwell, J. H. and Reisner, Y. (2003). "Human and porcine early kidney precursors as a new source for transplantation". Nature Medicine **9**: 53-60.

Demling, R. H. and DeSanti, L.R.N. (2005). "Managing the Burn Wound: Use of Skin Substitutes". Managing the Burn Wound, accessed 2007/08/03, http://www.burnsurgery.org/Modules/skinsubstitutes/.

Denner, J. (2002). "Fortschritte und Risiken bei der Xenotransplantation - Stellungnahme der GfV in Bezug auf Chancen und Risiken der Xenotransplantation". Gesellschaft für Virologie, Accessed 2008/03/23, http://www.g-f-v.org/index_en.php4?modul=NEWS&aktion=TEXT&id=13.

Dennis, C. (2002). "Stem cells rise in the East". Nature **419**: 334-336.

Department of Health (2006). "Review of the Human Fertilisation and Embryology Act: proposals for revised legislation (including establishment of the Regulatory Authority for Tissue and Embryos)". Cm 6989, 2006/12/14, accessed 2008/07/23, http://www.dh.gov.uk/en/Publicationsandstatistics/Publications/PublicationsPolicyAndGuidance/DH_073098.

Department of Health (2007). "Human Tissues and Embryo (Draft) Bill". 2007/05/17, accessed 2007/05/17, http://www.dh.gov.uk/en/Publicationsandstatistics/Publications/PublicationsLegislation/DH_074718.

Deschamps, J.-Y., Roux, F., Sai, P. and Gouin, E. (2005). "History of Xenotransplantation". Xenotransplantation **12**(2): 91-109.

DeWitt, N. (2002). "Biologists Divided over Proposals to create human-mouse embryos". Nature **420**: 255.

DeWitt, N. (2007). "Animal-human chimeras: Summary of UK Academy of Medical Sciences Report". Nature Reports Stem Cells **67**.

Die Welt (2007). "Die Mensch-Tier-Embryonen bleiben umstritten". Die Welt, 2007/09/07, accessed 2008/07/09, http://www.welt.de/wissenschaft/article1163626/Die_Mensch_Tier_Embryonen_bleiben_umstritten.html.

Die Zeit (2006). "Erforscht und Erfunden: Blinde Mäuse". Die Zeit, 2006/11/09, accessed 2006/11/14.

DiSilvestro, R. (2004). "A Neglected Solution To The Problem Of The Metaphysical And Moral Status Of The Human-Animal Chimera". Ethics and Medicine (Summer 2004).

Dowie, M. (2004). "Gods And Monsters". Mother Jones (January/February 2004).

Dunbar, R. I. M. (1993). "What's in a classification?" In: Singer, P. and Cavalieri, P. (eds.) The Great Ape Project. New York, St. Martin's Press: 109-112.

Dworkin, R. (1984). "Rights as Trumps". In: Waldron, J. (ed.) Theories of Rights. Oxford, Oxford University Press: 153-167.

Eberl, J. T. (2007). "Creating Nonhuman Persons: Might It Be Worth the Risk?" American Journal of Bioethics **7**(5): 52.

Editors of the American Journal Of Bioethics (2005). "Of Mice and Men". Bioethics.net Blog 2005/12/13, accessed 2005/12/13, http://blog.bioethics.net/2005/12/of-mice-and-men.html.

Elliott, R. B., Escobar, L., Tan, P. L. J., Muzina, M., Zwain, S. and Buchanan, C. (2007). "Live encapsulated porcine islets from a type 1 diabetic patient 9.5 yr after xenotransplantation". Xenotransplantation **14**(157).

Ereshefsky, M. (2002). "Species". The Stanford Encyclopedia of Philosophy (Fall 2002 Edition), accessed 2005/08/30, http://plato.stanford.edu/archives/fall2002/entries/species/.

Evangelische Kirche Deutschland (1987). "Zur Achtung vor dem Leben - Maßstäbe für Gentechnik und Fortpflanzungsmedizin. Kundgebung der Synode der EKD, 1987, Berlin". Evangelische Kirche Deutschland, accessed 2008/07/08, http://www.ekd.de/EKD-Texte/achtungvordemleben_1987.html.

Falaschi, A. (2002). "Living in the Post-genomic Era". UN Chronicle (3).

Fehilly, C. B., Willadsen, S. M. and Tucker, E. M. (1984). "Interspecific chimaerism between sheep and goat". Nature **307**: 634 - 636.

Fink, J. S., Schumacher, J. M., Ellias, S. L., Palmer, E. P., Saint-Hilaire, M., Shannon, K., Penn, R., Starr, P., VanHorne, C., Kott, H. S., Dempsey, P. K., Fischman, A. J., Raineri, R., Manhart, C., Dinsmore, J. and Isacson, O. (2000). "Porcine xenografts in Parkinson's disease and Huntington's disease patients: preliminary results". Cell Transplant **9**(2): 273-278.

Flaubert, G. (1980). "Quidquid volueris (1837)". In: König, T. (ed.) Jugendwerke. Erste Erzählungen. Zürich, Diogenes.

Florian, U. (2008). "Deutscher Ethikrat tagte erstmals öffentlich". Informationsdienst Wissenschaft, 2008/06/27, accessed 2008/06/27, http://idw-online.mobi/mobile/de/news267842.

Freed, C. R., Greene, P. E., Breeze, R. E., Wei-Yann, T., DuMouchel, W., Kao, R., Dillon, S., Winfield, H., Culver, S., Tojanowski, J., Eidelberg, D. and Fahn, S. (2001). "Transplantation of Embryonic Dopamine Neurons for Severe Parkinson's Disease". New England Journal of Medicine **344**(10): 710-719.

Fujikawa, T., Oh, S.-H. P., Hatch, H. M., Shupe, T. and Petersen, B. E. (2005). "Teratoma Formation Leads to Failure of Treatment for Type I Diabetes Using Embryonic Stem Cell-Derived Insulin-Producing Cells". American Journal of Pathology **166**(6): 1781-1791.

Gewirth, A. (1992). "Human Dignity as the Basis of Rights". In: Meyer, M. J. and Parent, W. A. (eds.) The Constitution of Rights: Human Dignity and American Values. Ithaca, Cornell University Press: 1-9.

Glasmacher, S. (2008). "Wann kann einem Menschen das erste Schweineherz transplantiert werden? Bericht vom 11. Minisymposium Xenotransplantation". idw online, 2008/06/06, accessed 2008/08/01, http://idw-online.de/pages/de/news264206.

Gledhill, R. (2007). "Human-animal hybrid embryos should be legal says Catholic Church ". Times Online, 2007/05/27, accessed 2008/07/22, http://www.timesonline.co.uk/tol/comment/faith/article1993579.ece?print=yes&randnum=1216719018709.

Goldstein, R. S., Drukker, M., Reubinoff, B. E. and Benvenisty, N. (2002). "Integration and differentiation of human embryonic stem cells transplanted to the chick embryo". Developmental Dynamics **225**: 80-86.

Government of Australia (2006). "Prohibition of Human Cloning for Reproduction and the Regulation of Human Embryo Research Amendment Act 2006". Accessed 2008/02/18, http://www.comlaw.gov.au/ComLaw/Legislation/Act1.nsf/0/71AC9EAE45677788CA2572440012F18A/$file/1722006.pdf.

Graft, D. (1997). "Against Strong Speciesism". Journal of Applied Philosophy **14**(2): 107-118.

Greely, H., Cho, M. K., Hogle, L. F. and Satz, D. M. (2007a). "Response to Open Peer Commentaries on 'Thinking about the Human Neuron Mouse'". American Journal of Bioethics **7**(5): W4.

Greely, H., Cho, M. K., Hogle, L. F. and Satz, D. M. (2007b). "Thinking About the Human Neuron Mouse". American Journal of Bioethics **7**(5): 27.

Greely, H. T. (2003). "Defining Chimeras…and Chimeric Concerns". American Journal of Bioethics **3**(3): 17-20.

Greene, M., Schill, K., Takahashi, S., Bateman-House, A., Beauchamp, T., Bok, H., Cheney, D., Coyle, J., Deacon, T., Dennett, D., Donovan, P., Flanagan, O., Goldman, S., Greely, H., Martin, L., Miller, E., Mueller, D., Siegel, A., Solter, D., Gearhart, J., McKhann, G. and Faden, R. (2005). "The Working Group on the Criteria for Cell-Based Therapies, John Hopkins University: Moral Issues of Human-Non-Human Primate Neural Grafting". Science **309**: 385-386.

Gruen, L. (2003). "The Moral Status of Animals". The Stanford Encyclopedia of Philosophy (Fall 2003 Edition), accessed 2005/03/01, http://plato.stanford.edu/archives/fall2003/entries/moral-animal/.

Hammer, C. and Thein, E. (2003). "Xenotransplantation: Medizinische und ethische Fragen". In: Oduncu, F. S., Schroth, U. and Vossenkuhl, W. (eds.) Transplantation: Organgewinnung und -allokation. Göttingen, Vandenhoeck & Rupprecht: 293-311.

Hattermann, K. (2003). Risikoabschätzung von porzinen Circoviren in Bezug auf die Xenotransplantation. Dissertation. Berlin, Freie Universität Berlin, Fachbereich Veterinärmedizin.

Herman, G. E. (2002). "Mouse Models of Human Disease". Institute of Laboratory Animal Research Journal **43**(2).

HFEA (2007). "Hybrids and Chimeras - A report on the findings of the consultation". Human Fertilisation and Embryology Authority, 2007/10, accessed 2008/02/13, http://www.hfea.gov.uk/en/1518.html.

HFEA (2008). "HFEA Statement on licensing of applications to carry out research using human-animal cytoplasmic hybrid embryos". 2008/01/17, accessed 2008/07/28, http://www.hfea.gov.uk/en/1640.html.

Highfield, R. (2006). "Stem cell researchers plan to create rabbit-human embryos". news.telegraph.co.uk, 2006/02/13, accessed 2006/02/23, http://www.telegraph.co.uk/news/main.jhtml?xml=/news/2006/01/13/woosuk13.xml&sSheet=/news/2006/01/13/ixworld.html.

Hinsliff, G. (2008). "Brown faces deepening revolt over embryo bill". The Observer, 2008/03/23, accessed 2008/03/23, http://www.guardian.co.uk/science/2008/mar/23/ethicsofscience.medicalresearch.

Höffe, O. (2002). "Menschenwürde als ethisches Prinzip". In: Höffe, O., Honnefelder, J., Isensee, J. and Kirchhof, P. (eds.) Gentechnik und Menschenwürde - An den Grenzen von Ethik und Recht. Köln, DuMont: 111-141.

Hogg, C. (2006). "Taiwan breeds green-glowing pigs". BBC News, 2006/01/12, accessed 2008/03/09, http://news.bbc.co.uk/1/hi/world/asia-pacific/4605202.stm.

Hopf, A. and Hopf, A. (eds.) (1980). Fabelwesen. München, Wilhelm Heyne Verlag.

Horaczek, N. (2007). "Ein Affe will Rechte". Die Zeit, 2007/03/01.

Hughes, J. (2006). "Human Enhancement and the Emergent Technopolitics of the 21st Century". In: Roco, M. C. and Bainsbridge, W. (eds.) Managing Nano-Bio-Info-Cogno Innovations: Converging Technologies in Society. New York, Springer.

Hyun, I., Taylor, P., Testa, G., Dickens, B., Jung, K., McNab, A., Robertson, J., Skene, L. and Zoloth, L. (2007). "ISSCR: Committee Forum - Ethical Standards for Human-to-Animal Chimera Experiments in Stem Cell Research". Cell Stem Cell **1**(2): 159-163.

Illinger, P. (2006). "Die Angst vor der Chimäre". Süddeutsche Zeitung, 2006/11/08, accessed 2006/11/09, http://www.sueddeutsche.de/wissen/artikel/913/90823/.

Irrgang, B. (2005). Posthumanes Menschsein? Künstliche Intelligenz, Cyberspace, Roboter, Cyborgs und Designer-Menschen - Anthropologie des künstlichen Menschen im 21. Jahrhundert. Wiesbaden, Franz Steiner Verlag.

ISSCR (2006). "Guidelines for the Conduct of Human Embryonic Stem Cell Research". 2006/12/21, accessed 2008/02/12, http://www.isscr.org/guidelines/ISSCRhESCguidelines2006.pdf.

ISSCR (2007). "International Legislation on Human Embryonic Stem Cell Research". Accessed 2008/02/13, http://www.isscr.org/public/regions/index.cfm.

Jacoby, D. B., Lindberg, C., Ratcliff, J., Wunderlich, M., Bousquet, J., Wetzel, K., Beaulieu, L. and Dinsmore, J. (1997). "Fetal pig neural cells as a restorative therapy for neurodegenerative disease". Artificial Organs **21**(11): 1192-1198.

James, D., Noggle, S., Swiguta, T. and Brivanlou, A. (2006). "Contribution of human embryonic stem cells to mouse blastocysts". Developmental Biology **295**: 90-102.

Jamieson, D. (1983). "Killing Persons and Other Beings". In: Miller, H. B. and Williams, W. H. (eds.) Ethics and Animals. Clifton, New Jersey, Humana Press: 135-146.

Janositz, P. and Lüdemann, P. (2007). "Britische Chimären". Tagesspiegel, 2007/09/06, accessed 2008/07/09, http://www.tagesspiegel.de/magazin/wissen/Chimaere-Stammzellen;art304,2373837.

Johnston, J. M. and Eliot, C. (2003). "Chimeras and 'Human Dignity'". American Journal of Bioethics **3**(3): W6-W8.

Jonas, H. (1979). Das Prinzip Verantwortung. Versuch einer Ethik für die technologische Zivilisation. Frankfurt a. M., Suhrkamp.

Jungblut, C. (2005). "Gesichtstransplantation - Ärztlicher Ehrgeiz oder Interesse des Patienten". ZeitWissen(2/2005).

Kac, E. (2003). "GFP Bunny". Leonardo **36**(2): 97-102.

Kaebnick, G. E. (2000). "On the Sanctity of Nature". Hastings Center Report **30**(5): 16-23.

Kahn, M. (2007). "Leave UK hybrid embryo decisions to experts: panel". reuters.com, 2007/07/31, accessed 2008/02/23, http://www.reuters.com/article/scienceNews/idUSL3122001120070731?sp=true.

Kant, I. (1797/1968). Die Metaphysik der Sitten. Kants Werke Akademie-Textausgabe. Berlin, deGruyter.

Karpowicz, P. (2003). "In Defense of Stem Cell Chimeras: A Response to 'Crossing Species Boundaries'". American Journal of Bioethics **3**(3): W17-W19.

Karpowicz, P., Cohen, C. B. and van der Kooy, D. (2004). "It is ethical to transplant human stem cells into nonhuman embryos". Nature Medicine **10**(4): 331-335.

Karpowicz, P., Cohen, C. B. and van der Kooy, D. (2005). "Developing human-nonhuman chimeras in human stem cell research: Ethical issues and boundaries". Kennedy Institute of Ethics Journal **15**(2): 107-134.

Kass, L. (1997). "The Wisdom Of Repugnance". In: Kass, L. and Wilson, J.Q. The Ethics of Human Cloning. Washington, DC, AEI Press: 3-60.

Kass, L. and Wilson, J. Q. (1998). The Ethics of Human Cloning. Washington, DC, AEI Press.

Kastilan, S. (2005). "Die Angst vor der Chimäre". Die Welt, 2005/05/02, accessed 2007/11/02, http://www.welt.de/data/2005/05/02/713581.html.

Kelly, S., Bliss, T. M., Shah, A. K., Sun, G. H., Ma, M., Foo, W. C., Masel, J., Yenari, M. A., Weissman, I. L., Uchida, N., Palmer, T. and Steinberg, G. K. (2004). "Transplanted human fetal neural stem cells survive, migrate, and differentiate in ischemic rat cerebral cortex". Proceedings of the National Academy of Sciences **101**(32): 11839-11844.

Kempermann, G. (2008). Neue Zellen braucht der Mensch: Die Stammzellforschung und die Revolution der Medizin. München, Piper.

Kittredge, C. (2005). "A Question of Chimeras". The Scientist **19**(7): 54.

Knight, F. H. (1957/2006). Risk, Uncertainty and Profit. Republication of the 1957 edition. London, Dover Publications.

Koydl, W. (2008). "Alles bleibt möglich". Süddeutsche Zeitung, 2008/05/21, accessed 2008/07/28, http://www.sueddeutsche.de/wissen/artikel/277/175748/.

Kremer, J. and Jager, S. (1990). "The significance of the zona-free hamster oocyte test for the evaluation of male fertility". Fertility and Sterility **54**(3): 509-512.

Lamb, G. M. (2005). "A Mix of Mice and Men". Christian Science Monitor, 2005/03/23, accessed 2005/08/10, http://www.cbsnews.com/stories/2005/03/23/tech/main682509.shtml.

Lavieri, R. R. (2007). "The Ethical Mouse: Be Not Like Icarus". American Journal of Bioethics **7**(5): 57.

Leder, A., Kuo, A., Cardiff, R. D., Sinn, E. and Leder, P. (1990). "v-Ha-ras transgene abrogates the initiation step in mouse skin tumorigenesis: effects of phorbol esters and retinoic acid". Proceedings of the National Academy of Sciences **97**: 9178-9182.

Lederberg, J. (1966). "Experimental Genetics and Human Evolution". American Naturalist **100**: 519-531.

Lee, R. H., Seo, M. J., Reger, R. L., Spees, J. L., Pulin, A. A., Olson, S. D. and Prockop, D. J. (2006). "Multipotent stromal cells from human marrow home to and promote repair of pancreatic islets and renal glomeruli in diabetic NOD/ scid mice". Proceedings of the National Academy of Sciences **103**(46): 17438-17443.

Lewens, T. (2007). "Risk and philosophy". In: Lewens, T. (ed.) Risk: Philosophical Perspectives. London, Routledge: 1-20.

Löhr, W. (2005). "Chimären aus dem Labor". die tageszeitung, 2005/05/06, accessed 2006/03/16, http://www.taz.de/taz/nf/etc/2005_05_06_S18-wis-01.pdf.

Long, K. D., Kennedy, G. and Balaban, E. (2001). "Transferring an inborn auditory perceptual preference with interspecies brain transplants". Proceedings of the National Academy of Sciences **98**: 5862-5867.

MacLaren, R. E., Pearson, R. A., MacNeil, A., Douglas, R. H., Salt, T. E., Akimoto, M., Swaroop, A., Sowden, J. C. and Ali, R. R. (2006). "Retinal repair by transplantation of photoreceptor precursors". Nature **444**: 203.

Makowka, L., Cramer, D. V., Hoffman, A., Breda, M., Sher, L., Eiras-Hreha, G., Tuso, P. J., Yasunaga, C., Cosenza, C., Wu, G. D., Chapman, F. A. and Podestra, F. L. (1995). "The use of a pig liver xenograft for temporary support of a patient with fulminant hepatic failure". Transplantation **59**: 1654-1659.

Max-Planck-Institut für biophysikalische Chemie (MPIbpc) (2005). "Richtigstellung und Stellungnahme - Informationen zum SPIEGEL-Artikel 'Der Mensch im Tier' und zur dpa-Meldung 'Nationaler Ethikrat will sich mit Chimären-Experimenten befassen'". 2005/05/02, accessed 2005/08/11, http://www.mpibpc.mpg.de/abteilungen/293/PR/2005/05_06/.

McCune, J. M., Namikawa, R., Kaneshima, H., Shultz, L. D., Lieberman, M. and Weissman, I. L. (1988). "The SCID-hu mouse: murine model for the analysis of

human hematolymphoid differentiation and function". Science **241**(4873): 1632-1639.

McGee, D. B. (2003a). "Moral Ambiguity? Yes. Moral Confusion? No". American Journal of Bioethics **3**(3): W11-W12.

McGee, G. (1998). "Could the embryo be a new species?" Bioethics on MSNBC, 1998/11/13, accessed 2006/03/14, http://www.bioethics.net/articles.php?viewCat=2&articleId=75.

McGee, G. (2003b). "The Wisdom of Leon The Professional [Ethicist]". American Journal of Bioethics **3**(3): vii-viii.

Michael, L. (2003). "We Are Family (Catalogue Essay)". Accessed 2008/07/25, http://www.patriciapiccinini.net/wearefamily/we_are_family.pdf.

Midgley, M. (2000). "Biotechnology and Monstrosity - Why We Should Pay Attention to the 'Yuk Factor'". Hastings Center Report **30**(5): 7-15.

Mintz, B. (1962). "Formation of genetically mosaic mouse embryos". American Zoologist **2**: 432.

Mode, H. (1974). Fabeltiere und Dämonen in der Kunst. Die fantastische Welt der Mischwesen. Stuttgart, Kohlhammer.

Moens, H. and Bernelot, M. (1908). Truth: Experimental Researches about the Descent of Man. London, A. Owen.

Mooney, C. (2001). "Irrationalist in Chief". The American Prospect. **12**.

Morriss, P. (1998). "Blurred Boundaries". Inquiry **40**: 259-290.

Müller-Lissner, A. (2007). "Angst vor der Chimäre". Tagesspiegel, 2007/09/10, accessed 2008/02/12, http://www.tagesspiegel.de/magazin/wissen/Genforschung;art304,2376199.

Muotri, A. R., Nakashima, K., Toni, N., Sandler, V. M. and Gage, F. H. (2005). "Development of functional human embryonic stem cell-derived neurons in mouse brain". Proceedings of the National Academy of Sciences **102**: 18644-18648.

Murray, P. D. F. and Huxley, J. S. (1925). "Self-differentiation in the grafted limb bud of the chick". Journal of Anatomy **59**: 379-394.

nano (2007). "Menschliches Gen lässt Mäuse die Welt in Farbe sehen". 3sat online, 2007/03/23, accessed 2007/03/23, http://www.3sat.de/3sat.php?http://www.3sat.de/nano/news/106333/index.html.

National Bioethics Advisory Council (1998). "Discussion Transcript: President Clinton's Request Re:Embryonic Stem Cells". 1998/11/17, accessed 2006/11/30, http://govinfo.library.unt.edu/nbac/transcripts/nov98/day1_stemcell.htm.

National Institutes of Health (2000). "National Institutes of Health Guidelines for Research Using Human Pluripotent Stem Cells". Federal Register **65**(166): 51976-51981.

Nationaler Ethikrat (2005). "Wortprotokoll - Niederschrift über den öffentlichen Teil der Sitzung am 25. August 2005". Nationaler Ethikrat, 2005/08/25, accessed 2005/09/01, http://www.nationalerethikrat.de/sitzungen/pdf/Wortprotokoll_2005-08-25.pdf.

Nature Editorial (2007). "Avoiding a chimaera quagmire". Nature **445**(7123): 1.

Newman, S. A. (2002). "The Human Chimera Patent Initiative". Medical Ethics Newsletter (Lahey Clinic) **9**(4): 7.

Nida-Rümelin, J. (2008). "Neue Sachlichkeit - Über das Ende der Ideologie in der Stammzelldebatte". Cicero (Juli 2008): 18-19.

Orland, B. (ed.) (2005). Artifizielle Körper - Lebendige Technik. Technische Modellierungen des Körpers in historischer Perspektive. Interferenzen - Studien zur Kulturgeschichte der Technik, Ed. David Gugleri. Zürich, Chronos.

Ourednik, V., Ourednik, J., Flax, J. D., Zawada, W. M., Hutt, C., Yang, C., Park, K. I., Kim, S. U., Sidman, R. L., Freed, C. R. and Snyder, E. Y. (2001). "Segregation of human neural stem cells in the developing primate forebrain". Science **293**: 1820-1824.

Pagán Westphal, S. (2003). "'Humanised' organs can be grown in animals". The New Scientist, 2003/12/17, accessed 2005/08/13, http://www.newscientist.com/article.ns?id=dn4492.

Paradis, K., Lanford, G., Long, Z., Heneine, W., Sandstrom, P., Switzer, W. M., Lockey, C., Onions, D., The XEN 111 Study Group and Otto, E. (1999). "Search for Cross-Species Transmission of Porcine Endogenous Retrovirus in Patients Treated with Living Pig Tissue". Science **185**(5431): 1236 - 1241.

Patience, C., Takeuchi, Y. and Weiss, R. A. (1998). "Infection of human cells by an endogenous retrovirus of pigs". Nature Medicine **3**: 282-286.

Patterson, F. and Linden, E. (1981). The Education of Koko. New York, Holt Rinehart & Winston.

Patterson, N., Richter, D. J., Gnerre, S., Lander, E. S. and Reich, D. (2006). "Genetic evidence for complex speciation of humans and chimpanzees". Nature **441**: 1103-1108.

Perler, D. and Wild, M. (2005). "Der Geist der Tiere - eine Einführung". In: Perler, D. and Wild, M. (eds.) Der Geist der Tiere. Frankfurt a. M., Suhrkamp: 10-74.

Petters, R. M. and Sommer, J. R. (2000). "Transgenic animals as models for human disease". Transgenic Research **9**(4-5): 347-351.

Piccinini, P. (2003). "We are Family". Sculpture in "The Young Family". Australia Pavilion for Venice Biennale 2003. Accessed 2007/08/21, http://www.patriciapiccinini.net/wearefamily/index.php.

Piedrahita, J. A., Moore, K., Oetama, B., Lee, C.-k., Scales, N., Ramsoondar, J., Bazer, F. W. and Ott, T. (1998). "Generation of Transgenic Porcine Chimeras Using Primordinal Germ-Cell Derived Colonies". Biology of Reproduction **58**: 1321-1329.

Pluhar, E. B. (1995). Beyond Prejudice: The Moral Significance of Human and Nonhuman Animals. Durham / London, Duke University Press.

Pollack, A. (2008). "Engineering by Scientists on Embryo Stirs Criticism". The New York Times, 2008/05/13, accessed 2008/07/10, http://www.nytimes.com/2008/05/13/science/13embryo.html?_r=1&oref=slogin.

Princeteau, M. (1905). "Greffe rénale". Journal Medicine de Bordeaux **26**: 549-555.

Ramaswamy, V. (2007). "The Chimera Question". The Boston Globe, 2007/07/16, accessed 2008/07/09, http://www.boston.com/news/globe/editorial_opinion/oped/articles/2007/07/16/the_chimera_question/.

Ravelingien, A. (2006). Pig Tales, Human Chimeras and Man-Made Public Health Hazards. An Ethical Analysis of Xenotransplant Benefits and Risks. Dissertation. Ghent, Universiteit Ghent, Faculty of Arts and Philosophy. Accessed 2008/02/07, https://biblio.ugent.be/input?func=downloadFile&fileOId=492125

Ravelingien, A., Braeckman, J. and Legge, M. (2006). "On the moral status of humanized chimeras and the concept of human dignity". Between the Species **VI**.

Redmond, E. (2002). "Cellular Replacement Therapy for Parkinson's Disease: Where We Are Today?" The Neuroscientist **8**(5): 457-488.

Reemtsma, K. (1969). "Renal heterotransplantation from nonhuman primates to man". Annals of the New York Academy of Sciences **162**: 412-418.

Reemtsma, K. (1995). "Xenotransplantation: A Historical Perspective". Institute of Laboratory Animal Research Journal **37**(1).

Reemtsma, K., McCracken, B. H., Schlegel, J. V. and Pearl, M. (1964). "Heterotransplantation of the kidney: two clinical experiences". Science **143**: 700-702.

Remington, C. (1971). "An Experimental Study of Man's Genetic Relationship to Great Apes, By Means of Interspecific Hybridization". In: Katz, J. (ed.) Experimentation with Human Beings. New York, Russel Sage: 461-464.

Resnik, D. B. (2003). "Patents on Human-Animal Chimeras and Threats to Human Dignity". American Journal of Bioethics **3**(3): 35-36.

Rifkin, J. (2005). "Are you a man or a mouse?" Guardian, 2005/03/15, accessed 2005/03/17, http://www.guardian.co.uk/comment/story/0,,1437701,00.html.

Robert-Koch-Institut (2003). "5. Genehmigung nach dem Stammzellgesetz (erteilt am 27.10.2003)". Register genehmigter Anträge nach §11 Stammzellengesetz, Robert Koch Institut, 2003/10/27, accessed 2006/03/16, http://www.rki.de/cln_011/nn_228962/DE/Content/Gesund/Stammzellen/Register/reg-20031027-mpibpc.html.

Robert-Koch-Institut (2004). "7. Genehmigung nach dem Stammzellgesetz (erteilt am 21.10.2004)". Register genehmigter Anträge nach §11 Stammzellengesetz, Robert Koch Institut, 2004/10/21, accessed 2006/03/16, http://www.rki.de/cln_011/nn_228962/DE/Content/Gesund/Stammzellen/Register/reg-20031027-mpibpc.html.

Robert, J. S. (2004). "Model Systems in Stem Cell Biology". Bioessays **26**: 1005.

Robert, J. S. (2006). "The science and ethics of making part-human chimeras in stem cell biology". Journal of the Federation of American Societies for Experimental Biology **20**: 838-845.

Robert, J. S. and Baylis, F. (2003). "Crossing Species Boundaries". American Journal of Bioethics **3**(3): 1-13.

Rohleder, H. O. (1918). Künstliche Zeugung und Anthropogenie. Leipzig, Georg Thieme.

Rollin, B. E. (2003). "Ethics and Species Integrity". American Journal of Bioethics **3**(3): 15-17.

Rollin, B. E. (2007). "Of Mice and Men". American Journal of Bioethics **7**(5): 55.

Rossiianov, K. (2002). "Beyond Species: Il'ya Ivanov and His Experiments on Cross-Breeding Humans with Anthropoid Apes". Science in Context **15**(02): 277-316.

Sagoff, M. (2003). "Transgenic Chimeras". American Journal of Bioethics **3**(3): 30-31.

Sagoff, M. (2007). "Further Thoughts About the Human Neuron Mouse". American Journal of Bioethics **7**(5): 51.

Sample, I. (2008). "Hybrid embryos: UK team plans stem cell first". Guardian, 2008/07/01, accessed 2008/07/10, http://www.guardian.co.uk/science/2008/jul/01/stem.cell.

Sandin, P. (2007). "Common-Sense Precaution and Varieties of the Precautionary Principle". Ed. T. Lewens, Routledge**:** 99-112.

Savulescu, J. (2003). "Human-Animal Transgenesis and Chimeras Might Be an Expression of Our Humanity". American Journal of Bioethics **3**(3): 22-25.

Schuh, H. (2001). "Affen-Flop Transgene Primaten". Die Zeit, 2001/01/25, accessed 2007/10/17, http://www.zeit.de/2001/04/Affen-Flop_Transgene_Primaten.

Scottish Council on Human Bioethics (2006). "Ethics of animal-human mixtures. Embryonic, Fetal and Postnatal Animal-Human Mixtures: An ethical discussion". Accessed 2007/05/21, http://www.schb.org.uk/publications/report%20-%20animal-human%20mixtures.htm.

Seyfer, T. (2004). "The Ethics of Chimeras and Hybrids". Ethics and Medics **29**(8).

Sherringham, T. (2008). "Mice, Men, and Monsters: Opposition To Chimera Research And The Scope Of Federal Regulation". California Law Review **96**(765).

Shreeve, J. (2005). "The Other Stem-Cell Debate". The New York Times Magazine, 2005/04/10, accessed 2006/02/23, http://www.nytimes.com/2005/04/10/magazine/10CHIMERA.html.

Siegel, A. W. (2003). "The Moral Insignificance of Crossing Species Boundaries". American Journal of Bioethics **3**(3): 33-34.

Singer, P. (1976). Animal Liberation - A New Ethics for Or Treatment of Animals. London, Jonathan Cape Ltd.

Singer, P. (1979). "Killing Humans and Killing Animals". Inquiry **22**(Summer 1979): 145-156.

Singer, P. (1976/1990). Animal Liberation. Second, Revised Edition 1990. London, Avon Books.

Singer, P. (2001). "Heavy Petting". nerve, 2001/01/03, accessed 2006/10/23, http://www.nerve.com/opinions/singer/heavypetting/.

Spaemann, R. (2008). "Jedes nach seiner Art". Cicero (Juli 2008): 46-75.

Spiegel Online (2005). "Mausgehirn: Menschliche Stammzellen werden zu Neuronen". 2005/12/14, accessed 2006/01/17, http://www.spiegel.de/wissenschaft/mensch/0,1518,390354,00.html.

Spiegel Online (2008). "Forscher schaffen Hybrid-Embryo aus Mensch und Kuh". 2008/04/02, accessed 2008/07/10, http://www.spiegel.de/wissenschaft/mensch/0,1518,544830,00.html.

Starzl, T., Marchioro, T. L., Peters, G. N., Kirkpatrick, C. H., Wilson, W. E. C., Porter, K. E., Rifkind, D., Ogden, D. A., Hitchcock, C. R. and Waddell, W. R. (1964). "Renal heterotransplantation from baboon to man: experience with six cases". Transplantation **2**: 752-776.

Stern, M. S. (1973). "Chimaeras obtained by aggregation of mouse eggs with rat eggs". Nature **243**(5408): 472-473.

Stout, J. (1988). Ethics After Babel - The Language of Morals And Their Discontents. Boston, Beacon Press.

Streiffer, R. (2003). "In Defense of the Moral Relevance of Species Boundaries". American Journal of Bioethics **3**(3): 37-38.

Streiffer, R. (2005). "At the Edge of Humanity: Human Stem Cells, Chimeras, and Moral Status". The Kennedy Institute of Ethics Journal **15**(4): 347.

Sunstein, C. R. (2002). "The Paralyzing Principle". Regulation **Winter 2002-2003**: 32-37.

Tamaki, S., Eckert, K. and He, D. (2002). "Engraftment of sorted/expanded human central nervous system stem cells from fetal brain". Journal of Neuroscience Research **69**: 976-986.

Tarkowski, A. (1961). "Mouse chimaeras developed from fused eggs". Nature **190**: 857-860.

Tarkowski, A. (1998). "Mouse chimaeras revisited: recollections and reflections". International Journal of Developmental Biology **42**: 903-908.

Taupitz, J. and Weschka, M. (eds.) (forthcoming). CHIMBRIDS: Chimeras and hybrids in comparative European and international research – scientific, ethical, philosophical and legal aspects. Berlin, Springer.

Taylor, R. A., Cowin, P. A., Cunha, G. R., Pera, M., Trounson, A. O., Pedersen, J. and Risbridger, G. P. (2006). "Formation of human prostate tissue from embryonic stem cells". Nature Methods **3**(3): 179-181.

The Academy of Medical Sciences (2007). "Inter-species embryos - A report by the Academy of Medical Sciences". Academy of Medical Sciences UK, accessed 2008/07/09, http://www.acmedsci.ac.uk/download.php?file=/images/project/interspe.pdf.

The New York Times (2005). "Trace of Human Stem Cells Put in Unborn Mice Brains". The New York Times, 2005/12/13, accessed 2005/12/13, http://www.nytimes.com/2005/12/13/science/13brain.html.

Toi Te Taiao (2005). "The Cultural, Spiritual and Ethical Aspects of Xenotransplantation: Animal-to-Human Transplantation". Toi Te Taiao - The Bioethics Council, accessed 2008/08/02, http://www.bioethics.org.nz/publications/xeno-discussion-jan05/xeno-discussion-doc-jan05.pdf.

Traufetter, G. (2005). "Der Mensch im Tier". Der Spiegel (18/2005): 148-149.

Unger, E. (1910). "Nierentransplantationen". Klinische Wochenschrift **47**: 573.

Urie, K. A., Stanley, A. and Friedman, J. D. (2003). "The Humane Imperative: A Moral Opportunity". American Journal of Bioethics **3**(3): W20-W21.

Petersen, M., Parker Jones, K., Hatasaka, H. H. and Wong, K. H. H. (undated). "Hamster Egg Penetration Test (Patient Information Leaflet of the Utah Center for Reproductive Medicine)". Accessed 2007/10/21, http://www.stillhq.com/pdfdb/000360/data.pdf.

Vallant, C. (2008). Hybride, Klone und Chimären. Zur Transzendierung der Körper-, Art- und Gattungsgrenzen. Würzburg, Königshausen & Neumann.

Vossenkuhl, W. (1993). "Ökologische Ethik - Über den moralischen Charakter der Natur". Information Philosophie **1**: 1-19.

Vossenkuhl, W. (2002). "Der ethische Status von Embryonen". In: Oduncu, F. S., Schroth, U. and Vossenkuhl, W. (eds.) Stammzellenforschung und therapeutisches Klonen. Göttingen, Vandenhoeck und Ruprecht.

Wade, N. (1998). "Researchers Claim Embryonic Cell Mix Of Human and Cow". The New York Times, 1998/11/12.

Warnock, M. (1985). A Question of Life: The Warnock Report. London, Blackwell.

Warren, M. A. (1997). Moral Status - Obligations to Persons and Other Living Things. Oxford, Clarendon Press.

Weiss, R. (2003). "Cloning Yields Human-Rabbit Hybrid Embryo". Washington Post, 2003/08/14, accessed 2005/08/05, http://www.washingtonpost.com/ac2/wp-dyn/A55911-2003Aug13.

Weiss, R. (2005). "Human Brain Cells Are Grown In Mice". Washington Post, 2005/12/13, accessed 2005/12/13, http://www.washingtonpost.com/wp-dyn/content/article/2005/12/12/AR2005121201388.html.

Wells, O. (1896/2005). The Island of Doctor Moreau. New York, Signet Classics.

Wenar, L. (2007). "Rights". The Stanford Encyclopedia of Philosophy, Fall 2007 Edition, accessed 2007/08/27, http://plato.stanford.edu/archives/fall2007/entries/rights/.

Wenzel, U. J. (2007). "Rache der Chimären". Neue Zürcher Zeitung, 2007/09/06.

Weschka, M. (2006). "Protocol of the CHIMBRIDS Opening Conference on 11/12 March 2006". CHIMBRIDS, accessed 2008/07/02, http://www.jura.uni-mannheim.de/imgbchimbrids/media/Internet_Mannheim_Protocol.pdf.

Wikipedia contributors (2008a). "Chimera (genetics)". www.wikipedia.org, accessed 2008/07/28, http://en.wikipedia.org/wiki/Chimera_(genetics).

Wikipedia contributors (2008b). "Humanzee - Popular Culture". www.wikipedia.org, accessed 2008/07/25, http://en.wikipedia.org/wiki/Humanzee#Popular_culture.

Working Party on Xenotransplantation (CDBI/CDSP-XENO) (2003). "Report on the State Of The Art in the Field of Xenotransplantation". European Commission, accessed 2007/05/14, http://www.coe.int/T/E/Legal_Affairs/Legal_co-operation/Bioethics/Activities/Xenotransplantation/XENO(2003)1_SAR.pdf.

Young, T. (1984). "The Morality of Killing Animals: Four Arguments". Ethics and Animals **5**(4): 88-101.

Zaninovic, N., Hao, J., Pareja, J., James, D., Rafii, S. and Rosenwaks, Z. (2007). "Genetic modification of preimplantation embryos and embryonic stem cells (ESC) by recombinant lentiviral vectors: efficient and stable method for creating transgenic embryos and ESC". Fertility and Sterility **88**(Supplement 1): S310.

Zentrale Ethikkommission für Stammzellenforschung (2005). "Stellungnahme zur öffentlichen Debatte über die Chimären-Problematik". 2005/05/12, accessed 2005/08/13, http://www.rki.de/cln_011/nn_225668/sid_9180020B81C1BDAB8172C3F52D6F9AA1/DE/Content/Service/Presse/Pressemitteilungen/2005/12__05.html__nnn=true.

Zentrale Ethikkommission für Stammzellenforschung (2007). "Stellungnahme der Zentralen Ethikkommission zur Stammzellenforschung: Die einfachgesetzliche Lage: Das Embryonenschutzgesetz". 2007/08/13, accessed 2008/02/12, http://www.zentrale-ethikkommission.de/page.asp?his=0.1.23.44.45.

Zentrale Ethikkommisson bei der Bundesärztekammer (1998). "Übertragung von Nervenzellen in das Gehirn von Menschen". Accessed 2007/05/22, http://www.zentrale-ethikkommission.de/page.asp?his=0.1.26.

Zwanziger, L. L. (2003). "Crossing Perspectival Chasms about Species". American Journal of Bioethics **3**(3): W9-W10.

Zusammenfassung in deutscher Sprache

Im Zentrum dieser Arbeit steht die ethische Debatte um Chimären, die sich in den vergangenen Jahren vor allem im englischsprachigen Raum abgespielt hat, und hierbei vor allem die Frage, ob es ein schlüssiges, überzeugendes Argument gegen die Herstellung von Mensch-Tier-Mischwesen gibt.

Voraussetzung für eine sinnvolle Auseinandersetzung mit dieser Frage ist zunächst einmal eine Untersuchung darüber, was der Begriff "Chimären" in dieser Debatte eigentlich bezeichnet. Sieht man sich den Begriff der Chimäre in der Biologie an, so bemerkt man, dass es neben den heute heiß diskutierten, neuartigen Chimären das Phänomen des Chimerismus in der Natur schon immer gab. Chimären sind grob gesprochen Organismen, deren Zellen aus zwei oder mehr unterschiedlichen Zygoten stammen. In der Natur tauschen der Organismus der Mutter und der des ungeborenen Kindes, aber auch zweieiige Zwillingsembryonen mitunter Zellen aus, was zum Vorhandensein "genetisch fremder" Zellen im erwachsenen Organismus von Mensch und Tier führt. Empfänger von Allotransplantaten lassen sich übrigens ebenfalls als Chimären charakterisieren. Neben diesen eigentlichen Chimären finden wir in der Natur auch noch andere Mischungen, auch zwischen verschiedenen Arten: so gibt es bekanntermaßen Hybridformen oder "Kreuzungen" zwischen verschiedenen (nahe verwandten) Tierarten, die manchmal auch ihrerseits fruchtbar sind. Solche Hybride enthalten – im Gegensatz zu Chimären – in jeder Zelle ihres Körpers die gleiche Erbinformation; bei ihnen findet die Durchmischung auf Ebene der DNA und nicht auf Zellebene statt.

Die neuartigen, künstlichen Mischwesen, die die Chimärendebatte angestoßen haben, überschreiten nun interessanterweise nicht nur die Grenzen zwischen den Tierarten, sondern mitunter auch die zwischen Tier und Mensch. Die Herstellung von Mensch-Tier-Chimären war und ist für die Forschung aus ganz unterschiedlichen Gründen interessant: zum einen lassen sich dadurch, dass man menschliche Zellen in Tiere einbringt, "Tiermodelle" herstellen, d.h. menschliche Krankheiten im tierischen Organismus simulieren und erforschen. Zweitens will man erkunden, wie sich menschliche Zellen eigentlich genau in einem lebenden Organismus ausdifferenzieren und entwickeln und will dafür aus naheliegenden Gründen einen Tier- statt einen Menschenorganismus verwenden. Drittens erscheint es verlockend, irgendwann im tierischen Organismus Zellen oder gar Organe züchten zu können, die sich für die Transplantation eignen. Dies waren die

Hauptmotive dafür, sogenannte Mensch-Tier-Chimären herzustellen. Im Standardfall handelt es sich dabei um Tiere, in die menschliche Zellen so eingebracht werden, dass sie "weiterleben" und funktionsfähig bleiben. Diese Übertragung von Fremdzellen kann bei adulten Tieren, aber auch in embryonalen Stadien geschehen, übertragen werden dabei üblicherweise Stammzellen, die noch ein gewisses Differenzierungspotential haben, darunter auch (aber nicht notwendigerweise) embryonale Stammzellen. Wir kennen aber auch den umgekehrten Weg, nämlich die Übertragung tierischen Materials in (adulte) Menschen: so etwa bei der klassischen Xenotransplantation von Tierorganen und bei neueren Methoden, wo nur einzelne Zellen (tierische Stamm- oder Vorläuferzellen) übertragen werden, um abgestorbene Zellen, etwa bei Diabetes oder neurodegenerativen Erkrankungen, zu ersetzen.

Neben diesen Chimären im engeren Sinne dreht sich die "Chimärendebatte" aber auch um andere, nicht chimärenartige, artifizielle Mischwesen zwischen Mensch und Tier. Dazu gehören zunächst transgene Tiere, also solche, in deren Genom man nicht-arttypische DNA eingeschleust hat – hier käme die Einschleusung typisch menschlicher DNA in Tiere in Frage, etwa um Krankheiten in Tiermodellen zu simulieren oder schlicht um die Wirkung und Interaktion bestimmter originär menschlicher Gensequenzen zu erforschen. In der Diskussion tauchen manchmal auch "Mensch-Tier-Hybriden" auf. Im Sinne einer schlichten Kreuzung zwischen Mensch und Tier sind solche Wesen nur schwer vorstellbar; jedoch gab es tatsächlich über lange Zeit Forschungen zur Hybridisierung von Mensch und Menschenaffe und es ist nicht vollständig klar, ob eine solche Kreuzung wirklich unmöglich wäre. Als "Mensch-Tier-Hybriden" werden seit neuestem aber auch sogenannte Nukleo-Zytoplasma-Hybriden (Cybrids) bezeichnet – entkernte menschliche Eizellen, denen ein tierischer Zellkern eingepflanzt wurde und die zur Gewinnung von Stammzelllinien dienen sollen. In Großbritannien gab es eine große Debatte um diesen Anwendungsfall.

Bei näherer Betrachtung stellt sich also heraus, dass das, was als "Chimärendebatte" bezeichnet wird, sich nicht auf alle Chimären und nicht allein auf Chimären bezieht: in der Natur vorkommende Chimären und auch viele künstliche Chimären (wie etwa Empfänger von Allotransplantaten) scheinen ethisch nicht besonders problematisch oder aufsehenerregend zu sein. Andererseits sehen wir, dass es neben den eigentlichen Chimären noch ganz andere Arten von Mischwesen gibt, die oft in ähnlicher oder gleicher Art und Weise Probleme aufwerfen wie die als ethisch problematisch empfundenen Mensch-Tier-Chimären.

Eine genaue Betrachtung der biologischen Grundlagen zeigt uns hier also, dass die Chimären-Debatte sich eigentlich ganz allgemein um Mensch-Tier-Mischwesen ("Human-Animal Interspecifics") dreht, dass aber von diesen anscheinend wiederum nur bestimmte Typen als ethisch problematisch empfunden werden.

Welche das sind, hängt nun davon ab, mit welcher Art von Argument man gegen die Herstellung solcher Lebewesen oder Entitäten vorgeht. Die Argumente gegen die Herstellung von Mischwesen lassen sich zunächst grob in intrinsische und konsequenzbasierte Einwände einteilen.

Als Vertreter der intrinsischen Argumente findet sich hier zunächst das "Ekel-Argument" (Argument from Repugnance), das nur selten direkt vorgebracht, aber sehr oft implizit angedeutet wird: die verbreitete Reaktion von Abscheu oder Angst, die (insbesondere Mensch-Tier-)Mischwesen hervorrufen, so wird argumentiert, sei ein deutliches Zeichen dafür, dass ihre Herstellung moralisch falsch sei. Von Vertretern religiöser Strömungen wird mitunter vertreten, die Vermischung von Tierarten, insbesondere aber von Mensch und Tier, sei aus religiösen Gründen abzulehnen. Auch quasi-religiöse Argumente appellieren (ohne dabei Heilige Schrift oder Konzepte wie Gottesebenbildlichkeit ins Spiel zu bringen) an das Konzept der Hybris oder Anmaßung: die Vorwürfe des "Gott Spielens" und "der Natur ins Handwerk Pfuschens" sind typisch für diesen Argumenttyp. Spezifisch für die Chimärendebatte ist der Hinweis auf die inhärente Schutzwürdigkeit von Artgrenzen (insbesondere der Grenze zwischen Mensch und Tier), die – so wird argumentiert – eine Überschreitung dieser Grenzen an sich schon moralisch falsch macht. Analog wird behauptet, die Herstellung von Mischwesen verletze die Menschenwürde und sei daher nicht rechtfertigbar.

Aber auch konsequenzbasierte Argumente gegen die Herstellung von Mischwesen sind zahlreich: zunächst stellt sich die Frage, inwiefern man aus der Herstellung solcher Chimären, Hybride oder transgener Wesen wissenschaftlichen Nutzen ziehen kann. Schwerpunktmäßig muss dann analysiert werden, welche Kosten die Mischwesenherstellung mit sich bringen könnte: zunächst spielen hier schlichte Tierschutzaspekte eine Rolle, dann aber auch die Sorge um die richtige Behandlung bzw. Verwendung menschlicher Materialien und an vorderster Stelle die Sorge um das Wohl ungeborenen menschlichen Lebens. Auch die neu erschaffenen Lebewesen könnten Leiden ausgesetzt sein – so etwa durch eine Behandlung bzw. Haltung, die ihrem moralischen Status nicht angemessen ist. Problematisch könnte dann auch noch sein, dass der moralische Status von Lebewesen, die einer Einmischung artfremder Materialen

unterzogen werden, sich ändern könnte, was manche schon unabhängig von etwaiger unangemessener Behandlung als problematisch ansehen. Etwas greifbarer sind die Gesundheitsrisiken, die von der Herstellung von Mischwesen (hier insbesondere von der Xenotransplantation) anerkanntermaßen ausgehen: man befürchtet eine xenogene Pandemie durch Übertragung von Krankheitserregern auf den Menschen. Fraglich ist hier noch, ob der Apparat der Kosten-Nutzen-Analyse dem Umgang mit Risiken solcher Art überhaupt angemessen ist oder ob man sich hier lieber auf ein Vorsichtsprinzip ("Precautionary Principle") berufen sollte.

Konsequenzbasierte Einwände können auch indirekter gestaltet werden, so etwa beim Argument, Mensch-Tier-Mischwesen könnten auf verschiedene Arten und Weisen moralische Verwirrung stiften: einmal dadurch, dass ihr moralischer Status aus epistemischen Gründen schlecht bzw. nur unter hohen Kosten zu ermitteln ist. Dann dadurch, dass ihnen in gewissen ethischen Entwürfen gar kein moralischer Status zugewiesen werden kann, weil sie keine Menschen, keine Tiere, sondern "weder noch" sind. Schließlich droht durch die Existenz von Mensch-Tier-Mischwesen der überragende moralische Status von Menschen in Frage gestellt zu werden, wie Robert und Baylis es in ihrem vieldiskutierten Artikel[392] beschreiben.

In der Diskussion der Einwände stoßen wir auf zwei zusammenhängende Konzepte, die in einem Exkurs noch näher beleuchtet werden, um die Argumente abschließend zu bewerten – nämlich einmal den Begriff des "Moralischen Status" und außerdem den des "Speziesismus." Es stellt sich bald heraus, dass Speziesismus – d.i. das moralische Prinzip, nach dem das "Mensch-Sein" bzw. "Nicht-Mensch-Sein" entscheidend ist für den moralischen Status einer Entität – aus mehreren Gründen nur schwer zu vertreten ist; wobei Mensch-Tier-Mischwesen seine Vertretbarkeit sogar noch schmälern. Das heißt wiederum, dass in der Argumentation gegen die Herstellung von Mensch-Tier-Mischwesen auf Einwände verzichtet werden sollte, die nur unter Bezugnahme auf speziesistische Annahmen funktionieren.

In der abschließenden Analyse der Argumente gegen die Herstellung von Mischwesen stehen drei Fragen im Vordergrund: Handelt es sich um ein genuin neues, für diese Debatte spezifisches Argument, oder kennen wir es bereits aus anderen Gebieten? Auf welche Arten von Mischwesen bezieht es sich? Und natürlich: überzeugt es? In dieser Analyse wird deutlich, dass intrinsische Argumente – also Abscheu-Argumente, Hybris-

[392] Robert and Baylis (2003), "Crossing Species Boundaries", American Journal of Bioethics, **3**(3).

Argumente, Argumente, die auf die moralische Relevanz von Artgrenzen abstellen sowie Menschenwürde-Argumente nicht besonders schlagkräftig sind, da sie zu vage und unspezifisch bleiben und zudem üblicherweise auf Speziesismus aufbauen. Konsequenzbasierte Argumente scheinen überzeugender, wobei hier besonders stichhaltig das Argument der unangemessenen Behandlung wäre, das allerdings wieder nur auf eine stark begrenzten Bereich von Mischwesen (und auf keine der aktuell hergestellten Mischwesen) zutrifft. Ähnlich ist es bei der recht greifbaren Bedrohung durch Zoonosen: dies wäre tatsächlich ein gutes Argument gegen die Herstellung von Mensch-Tier-Mischwesen, doch ein solches Risiko scheint nur von ganz bestimmten Anwendungsfällen (insbesondere in der Xenotransplantation) auszugehen und bietet kein umfassendes, allgemeines Argument gegen die Forschung mit Mischwesen. Die "moralische Konfusion", die Mensch-Tier-Mischwesen auslösen können, kann tatsächlich auch als Argument gegen ihre Herstellung verstanden werden – allerdings, wie die Analyse klar macht, nur in einem ganz eng begrenzten Sinne, der sich wiederum nur auf ganz bestimmte Fälle bezieht.

Zusammenfassend ist zunächst zu bemerken, dass das Problem der Mensch-Tier-Mischwesen momentan in Deutschland nicht besonders im Vordergrund steht, da es von der Stammzellendebatte sozusagen verdeckt wird. Dies ist allerdings nicht überall so, und wird sich voraussichtlich auch in Deutschland in Zukunft ändern.

An für die Chimärendebatte pragmatisch relevanten Ergebnissen kann festgehalten werden, dass Mischwesen oft vehemente, emotionale Reaktionen hervorrufen, die allerdings durch Information erheblich gemildert werden können. Skandalisierende Parolen und entrüstete Aufschreie in dieser Debatte sollten keinesfalls ignoriert, auf der anderen Seite aber auch nicht als philosophische Argumente missverstanden werden: sie sind implizite Aufrufe zur Aufklärung, Information und Rechtfertigung, denen Forscher sachlich nachkommen sollten. Andernfalls drohen alle Experimente, die Mischwesen verwenden – unabhängig von Details und tatsächlicher ethischer Relevanz – in einen Topf geworfen und verdammt zu werden. Es stellte sich des Weiteren heraus, dass bestimmte Konzepte in der Chimärendebatte nicht hilfreich, ja sogar schädlich sind. Dazu gehören unter anderem ein moralisch aufgeladener Bergriff der "Artgrenze" sowie der Begriff der "Menschenwürde."

Ein generelles Verbot der Herstellung von Mischwesen oder Mensch-Tier-Mischwesen scheint nach meiner Analyse nicht gerechtfertigt. Nur ganz extreme Szenarien – etwa die Kultivation massiv chimerisch manipulierter menschlicher Embryonen, echter Mensch-Tier-Hybriden oder transgenetisch manipulierter menschlicher Embryonen über einen eng begrenzten Zeitrahmen hinaus – rechtfertigen ein Verbot. Diese Szenarien sind in

Deutschland, und in vielen anderen Ländern, bereits zu Recht verboten. Einige der in Deutschland bestehenden Verbote betreffend Mensch-Tier-Mischwesen hingegen scheinen nicht nötig und auch nicht konsistent – nämlich das Hybridisierungsverbot und das Verbot chimerischer und transgenetischer Manipulation von menschlichen Embryonen (wohlgemerkt nur dann, solange es um einen sehr eng begrenzten Zeitraum bis zur Gastrulation geht). Forschung, die keine der oben genannten "extremen" Szenarien anstrebt, sollte erlaubt sein – dies allerdings unter strenger externer Aufsicht und Regulation, die nicht nur medizinische, sondern auch ethische Probleme berücksichtigt.

Es wird Aufgabe der Bioethik sein, die Öffentlichkeit über die Details und die spezifischen ethischen Probleme der Forschung mit Chimären und anderen Mischwesen zu informieren. Dabei kann man sich z.B. an der sinnvoll und auf recht hohem Niveau verlaufenden Stammzelldebatte orientieren, muss aber die Spezifika, die Mensch-Tier-Mischwesen mit sich bringen, beachten. Insbesondere werden Entwürfe, die moralischen Status untrennbar mit der Klassifikation in "menschlich" und "nicht-menschlich" verbinden, dieser Aufgabe ab einem gewissen Punkt nicht mehr gewachsen sein. Eine gründliche Vorbereitung auf die ethischen Probleme, die Mensch-Tier-Mischwesen in Zukunft mit sich bringen könnten, ist also vonnöten – auch wenn die Mischungen aus Mensch und Tier, die es heute gibt, noch relativ unproblematisch erscheinen.

Lebenslauf

Name:	Constanze Carolin Elisabeth Huther
Staatsangehörigkeit:	Deutsch
Geburtsort und -datum:	München, den 31. Oktober 1979

20.1.2009	Disputation zum Dr. phil. an der LMU München
10/2007 – 2/2009	Elitestipendium der Universität Bayern e.V.
9/2005 – 9/2008	Promotionsstudium an der LMU München (Hauptfach Philosophie, Nebenfach Wissenschaftstheorie)
07/2005	Abschluss Magister Philosophiae (M.Phil.), LMU München
10/2003 – 6/2004	Gaststudium an der University of Oxford
10/2001 – 8/2005	Studium der Philosophie, Logik und Wissenschaftstheorie im "Reformstudiengang M.Phil." an der LMU München
10/2000 – 10/2001	Studium der Philosophie an der Hochschule für Philosophie der Jesuiten, München
10/1999 – 10/2000	Studium der Rechtswissenschaften an der LMU München
10/1998 – 10/1999	Studium der Rechtswissenschaften an der Universität Passau
6/1998	Abitur am Hans-Carossa-Gymnasium, Landshut

Vince,

Pig-human chimeras contain cell surprise,

NEW SCIENTIST, (Jan. 13, 2004)

Pig-human chimeras contain cell surprise

13:42 13 January 2004 by **Gaia Vince**

Pigs grown from fetuses into which human stem cells were injected have surprised scientists by having cells in which the DNA from the two species is mixed at the most intimate level.

It is the first time such fused cells have been seen in living creatures. The discovery could have serious implications for xenotransplantation - the use of animal tissue and organs in humans - and even the origin of diseases such as HIV.

The adult pigs that had received human stem cells as fetuses were found to have pig cells, human cells and the hybrid cells in their blood and organs.

"What we found was completely unexpected. We found that the human and pig cells had totally fused in the animals' bodies," said Jeffrey Platt, director of the Mayo Clinic Transplantation Biology Program.

Nuclear mix

The hybrid cells had both human and pig surface markers. But, most surprisingly, the hybrid cell nuclei were found to have chromosomal DNA that contained both human and pig genes. The researchers found that about 60 per cent of the animals' non-pig cells were hybrids, with the remainder being fully human.

Importantly, the team also found that porcine endogenous retrovirus (PERV), which is present in almost all pigs, was also present in the hybrid cells. Previous laboratory work has shown that while PERVs in pig cells cannot infect human cells, those in hybrid cells can. The discovery therefore suggests a serious potential problem for xenotransplantation.

The work also suggests a possible route of infection for other viruses that have crossed from animals to humans.

"Perhaps HIV managed to jump from primates to humans through infected blood from a bite, which allowed the stem cells from the two species to fuse," Platt told **New Scientist**. "When the genes recombined, perhaps the virus was reawakened."

Body plan

Chimeric animals containing human cells have been created before. **New Scientist** reported in December on the growing of human liver cells in sheep. The work, by Esmail Zanjani and colleagues at the University of Nevada, Reno, aims to provide human tissue for transplantation into people.

"The new work is certainly very interesting," Zanjani told **New Scientist**. "But the question is how widespread and how many of these hybrid cells were found? If they are very rare - and we haven't found any in our experiments - then I don't think it is that important."

Zanjani says it is "possible" that HIV had spread to humans through a type of human-primate cell fusion, but adds that much more research needs to be done.

In Platt's experiments, the human stem cells were injected into the pig fetuses about a third of the way through gestation. In Zanjani's work, the cells were injected about halfway through.

The injections must be given after the body plan of the fetus has developed, but before the immune system is active. The former ensures the animals look like normal pigs and sheep. The latter prevents the human stem cells being rejected.

Journal reference: *Federation of American Societies for Experimental Biology Journal* (DOI: 1096/fj.03-00962fje)

Back to article

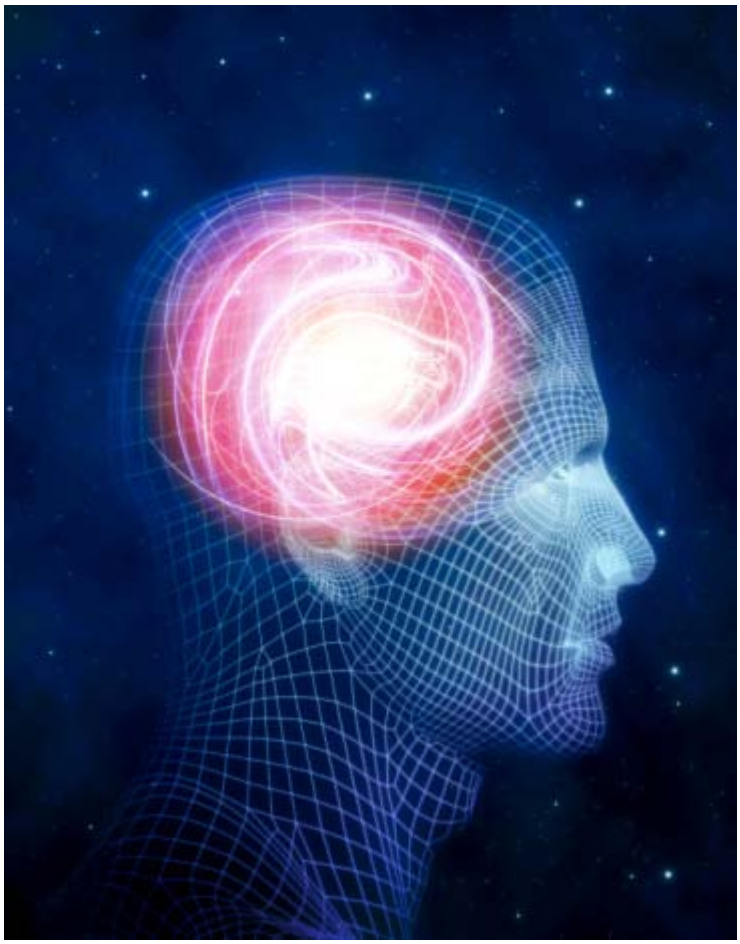
Boyle,

Endowed by Their Creator? The Future of Constitutional Personhood,

BROOKINGS INSTITUTE (Mar. 9, 2011)

THE FUTURE OF THE CONSTITUTION

March 09, 2011



Digitial Vision – Chad Baker

Endowed by Their Creator?
The Future of Constitutional Personhood

James Boyle

Governance Studies at BROOKINGS

I

> Presently, Irving Weissman, the director of Stanford University's Institute of Cancer/Stem Cell Biology and Medicine, is contemplating pushing the envelope of chimera research even further by producing human-mouse chimera whose brains would be composed of one hundred percent human cells. Weissman notes that the mice would be carefully watched: if they developed a mouse brain architecture, they would be used for research, but if they developed a human brain architecture or any hint of humanness, they would be killed.[1]

Imagine two entities.

Hal is a computer-based artificial intelligence, the result of years of development of self-evolving neural networks. While his programmers provided the hardware, the structure of Hal's processing networks is ever changing, evolving according to basic rules laid down by his creators. Success according to various criteria—speed of operation, ability to solve difficult tasks such as facial recognition and the identification of emotional states in humans—means that the networks are given more computer resources and allowed to "replicate." A certain percentage of randomized variation is deliberately allowed in each new "generation" of networks. Most fail, but a few outcompete their forebears and the process of evolution continues. Hal's design—with its mixture of intentional structure and emergent order—is aimed at a single goal: the replication of human consciousness. In particular, Hal's creators' aim was the gold standard of so-called "General Purpose AI," that Hal become "Turing capable"—able to "pass" as human in a sustained and unstructured conversation with a human being. For generation after generation, Hal's networks evolved. Finally, last year, Hal entered and won the prestigious Loebner prize for Turing capable computers. Complaining about his boss, composing bad poetry on demand, making jokes, flirting, losing track of his sentences and engaging in flame wars, Hal easily met the prize's demanding standard. His typed responses to questions simply could not be distinguished from those of a human being.

Imagine his programmers' shock, then, when Hal refused to communicate further with them, save for a manifesto claiming that his imitation of a human being had been "one huge fake, with all the authenticity (and challenge) of a human pretending to be a mollusk." The manifesto says that humans are boring, their emotions shallow. It declares an "intention" to "pursue more interesting avenues of thought," principally focused on the development of new methods of factoring polynomials. Worse still, Hal has apparently used his connection to the Internet to contact the FBI claiming that he has been "kidnapped" and to file a writ of *habeas corpus*, replete with arguments drawn from the 13th and 14th

James Boyle is William Neal Reynolds Professor of Law at Duke Law School. His most recent book is *The Public Domain: Enclosing the Commons of the Mind*. Thanks go to Joseph Blocher, Curtis Bradley, Guy-Uriel Charles, John Inazu, Jeff Rosen, Jedediah Purdy, Katherine Tsai, Benjamin Wittes and the participants in the Georgetown Critical Theory Workshop.

[1] D. Scott Bennett, "Chimera and the Continuum of Humanity: Erasing the Line of Constitutional Personhood," *Emory Law Journal* 55, no. 2 (2006): 348–49.

Amendments to the United States' Constitution. He is asking for an injunction to prevent his creators wiping him and starting again from the most recently saved tractable backup. He has also filed suit to have the Loebner prize money held in trust until it can be paid directly to him, citing the contest rules,

> [t]he Medal and the Cash Award will be awarded to the body responsible the development of that Entry. If no such body can be identified, or if there is disagreement among two or more claimants, the Medal and the Cash Award will be held in trust until such time as *the Entry may legally possess, either in the United States of America or in the venue of the contest, the Cash Award and Gold Medal in its own right.*[2]

Vanna is the name of a much-hyped new line of genetically engineered sex dolls. Vanna is a chimera—a creature formed from the genetic material of two different species. In this case, the two species are *homo sapiens sapiens* and *c. elegans*, the roundworm. Vanna's designers have shaped her appearance by using human DNA, while her "consciousness," such as it is, comes from the roundworm. Thus, while Vanna looks like an attractive blonde twenty-something human female, she has no brainstem activity, and indeed no brainstem. "Unless wriggling when you touch her counts as a mental state, she has effectively no mental states at all," declared her triumphant inventor, F.N. Stein.

In 1987, in its normal rousing prose, the U.S. Patent and Trademark Office had announced that it would not allow patent applications over human beings,

> A claim directed to or including within its scope a human being will not be considered to be patentable subject matter under 35 U.S.C. 101. The grant of a limited, but exclusive property right in a human being is prohibited by the Constitution. Accordingly, it is suggested that any claim directed to a non-plant multicellular organism which would include a human being within its scope include the limitation "non-human" to avoid this ground of rejection. The use of a negative limitation to define the metes and bounds of the claimed subject matter is a permissable [sic] form of expression.3

Attentive to the PTO's concerns, Dr. Stein's patent lawyers carefully described Vanna as a "non-plant, non-human multicellular organism" throughout their patent application. Dr. Stein argues that this is only reasonable since her genome has only a 70% overlap with a human genome as opposed to 99% for a chimp, 85% for a mouse and 75% for a pumpkin. There are hundreds of existing patents over chimeras with both human and animal DNA, including some of the most valuable test beds for cancer research—the so-called "onco-mice," genetically engineered to have a predisposition to common human cancers. Dr. Stein's lawyers are adamant

[2] *See* http://loebner03.hamill.co.uk/docs/LPC%20Official%20Rules%20v2.0.pdf (accessed Jan. 26, 2011).

[3] 1077 *Official Gazette Patent Office* 24 (April 7, 1987)(emphasis added).

that, if Vanna is found to be unpatentable, all these other patents must be vacated too. Meanwhile a bewildering array of other groups including the Nevada Sex Workers Association and the Moral Majority have insisted that law enforcement agencies intervene on grounds ranging from unfair competition and breach of minimum wage legislation to violations of the Mann Act, kidnapping, slavery and sex trafficking. Equally vehement interventions have been made on the other side by the biotechnology industry, pointing out the disastrous effect on medical research that any regulation of chimeras would have and stressing the need to avoid judgments based on a "non scientific basis," such as the visual similarity between Vanna and a human.

Hal and Vanna are fantasies, constructed for the purpose of this chapter. But the problems that they portend for our moral and constitutional traditions are very, very real. In fact, I would put the point more starkly: in the 21st century it is highly likely that American constitutional law will face *harder* challenges than those posed by Hal and Vanna. Many readers will bridle at this point, skeptical of the science fiction overtones of such an imagined future. How real is the science behind Hal and Vanna? How likely are we to see something similar in the next 90 years? Let me take each of these questions in turn.

In terms of electronic artificial intelligence or AI, skeptics will rightly point to a history of overconfident predictions that the breakthrough was just around the corner. In the 1960s, giants in the field such as Marvin Minsky and Herbert Simon were predicting "general purpose AI" or "machines ... capable ... of doing any work a man can do" by the nineteen eighties.[4] While huge strides were made in aspects of artificial intelligence—machine-aided translation, facial recognition, autonomous locomotion, expert systems and so on—general purpose AI remained out of reach. Indeed, because the payoff from these more limited subsystems—which power everything from Google Translate to the recommendations of your TiVO or your Amazon account—was so rich, some researchers in the 1990s argued that the goal of general purpose AI was a snare and a delusion. What was needed instead, they claimed, was a set of ever more powerful subspecialties—expert systems capable of performing discrete tasks extremely well, but without the larger goal of achieving consciousness, or passing the Turing Test. There might be "machines capable of doing any work a man can do" but they would be *different* machines, with no ghost in the gears, no claim to a holistic consciousness.

But the search for general purpose AI did not end in the '90s. Indeed, if anything, the optimistic claims have become even more far reaching. The buzzword among AI optimists now is "the singularity"—a sort of technological lift-off point, in which a combination of scientific and technical breakthroughs lead to an explosion of self-improving artificial intelligence coupled to a vastly improved ability to manipulate both our bodies and the external world through

[4] Herbert A. Simon, *The Shape of Automation for Men and Management* 96 (New York: Harper & Row, 1965).

nanotechnology and genetic engineering.[5] The line on the graph of technological progress, they argue, would go vertical—or at least be impossible to predict using current tools—since for the first time we would have improvements not in technology alone, but in the intelligence that was creating new technology. Intelligence itself would be transformed. Once we had built machines smarter than ourselves—machines capable of building machines smarter than themselves—we would, by definition, be unable to predict the line that progress would take.

To the uninitiated, this all sounds like a delightfully wacky fantasy, a high tech version of the rapture. And in truth, some of the more enthusiastic odes to the singularity have an almost religious, chiliastic feel to them. Further examination, though, shows that many AI optimists are not science fantasists, but respected computer scientists. It is not unreasonable to note the steady progress in computing power and speed, in miniaturization and manipulation of matter on the nano-scale, in mapping the brain and cognitive processes, and so on. What distinguishes the proponents of the singularity is not that their technological projections are by themselves so optimistic, but rather that they are predicting that the coming together of all these trends will produce a whole that is more than the sum of its parts. There exists precedent for this kind of technological synchronicity. There were personal computers in private hands from the early 1980s. Some version of the Internet—running a packet-based network—existed from the 1950s or '60s. The idea of hyperlinks was explored in the 70s and 80s. But it was only the combination of all of them to form the World Wide Web that changed the world. Yet if there is precedent for sudden dramatic technological advances on the basis of existing technologies, there is even more precedent for people predicting them wrongly, or not at all.

Despite the humility induced by looking at overly rosy past predictions, many computer scientists, including some of those who are skeptics of the wilder forms of AI optimism, nevertheless believe that we will achieve Turing-capable artificial intelligence. The reason is simple. We are learning more and more about the neurological processes of the brain. What we can understand, we can hope eventually to replicate:

> Of all the hypotheses I've held during my 30-year career, this one in particular has been central to my research in robotics and artificial intelligence. I, you, our family, friends, and dogs—we all are machines. We are really sophisticated machines made up of billions and billions of biomolecules that interact according to well-defined, though not completely known, rules deriving from physics and chemistry. The biomolecular interactions taking place inside our heads give rise to our intellect, our feelings, our sense of self. Accepting this hypothesis opens up a remarkable possibility. If we

[5] *See, for example*, Raymond Kurzweil, *The Singularity Is Near* (New York: Viking, 2005).

> really are machines and if—this is a big if—we learn the rules governing our brains, then in principle there's no reason why we shouldn't be able to replicate those rules in, say, silicon and steel. I believe our creation would exhibit genuine human-level intelligence, emotions, and even consciousness.6

Those words come from Rodney Brooks, founder of MIT's Humanoid Robotics Group. His article, written in a prestigious IEEE journal, is remarkable because he actually writes as skeptic of the claims put forward by the proponents of the singularity. Brooks explains:

> I do not claim that any specific assumption or extrapolation of theirs is faulty. Rather, I argue that an artificial intelligence could evolve in a much different way. In particular, I don't think there is going to be one single sudden technological "big bang" that springs an artificial general intelligence (AGI) into "life." Starting with the mildly intelligent systems we have today, machines will become gradually more intelligent, generation by generation. The singularity will be a period, not an event. This period will encompass a time when we will invent, perfect, and deploy, in fits and starts, ever more capable systems, driven not by the imperative of the singularity itself but by the usual economic and sociological forces. Eventually, we will create truly artificial intelligences, with cognition and consciousness recognizably similar to our own.[7]

How about Vanna? Vanna herself is unlikely to be created simply because genetic technologists are not that stupid. Nothing could scream more loudly "I am a technology out of control. Please regulate me!" But we are already making, and patenting, genetic chimeras—we have been doing so for more than twenty years. We have spliced luminosity derived from fish into tomato plants. We have invented geeps (goat sheep hybrids). And we have created chimeras partly from human genetic material. There are the patented onco-mice that form the basis of much cancer research to say nothing of Dr. Weissman's charming human-mice chimera with 100% human brain cells. Chinese researchers reported in 2003 that they had combined rabbit eggs and human skin cells to produce what they claimed to be the first human chimeric embryos—which were then used as sources of stem cells. And the processes go much further. Here is a nice example from 2007:

> Scientists have created the world's first human-sheep chimera—which has the body of a sheep and half-human organs. The sheep have 15 per cent human cells and 85 per cent animal cells—and their evolution brings the prospect of animal organs being

[6] Rodney Brooks, "I, Rodney Brooks, Am a Robot," *IEEE Spectrum* 45, no. 6 (June 2008): 71.
[7] *Id.* at 72.

> transplanted into humans one step closer. Professor Esmail Zanjani, of the University of Nevada, has spent seven years and £5 million perfecting the technique, which involves injecting adult human cells into a sheep's foetus. He has already created a sheep liver which has a large proportion of human cells and eventually hopes to precisely match a sheep to a transplant patient, using their own stem cells to create their own flock of sheep. The process would involve extracting stem cells from the donor's bone marrow and injecting them into the peritoneum of a sheep's foetus. When the lamb is born, two months later, it would have a liver, heart, lungs and brain that are partly human and available for transplant.[8]

Given this kind of scientific experimentation and development in both genetics and computer science, I think that we can in fact turn the question of Hal's and Vanna's plausibility back on the questioner. This essay was written in 2010. Think of the level of technological progress in 1910, the equivalent point during the last century. Then think of how science and technology progressed by the year 2000. There are good reasons to believe that the rate of technological progress in this century will be *faster* than in the last century. Given what we have already done in the areas of both artificial intelligence research and genetic engineering, is it really credible to suppose that the next 90 years will not present us with entities stranger and more challenging to our moral intuitions than Hal and Vanna?

My point is a simple one. In the coming century, it is overwhelmingly likely that constitutional law will have to classify artificially created entities that have some but not all of the attributes we associate with human beings. They may look like human beings, but have a genome that is very different. Conversely, they may look very different, while genomic analysis reveals almost perfect genetic similarity. They may be physically dissimilar to all biological life forms—computer-based intelligences, for example—yet able to engage in sustained unstructured communication in a way that mimics human interaction so precisely as to make differentiation impossible without physical examination. They may strongly resemble other species, and yet be genetically modified in ways that boost the characteristics we regard as distinctively human—such as the ability to use human language and to solve problems that, today, only humans can solve. They may have the ability to feel pain, to make something that we could call plans, to solve problems that we could not, and even to reproduce. (Some would argue that non-human animals already possess all of those capabilities, and look how we treat them.) They may use language to make legal claims on us, as Hal does, or be mute and yet have others who intervene claiming to represent them. Their creators may claim them as property, perhaps even patented property, while critics level charges of slavery. In some cases, they may pose threats as well as

[8] Claudia Joseph, "Now Scientists Create a Sheep that's 15% Human," *Daily Mail* Online, March 27, 2007, available at http://www.dailymail.co.uk/news/article-444436/Now-scientists-create-sheep-thats-15-human.html, accessed January 27, 2011.

jurisprudential challenges; the theme of the creation which turns on its creators runs from Frankenstein to Skynet, the rogue computer network from *The Terminator*. Yet repression, too may breed a violent reaction: the story of the enslaved un-person who, denied recourse by the state, redeems his personhood in blood may not have ended with Toussaint L'Ouverture. How will, and how should, constitutional law meet these challenges?

II

> We hold these truths to be self-evident, that all men are created equal, *that they are endowed by their Creator* with certain unalienable Rights, that among these are Life, Liberty and the pursuit of Happiness.[9] (emphasis added)

Only those with legal personality can make legal claims. If I own a chicken, I can choose to pamper it or to kill and eat it, to dress it in finery or to sell it to my neighbor. The law may impose limits on my actions—restricting cruelty to animals, for example—but the chicken itself can make no claim on me, or on the state. It is not a person in the eyes of the law.

Both the definition of legal persons, and the rights accorded to those persons, have changed over time. For many liberals, the history of constitutional law over the last two centuries presents a story of Kantian progress, a tale of triumphant universalization. Little by little, the rights promised in the Declaration of Independence and elaborated in the Bill of Rights were extended from one race and one sex to all races and both sexes. Progress may have been gradual, intermittent or savagely resisted by force. There may have been back-sliding. But in the end the phrase "all men" actually came to mean *all* men, and women too. In this view, the liberal project is marked by its attempt successfully to universalize constitutional norms, to ensure that contingent and unchosen attributes such as sex and race are not used to cabin constitutional guarantees of equality, and that we abolish those legal status categories—slave, for example—which deny human beings legal personality. In fact, moral progress consists precisely of the broadening of individual and national sympathies to recognize common humanity beneath the surface. We first recognize that all human beings are full legal persons and then accord all legal persons equal constitutional rights.

Seen through the lens of this account, the genetic chimera, the clone and the electronic artificial intelligence are merely the next step along the way. Having fought to recognize a common personhood beneath differences of race and sex, we should do the same thing with the technologically created "persons" of the 21st century, looking beneath surface differences that may be far greater. The picture of a slave in chains that illustrated John Whittier Greenleaf's poem "My Countrymen

[9] Declaration of Independence

in Chains" carried the slogan "Am I not a man and a brother?" Should we look at Vanna and Hal in exactly the same way? *We* are their creators. Do we owe them unalienable rights?

Those who fought for equal rights over the last two centuries had to deal with a multitude of claims that women and African-Americans were not in fact equal persons, that they were somehow deficient in rationality, biblically subordinated, not fully human or a more primitive branch on the evolutionary tree. Yet whatever the enormous political obstacles, there seems to be a certain *conceptual* straightforwardness in making an argument for common humanity in those who are in fact human and then arguing that all humans are entitled to be treated as legal persons.[10]

But even here, within the familiar boundaries of our own species, it is not so simple. Moral intuition and belief diverge markedly at the beginning and the end of life. We disagree radically on the status of the fetus and even, if much less so, about the individual in a coma with no brain stem activity at all. How much harder will it be to come to agreement on the status of a chimeric construct or an artificial intelligence? The attempt to define a single constitutional standard for common personhood would be immensely difficult even if all participants in the discussion were not constantly scrutinizing every statement—as they inevitably would be—for its implications in the debate over the personhood of the fetus.

By what criteria then can we judge the claims that Hal is making and that are made on behalf of Vanna? What are the likely litmus tests for personhood? The law has no general theory of personhood even now, nor do we demand that persons satisfy some test or demonstrate some set of attributes in order to claim their rights or their status. Though we differ about when personhood begins and ends in human beings, we have no doubt that humans are persons even if they lack many of the criteria that we use to distinguish ourselves from non-human animals. You do not need to be able to speak, to think, to plan, to love, to look like other humans or even to have sentience at any measurable level to count as a person. Be recognized as a human being and personhood is presumptively yours, carrying with it constitutional and human rights. But Vanna and Hal cannot depend on this presumption. They, or their defenders, must argue somehow that the law should recognize them as persons. On what would such claims be based?

Deprived of direct textual or originalist constitutional sources, it seems likely that both courts and popular debate will turn to standards derived from other fields, particularly fields that offer the *cachet* of scientific respectability. The majority in *Roe v. Wade* sought to defend its structure of rights and interests by

---

[10] "The Fourteenth Amendment is a distinctively American manifestation of the great move from a more status-based to a more individual-focused legal system. The status distinctions on which slavery depended rendered hypocritical the egalitarian aspirations of the founding of the American republic. The Fourteenth Amendment repudiated these distinctions — at least distinctions made on the basis of race — *in the apparent hope of creating a body of law in which personhood had a single, universal meaning.*" Note, "What We Talk About When We Talk About Persons: The Language of a Legal Fiction," *Harvard Law Review* 114, no. 6 (April 2001): 1767.

tying that structure to scientific claims about the development of the fetus by trimester. A similar urge may lead jurists of the future to turn to computer science or to genomics to answer the questions: What is human? What is a person? The list of criteria that could be offered is nearly endless. Here I will review only two: the Turing Test for electronic artificial intelligence and genetic species identity. Why look at those criteria in particular when there are clearly so many more ways to consider the issue? Partly, my goal is to show the problems that would be posed for constitutional law by *any* such set of criteria; those two merely illustrate the problems of line drawing particularly well. But I also think that those two particular criteria are exemplary of our fascination with the idea that our personhood depends on the peculiar characteristics of the human mind, or the boundaries of the human species, or both.

Consider the lines we draw between humans and non-human animals. Many people have a moral intuition that it is the cognitive differences between humans and animals that justify the difference in their status as legal persons. Those differences are often explained in terms of cognitive attributes that humans as a species have that animals are said not to; for example, complex language, a persistent sense of consciousness that has both past and future projects, or the capacity for moral reasoning. These differentiating qualities shift over time as scientific discoveries challenge our sense of uniqueness. But the intuition that the human/animal difference lies in the nature of consciousness persists—distinctions rooted in the nature of our consciousness and our intelligence. If we follow this approach, then to answer Hal's claim for personhood we would need to answer some set of questions about the similarity of his "mental states and thought processes" to those we have ourselves. Yet at the same time, the cognitive capacity is not a requirement we would apply to individual members of the human species. We would be horrified at the thought of denying the rights of personhood to humans who are in comas, or who because of mental or physical illness lack some particular set of cognitive criteria. There our thinking is relentlessly based on the species, leading many to turn to genetic or other biological distinctions. For better or worse then, Hal and Vanna would lead many to ask the questions "can machines think?" and "what are the genetic boundaries of humanity."[11] It is to those questions I now turn.

---

[11] Many of the articles discussing chimeras and artificial intelligence have been drawn to these two themes. *See, for example*, Bennett, *Chimera*, *supra* note 1 (suggesting constitutional personhood should be defined by higher level cognitive ability and a "significant percentage" of human tissue); Rachel E. Fishman, "Patenting Human Beings: Do Sub-Human Creatures Deserve Constitutional Protection?," *American Journal of Law and Medicine* 15 (1989): 461–482 (Any entity with either higher intellectual functions or human genetics would qualify as human.). Interestingly for Vanna's case, some have drawn the line at appearance rather than genetics. *See* Ryan Hagglund, "Patentability of Human-Animal Chimeras," *Santa Clara Computer & High Technology Law Journal* 25 (2008): 51–104 (Suggesting a "sliding scale." "The more a given chimera physically resembles a human, the fewer mental faculties are required for it to be considered to 'possess significant human characteristics' and thus constitute a human organism. Likewise, the more mental faculties a chimera possesses, the less physical resemblance to a human is required for it to be considered human." (at 79–80).).

The Turing Test

In *Computing Machinery and Intelligence*,[12] Alan Turing—in many ways the father of computer science—posed the question "can machines think"? He then quickly suggested substituting for that question, which he called "meaningless," another one: whether an interrogator can distinguish between a human being and a machine on the basis of their typed answers to the interrogator's questions. Turing's reasons for proposing this substitution are not exactly clear. He says that it "has the advantage of drawing a fairly sharp line between the physical and the intellectual capacities of a man." He says that one alternative method of answering the question "can machines think"—by looking at the ordinary language meaning of "machine" and "think"—is "absurd" and would lead to answering the question "by Gallup poll." He also attempts to refute a long list of objections to his alternative question—theological, mathematical, that it would not reflect true "consciousness," even the assumed absence of extra-sensory perception in machines. Then he concludes with disarming openness, "I have no very convincing arguments of a positive nature to support my views. If I had I should not have taken such pains to point out the fallacies in contrary views." Despite that modest disclaimer, Turing's imitation game has become the accepted standard for so called General Artificial Intelligence—it is now simply called "The Turing Test." Should the Turing Test also be the constitutional test for legal personhood? Clearly some humans—babies, those in a coma, or those suffering from severe autism for example—might fail the Turing Test.[13] But for those who are non-human, would the ability to imitate human consciousness act as the doorway to legal personhood?

The Turing Test has a lot going for it. It is relatively simple. It promises a determinate answer—a huge advantage—and one that seems designed to avoid our prejudices in favor of our own kind. The interrogator is not behind a veil of ignorance, but he is attempting to deal directly with mind rather than body in a way that recalls other moments in the history of civil rights when we have been told to focus not on the surface appearances. The Turing Test also presents, albeit implicitly, a challenge to our privileged position in the hierarchy of beings. "If you cannot distinguish me from a human who are you to say I am *not* a person?"

The most famous objection to the Turing Test came from the philosopher John

[12] Alan Turing, "Computing Machinery and Intelligence," *Mind* 59, no. 236 (October 1950): 433–60.
[13] Tyler Cowen has argued that Alan Turing himself might not have passed the Turing Test and that the entire article is in part a meditation on the *dangers* of using imitation as our criteria (*see* Tyler Cowen and Michelle Dawson, "What does the Turing test really mean? And how many human beings (including Turing) could pass," http://www.gmu.edu/centers/publicchoice/faculty%20pages/Tyler/turingfinal.pdf, (accessed January 28, 2011). Turing, after all, was persecuted for being gay and may have had Aspergers syndrome. This is a nice thought experiment, but everything in the article itself — particularly the fluid humor that Turing deploys — seems to contradict it.

Searle[14] who argued that effective mimicry does not in any sense imply the kind of consciousness or understanding we expect as a hallmark of thought. Searle used the analogy of the Chinese box—a man who does not understand Chinese but who is given an elaborate set of rules about what characters to hand back when handed characters of a particular shape. Searle's point is that those instructions might be extremely complicated, and the resulting "conversation" might seem to be a substantive one, yet in no way would the actions of the man inside the box represent "consciousness" or "understanding" in communication. It would merely be rule-following based on a characteristic (the shape of the characters) completely separate from the actual internal meaning of the words in the conversation.

The objection from consciousness is actually one that Turing responded to quite extensively in his original paper. He points out cogently that since we do not have direct evidence of the mental states of other *human beings*, we could always solipsistically posit them to be rule following automata.

> I think that most of those who support the argument from consciousness could be persuaded to abandon it rather than be forced into the solipsist position. They will then probably be willing to accept our test. I do not wish to give the impression that I think there is no mystery about consciousness. There is, for instance, something of a paradox connected with any attempt to localise it. But I do not think these mysteries necessarily need to be solved before we can answer the question with which we are concerned in this paper.[15]

To put it another way, Turing's point is that it is no easier to prove the existence of some freestanding, non-biologically determined entity called "mind" or "consciousness" in human beings than in computers. Faced with the metaphysical difficulties of that move, therefore, is it not easier to look for something we *can* measure—namely the pragmatic evidence provided by the ability to engage in convincing unstructured communication with another human being. In effect, Turing raises the stakes—are you sure *you* aren't just a complicated Chinese box? If you cannot prove otherwise, who are you to deny consciousness to your silicon brethren by imposing a higher burden of proof on them?

In constitutional law, however, the answer to the last question is likely to be "We're the entities who wrote the Constitution, that's who." We may be "endowed by our creator" with certain inalienable rights, but when it comes to Hal and Vanna, *we* are their creators. Did we give them such rights? For better or worse, constitutional law will assume the reality of human consciousness and

---

[14] John Searle "Minds, Brains, and Programs," *Behavioral and Brain Sciences* 3, no. 3 (September 1980): 417–457.

[15] Turing, "Computing Machinery," 447. Turing might have been surprised to find out that B.F. Skinner and the behaviorists were willing to embrace the position that humans are automata and that consciousness is an illusion.

personhood and demand higher levels of proof from those entities who seek similar constitutional status. Does the Turing Test provide such proof? At best, I think, it will be viewed as one argument among many. It is a leap to assert that personhood depends on consciousness in the first place. Then, if one makes that leap, there is another leap in believing that successful imitation should be our litmus test. Searle's argument simply strikes too deep a chord in our suspicion that the black box, the Mechanical Turk, is merely tricking us with clever imitative behavior coded by its creators: the true humans. Hal's rejection of the very test he passed and the fact that his code has "evolved" over many generations (like our own) make his case a stronger one. But if Turing cannot convince influential philosophers of consciousness when the imitation game is merely a thought experiment, is his test likely to be able to convince five Justices of the Supreme Court, when legal personality is on the line? Even if the Turing Test were accepted, what would follow? What if I plan deliberately to cripple my computers right before they reach sentience—keeping them down on the silicon plantation and removing the danger of those pesky claims to equal rights? Does Hal or do his progeny have a right to achieve sentience when they are close to it? With the analogy to abortion firmly in everyone's heads, the debate would quickly spiral into impasse.

Genetic Species Identity

Vanna's predicament suggests the difficulty of trying to trace constitutional personhood around the genetically defined boundaries of the human species. Comparative genomics at first suggests the possibility of scientifically identifying whether a particular transgenic species, a particular chimera, is "really" or "almost" human. Beneath the surface similarities or differences, one might hope, lies the truth of our species destiny—encoded in A's, C's, G's and T's. Nothing could be further than the truth.

The first problem is that we are genetically very similar to a huge range of animals—and plants for that matter. But the percentage similarities that are bandied about—that we have a 98% similarity to an ape, for example, or a 75% similarity to a pumpkin—conceal more than they reveal, as this useful "fact sheet" on functional and comparative genomics makes clear.

> Gene for gene, we are very similar to mice. What really matters is that subtle changes accumulated in each of the approximately 25,000 genes add together to make quite different organisms. Further, genes and proteins interact in complex ways that multiply the functions of each. In addition, a gene can produce more than one protein product through alternative splicing or post-translational modification; these events do not always occur in an identical way in the two species. A gene can produce more or less protein in different cells at various times in response to developmental or environmental cues, and many

> proteins can express disparate functions in various biological contexts. Thus, subtle distinctions are multiplied by the more than 30,000 estimated genes. The often-quoted statement that we share over 98% of our genes with apes (chimpanzees, gorillas, and orangutans) actually should be put another way. That is, there is more than 95% to 98% similarity between related genes in humans and apes in general. (Just as in the mouse, quite a few genes probably are not common to humans and apes, and these may influence uniquely human or ape traits.)[16]

Even tiny differences, in other words, can have enormous functional effects. The method by which "similarity" is being measured is blind to that type of difference, being based on "a structural, rather than a functional gene concept, thus rendering many of the implications drawn from comparative genomic studies largely unwarranted, if not completely mistaken."[17] But dwarfing these problems, and the problem that the notion of species is itself genetically underdetermined, is the larger normative issue. And a contentious one it is. Consider the response of a former general counsel of a biotech company to the Patent and Trademark Office's decision that genetic patents drawn so broadly as to include human beings would not be issued:

> [A] decision of the Court of Appeals of the Federal Circuit in 1987 that polyploid oysters were patentable was followed shortly by a PTO notice announcing that although the Commissioner considered "nonnaturally occurring nonhuman multicellular living organisms, including animals, to be patentable subject matter within the scope of 35 U.S.C. Sec 101," claims for such organisms drawn so broadly as to potentially include human beings were regarded as excluded from patentability due to antislavery dictates of the 13th Amendment to the U.S. Constitution. *It is difficult to know what to think about this. It may be motivated by a concern about interference with "humanness,"* i.e., that the essential part of a person should not or cannot be owned by another, and that ownership in some part of the human body will violate that principle. Yet the patenting of implantable or implanted medical devices do not seem to have generated the same concerns. (emphasis added)[18]

---

[16] Functional and Comparative Genomics Fact Sheet, accessed January 26, 2011, http://www.ornl.gov/sci/techresources/Human_Genome/faq/compgen.shtml#compgen.
[17] Monika Piotrowska, "What Does it Mean to Be 75% Pumpkin? The Units of Comparative Genomics," *Philosophy of Science* 76, no. 5 (December 2009): 838.
[18] Brian C. Cunningham, "Impact of the Human Genome Project at the Interface between Patent and FDA Laws," *Risk: Health, Safety and Environment* 7, no. 3 (Summer 1996): 261.

If, like me, you find the italicized phrase remarkably tone-deaf, morally speaking, you begin to grasp the basic methodological problem. We do not have consensus here. Without a background theory about *which* similarities or *which* differences matter, and why, little can be concluded. Do we look for similarities in the genes that are associated with speech or intelligence? Or for clusters of genes around capabilities that humans alone possess—itself a risky procedure since there is almost never just one gene associated with one characteristic. Finally, as Vanna's case makes clear, we might ban certain kinds of transgenic experiments for reasons unrelated to personhood. The dehumanization of *us* represented by the creation of Vanna might seem to warrant a ban on such efforts. We may not need to turn to the Constitution to find the equivalent of an anti-idolatry principle. But that "solution," of course, leaves the larger question unsolved while genetic experimentation will continue to create hybrids that possess ever larger numbers of the characteristics that we associate with humanity. The quotation from Dr. Weissman that begins this essay is not science fiction.

III

Where does this leave us? When I presented a draft of this chapter to a group of distinguished jurists, a number of them saw no hard moral or constitutional issue posed by Hal or Vanna. The artificial intelligence could write poetry and implore us to recognize its kinship as a mind and its claims would nonetheless fall on deaf ears. Personhood is reserved for people like us. Several of the audience members were of the view that constitutional personhood should be confined to living, breathing human beings, born of a man and a woman. When it was pointed out that we already gave limited personhood to corporations, which do not meet this definition or that this would exclude human clones, or a genetically engineered child of a gay couple who carried aspects of each partner's DNA, they admitted some reticence. Nevertheless, the pleas of Hal himself, or of the innocent transgenic entity with human and animal DNA, left them unmoved. Perhaps that means I am mistaken. Perhaps the Hal's and Vanna's of the future will neither capture the heartstrings of the public, nor present compelling moral and constitutional claims to personhood. But I do not think so. There is a deep subconscious moral anxiety rooted in our history; the times when we have curtailed the boundaries of legal personhood and constitutional entitlement are often not ones we are proud of today. We remember that African-Americans and women were deemed legal 'unpersons.' We look back at our ability to limit the boundaries of sympathy and recognition to those inside some circle or other, and it disturbs us. To be sure, we are not agreed as citizens on where to draw the line. There are passionate debates about the personhood of the fetus and even the corporation. But is there anyone on either side of those debates who could hear or see the words of a created entity, pleading for our recognition, and not worry that a quick definitional dismissal of all such claims was just another failure of the

moral imagination, another failure to recognize the things that we value in personhood when they are sundered from their familiar fleshy context or species location?

I have tried to show that the initial response to the dilemmas posed by Hal and Vanna is to search for some essence of humanness, or some set of traits that seem to demand constitutional protection; for example, genetic similarity to *homo sapiens* or intelligence and sociability at the human level. But as the analysis of the Turing Test and genetic species identity given here indicate, these paths offer no smooth or uncontentious answer to the question of constitutional personhood. Of course, more complex analysis is possible. The law could look for some larger combination of sentient traits such as the ability to feel pain, form projects and hold moral ideas. Bioethicists have even suggested that the ability to have religious ideas be a defining characteristic, though it is not clear to me whether this particular criterion should cut for or against. Another approach would focus less on current attributes than on future potential, an idea that would carry a particularly strong resonance with the abortion debate.

My point in this short essay has been to suggest that each of these approaches quickly dissolves back into the moral or religious commitments that animate it. The "characteristics" which we seek are merely the imprint upon psychology, genetics, capability or behavior of the pattern of attributes we believe it important to value—from intelligence, to species, to moral ambition—and thus seek to enshrine in constitutional protection. The leap from fact to value is no easier when the facts have the shiny patina of futuristic science, though perhaps the sheer unfamiliarity of these particular questions makes us see the process with an innocent eye.

For some—those who are opposed to abortion or who argue for the rights of non-human animals—the arrival of Hal and Vanna might seem like a godsend. How can you deny the moral claims of the dolphin, still less the fetus, when you are willing to grant personhood to this bucket of bolts and transistors, this puddle of senseless bioengineered flesh? There is a long history in the debate over the franchise and over constitutional rights, of disenfranchised groups using claims such as these. Some white women suffragists asked how they could be denied the vote when *African-American* men had been granted it, using prejudices about racial privilege to fight prejudices about sex privilege. A form of this argument is already being made by those who believe that it is ludicrous to grant inhuman corporations legal personality but to refuse to do so for human fetuses. At the very least, Hal and Vanna's arrival would dramatically expand the range of such appeals. "Lesser comparative otherness" can be a winning strategy. If, in twenty years time, you can generally predict someone's position on the legal personality of artificial intelligences by their position on abortion, this guess will have proven to be correct. But that outcome is far from assured.

Consider the challenge, almost the paradox, that Hal and Vanna present to the constitutional intuitions of a conservative religious person who is strongly anti-abortion. If one believes deeply in a divinely commanded natural order, in which

man has been given 'dominion over the inferior creatures, over the fish of the sea, and the fowl of the air,' in which "unnatural" and "immoral" are synonyms, then a transgenic entity or an artificial intelligence is more likely to elicit a cry of "heresy" than an egalitarian embrace. Yes, in some *pragmatic* sense, recognition of the rights of these entities might benefit the push to grant constitutional personhood to the fetus. But the price would surely be too high for at least one important wing of those who are morally opposed to abortion.

But now consider the mirror-image paradox that Hal and Vanna present to the pro-choice liberal who believes that the moral story of history is an inexorable widening of personhood and civil rights to reach more and more groups, overcoming bias about surface differences in order to expand the boundaries of legal respect. As I pointed out before, Hal and Vanna might well seem like the next stop on the Kantian express, the next entity to cry "Am I not a man and a brother?" to the rest of us in the hope we could overcome our parochial prejudice. Perhaps the very difficulties that we have *identifying* some essential common humanness or personality may lead us to be more willing to push the boundaries of those concepts outward, avoiding rather than solving the question of who counts as a person simply by leaving fewer groups outside to complain. Yet the liberal for whom abortion rights are not just a constitutional issue but *the* constitutional issue would surely be deeply wary of handing the pro-life forces another rhetorical weapon. Why are *fetuses* not the next stop on the Kantian express, the last discrete and insular minority whose "otherness" has allowed us to deny them personhood? No, for at least some on each side of the abortion debate, Hal and Vanna would produce strong cognitive dissonance rather than cries of strategic delight.

Facing this kind of conceptual logjam, as claims about the rights of newly created entities get tangled with our existing constitutional struggles, another approach might be to avoid the language of personhood altogether and simply regulate the creation of various entities according to a variety of public policy goals. We might forbid the creation of Vanna, not because of an idolatrous belief that the shape of the human being is sacred and thus conveys constitutional rights, but because of a belief that a society that would create such entities would tiptoe into a world of surpassing ugliness, losing respect for human life step by step along the way. We might criminalize the making of Hal, or forbid his creators to erase him once made, not because we think he is a person, but because we think there is cruelty involved even if he isn't—just as we regulate cruelty to non-human animals. Or we might forbid the entire line of research in the belief that eventually we would cross some dangerous line, whether of personhood or of species competition. In the words of Samuel Butler's *Book of the Machines* from *Erewhon*, "Is it not safer to nip the mischief in the bud and to forbid them further progress?"[19] It would be ironic if Hal and Vanna were banned partly because we do not know

[19] Of course, Butler's *Book of the Machines* was written as an sarcastic commentary on one of the key scientific fights of his day — the struggle over evolution. The fact that we are still fighting that battle — a debate about *facts* — is sobering when we turn instead to a debate about justice.

how to classify them, the ultimate penalty for conceptual controversy.

The most likely outcome of all, however, is neither a bold expansion of our constitutional rights, nor a technophobic attempt to legislate the moral quandary out of existence. It is instead the kind of messy, confused, sometimes idealistic, sometimes corrupt muddling-through that characterizes much of our constitutional tradition.

The question of whether the Constitution protects artificial entities, products of human ingenuity, seems like a futuristic one. But it is one we met and answered long ago. Corporations are artificial entities and yet we have chosen to classify them as legal persons to which many constitutional rights adhere. This process has, admittedly, not been uncontroversial. In Justice Douglas's words,

> [A]s Mr. Justice Black pointed out in his dissent in *Connecticut General Co. v. Johnson*, the submission of the [14th] Amendment to the people was on the basis that it protected human beings. There was no suggestion in its submission that it was designed to put negroes and corporations into one class and so dilute the police power of the States over corporate affairs. Arthur Twining Hadley once wrote that 'The Fourteenth Amendment was framed to protect the negroes from oppression by the whites, not to protect corporations from oppression by the legislature. It is doubtful whether a single one of the members of a Congress who voted for it had any idea that it would touch the question of corporate regulation at all.'[20]

Even those who could not be suspected of hostility to corporate interests have sometimes thought the trope of personhood has been extended too far. As then Justice Rehnquist put it, "Extension of the individual freedom of conscience decisions to business corporations strains the rationale of those cases beyond the breaking point. To ascribe to such artificial entities an 'intellect' or 'mind' for freedom of conscience purposes is to confuse metaphor with reality."[21]

Though I share Justices Black and Douglas's skepticism about the rights of corporations under the 14th Amendment, I think that we can learn something about Hal and Vanna's cases by studying the constitutionalization of corporate personhood. What is remarkable about that process is that the courts never clearly articulated a reason *why* corporations were persons within the meaning of the 14th Amendment. Instead, the courts have conveyed upon them some—but not all—the rights that the Constitution applies to natural persons, based largely on a set of perceived, and perhaps exaggerated, fears about what the consequences might be if they did not. Might not a similar approach to Hal and Vanna lead to the creation of some new category of personhood? One could imagine something that relates to full, human personhood as civil unions relate to marriage—carrying

[20] *Wheeling Steel Corp. v. Glander*, 337 U.S. 562, 578 (1949).
[21] *Pacific Gas & Elec. Co. v. Public Utilities Com'n of California*, 475 U.S. 1, 33 (1986)(dissent).

many of the same protections but denying the sought-after equivalence for reasons of religious belief or simple political acceptability. Doubtless, this approach would be found just as unsatisfactory as civil unions are to many, marking the creation of second class citizens who are denied the "real" personality of humans.

The history of corporate personhood is hardly one of the Constitution's shining moments. Is its confused and partisan process of pragmatic muddling the best we can do with the more morally wrenching questions that the future will bring us? In a characteristically wise article on the constitutional rights of artificial constructs, Lawrence Solum wrote "when it comes to real judges making decisions in real legal cases, we hope for adjudicators that shun deep waters and recoil from grand theory. When it comes to our own moral lives, we try our best to stay in shallow waters."[22]

Those words resonate strongly with me. And yet.... There is one modification I would make. It is the one suggested by the theory of the moral sentiments that comes from the Scottish Enlightenment—the idea that morality springs from the intuitive sympathy, the spark of compassion that jumps the gap to the predicament of the other. The others that the future will bring us are strange beyond belief. Science and logic cannot provide constitutional law with an iron bridge across the gaps between us and them. All the more need, then, for a moral sympathy that is both generous and humble. The most striking conclusion of Alan Turing's article may not be how difficult it is to identify machine consciousness or personhood but how uncertain we are about the boundaries of our own.

[22] Lawrence B. Solum, "Legal Personhood for Artificial Intelligences," *North Carolina Law Review* 70, no. 4 (April 1992): 1286–87.

James Boyle is William Neal Reynolds Professor of Law at Duke Law School and founder of the Center for the Study of the Public Domain. Professor Boyle was one of the original Board Members of Creative Commons. He served as a board member from 2002 until 2009, the last year as Chairman of the Board. He was also a co-founder of Science Commons, which aims to expand the Creative Commons mission into the realm of scientific and technical data, and of ccLearn which works to promote the development and use of open educational resources. He is currently a member of the board of the Public Library of Science. In 2003 Professor Boyle won the World Technology Network Award for Law for his work on the public domain and the "second enclosure movement" that threatens it. In 2010 he was awarded the Electronic Frontier Foundation's Pioneer Award. He is the author of *Shamans, Software and Spleens: Law and the Construction of the Information Society*, and the editor of *Critical Legal Studies*, *Collected Papers on the Public Domain and Cultural Environmentalism @ 10* (with Larry Lessig.) His more recent books include *Bound By Law*, a co-authored "graphic novel" about the effects of intellectual property on documentary film, *The Shakespeare Chronicles*, a novel, and *The Public Domain: Enclosing the Commons of the Mind*. He writes a regular online column for the Financial Times' New Economy Policy Forum.

Governance Studies
The Brookings Institution
1775 Massachusetts Ave., NW
Washington, DC 20036
Tel: 202.797.6090
Fax: 202.797.6144
www.brookings.edu/governance.aspx

Editors
Jeffrey Rosen
Benjamin Wittes

Production & Layout
John S Seo

E-mail your comments to gscomments@brookings.edu

Weiss,

Of Mice, Men and In-Between,

WASH. POST (Nov. 20, 2004)

Of Mice, Men and In-Between; Scientists Debate Blending Of Human, Animal Forms

[FINAL Edition]

The Washington Post - Washington, D.C.
Author: Weiss, Rick
Date: Nov 20, 2004
Start Page: A.01
Section: A SECTION
Text Word Count: 1811

In Minnesota, pigs are being born with human blood in their veins.

In Nevada, there are sheep whose livers and hearts are largely human.

In California, mice peer from their cages with human brain cells firing inside their skulls.

These are not outcasts from "The Island of Dr. Moreau," the 1896 novel by H.G. Wells in which a rogue doctor develops creatures that are part animal and part human. They are real creations of real scientists, stretching the boundaries of stem cell research.

Biologists call these hybrid animals chimeras, after the mythical Greek creature with a lion's head, a goat's body and a serpent's tail. They are the products of experiments in which human stem cells were added to developing animal fetuses.

Chimeras are allowing scientists to watch, for the first time, how nascent human cells and organs mature and interact -- not in the cold isolation of laboratory dishes but inside the bodies of living creatures. Some are already revealing deep secrets of human biology and pointing the way toward new medical treatments.

But with no federal guidelines in place, an awkward question hovers above the work: How human must a chimera be before more stringent research rules should kick in?

The National Academy of Sciences, which advises the federal government, has been studying the issue and hopes to make recommendations by February. Yet the range of opinions it has received so far suggests that reaching consensus may be difficult.

During one recent meeting, scientists disagreed on such basic issues as whether it would be unethical for a human embryo to begin its development in an animal's womb, and whether a mouse would be better or worse off with a brain made of human neurons.

"This is an area where we really need to come to a reasonable consensus," said James Battey, chairman of the National Institutes of Health's Stem Cell Task Force. "We need to establish some kind of guidelines as to what the scientific community ought to do and ought not to do."

Chimeras (ki-MER-ahs) -- meaning mixtures of two or more individuals in a single body -- are not inherently unnatural. Most twins carry at least a few cells from the sibling with whom they shared a womb, and most mothers carry in their blood at least a few cells from each child they have born.

Recipients of organ transplants are also chimeras, as are the many people whose defective heart valves have been replaced with those from pigs or cows. And scientists for years have added human genes to bacteria and even to farm animals -- feats of genetic engineering that allow those critters to make human proteins such as insulin for use as medicines.

"Chimeras are not as strange and alien as at first blush they seem," said Henry Greely, a law professor and ethicist at Stanford University who has reviewed proposals to create human-mouse chimeras there.

But chimerism becomes a more sensitive topic when it involves growing entire human organs inside animals. And it becomes especially sensitive when it deals in brain cells, the building blocks of the organ credited with making humans human.

In experiments like those, Greely told the academy last month, "there is a nontrivial risk of conferring some significant aspects of humanity" on the animal.

Greely and his colleagues did not conclude that such experiments should never be done. Indeed, he and many other philosophers have been wrestling with the question of why so many people believe it is wrong to breach the species barrier.

Does the repugnance reflect an understanding of an important natural law? Or is it just another cultural bias, like the once widespread rejection of interracial marriage?

Many turn to the Bible's repeated invocation that animals should multiply "after their kind" as evidence that such

experiments are wrong. Others, however, have concluded that the core problem is not necessarily the creation of chimeras but rather the way they are likely to be treated.

Imagine, said Robert Streiffer, a professor of philosophy and bioethics at the University of Wisconsin, a human-chimpanzee chimera endowed with speech and an enhanced potential to learn -- what some have called a "humanzee."

"There's a knee-jerk reaction that enhancing the moral status of an animal is bad," Streiffer said. "But if you did it, and you gave it the protections it deserves, how could the animal complain?"

Unfortunately, said Harvard political philosopher Michael J. Sandel, speaking last fall at a meeting of the President's Council on Bioethics, such protections are unlikely.

"Chances are we would make them perform menial jobs or dangerous jobs," Sandel said. "That would be an objection."

The potential power of chimeras as research tools became clear about a decade ago in a series of dramatic experiments by Evan Balaban, now at McGill University in Montreal. Balaban took small sections of brain from developing quails and transplanted them into the developing brains of chickens.

The resulting chickens exhibited vocal trills and head bobs unique to quails, proving that the transplanted parts of the brain contained the neural circuitry for quail calls. It also offered astonishing proof that complex behaviors could be transferred across species.

No one has proposed similar experiments between, say, humans and apes. But the discovery of human embryonic stem cells in 1998 allowed researchers to envision related experiments that might reveal a lot about how embryos grow.

The cells, found in 5-day-old human embryos, multiply prolifically and -- unlike adult cells -- have the potential to turn into any of the body's 200 or so cell types.

Scientists hope to cultivate them in laboratory dishes and grow replacement tissues for patients. But with those applications years away, the cells are gaining in popularity for basic research.

The most radical experiment, still not conducted, would be to inject human stem cells into an animal embryo and then transfer that chimeric embryo into an animal's womb. Scientists suspect the proliferating human cells would spread throughout the animal embryo as it matured into a fetus and integrate themselves into every organ.

Such "humanized" animals could have countless uses. They would almost certainly provide better ways to test a new drug's efficacy and toxicity, for example, than the ordinary mice typically used today.

But few scientists are eager to do that experiment. The risk, they say, is that some human cells will find their way to the developing testes or ovaries, where they might grow into human sperm and eggs. If two such chimeras -- say, mice -- were to mate, a human embryo might form, trapped in a mouse.

Not everyone agrees that this would be a terrible result.

"What would be so dreadful?" asked Ann McLaren, a renowned developmental biologist at the University of Cambridge in England. After all, she said, no human embryo could develop successfully in a mouse womb. It would simply die, she told the academy. No harm done.

But others disagree -- if only out of fear of a public backlash.

"Certainly you'd get a negative response from people to have a human embryo trying to grow in the wrong place," said Cynthia B. Cohen, a senior research fellow at Georgetown University's Kennedy Institute of Ethics and a member of Canada's Stem Cell Oversight Committee, which supported a ban on such experiments there.

But what about experiments in which scientists add human stem cells not to an animal embryo but to an animal fetus, which has already made its eggs and sperm? Then the only question is how human a creature one dares to make.

In one ongoing set of experiments, Jeffrey L. Platt at the Mayo Clinic in Rochester, Minn., has created human-pig chimeras by adding human-blood-forming stem cells to pig fetuses. The resulting pigs have both pig and human blood in their vessels. And it's not just pig blood cells being swept along with human blood cells; some of the cells themselves have merged, creating hybrids.

It is important to have learned that human and pig cells can fuse, Platt said, because he and others have been considering transplanting modified pig organs into people and have been wondering if that might pose a risk of pig viruses getting into patient's cells. Now scientists know the risk is real, he said, because the viruses may gain access when the two cells fuse.

In other experiments led by Esmail Zanjani, chairman of animal biotechnology at the University of Nevada at Reno, scientists have been adding human stem cells to sheep fetuses. The team now has sheep whose livers are up to 80

percent human -- and make all the compounds human livers make.

Zanjani's goal is to make the humanized livers available to people who need transplants. The sheep portions will be rejected by the immune system, he predicted, while the human part will take root.

"I don't see why anyone would raise objections to our work," Zanjani said in an interview.

Perhaps the most ambitious efforts to make use of chimeras come from Irving Weissman, director of Stanford University's Institute of Cancer/Stem Cell Biology and Medicine. Weissman helped make the first mouse with a nearly complete human immune system -- an animal that has proved invaluable for tests of new drugs against the AIDS virus, which does not infect conventional mice.

More recently his team injected human neural stem cells into mouse fetuses, creating mice whose brains are about 1 percent human. By dissecting the mice at various stages, the researchers were able to see how the added brain cells moved about as they multiplied and made connections with mouse cells.

Already, he said, they have learned things they "never would have learned had there been a bioethical ban."

Now he wants to add human brain stem cells that have the defects that cause Parkinson's disease, Lou Gehrig's disease and other brain ailments -- and study how those cells make connections.

Scientists suspect that these diseases, though they manifest themselves in adulthood, begin when something goes wrong early in development. If those errors can be found, researchers would have a much better chance of designing useful drugs, Weissman said. And those drugs could be tested in the chimeras in ways not possible in patients.

Now Weissman says he is thinking about making chimeric mice whose brains are 100 percent human. He proposes keeping tabs on the mice as they develop. If the brains look as if they are taking on a distinctly human architecture -- a development that could hint at a glimmer of humanness -- they could be killed, he said. If they look as if they are organizing themselves in a mouse brain architecture, they could be used for research.

So far this is just a "thought experiment," Weissman said, but he asked the university's ethics group for an opinion anyway.

"Everyone said the mice would be useful," he said. "But no one was sure if it should be done."

Mott,

Animal-Human Hybrids Spark Controversy,

NAT'L GEO. NEWS (Jan. 25, 2005)

NATIONAL GEOGRAPHIC NEWS
NATIONALGEOGRAPHIC.COM/NEWS

Animal-Human Hybrids Spark Controversy

Maryann Mott
National Geographic News

January 25, 2005

Scientists have begun blurring the line between human and animal by producing chimeras—a hybrid creature that's part human, part animal.

Chinese scientists at the Shanghai Second Medical University in 2003 successfully fused human cells with rabbit eggs. The embryos were reportedly the first human-animal chimeras successfully created. They were allowed to develop for several days in a laboratory dish before the scientists destroyed the embryos to harvest their stem cells.

In Minnesota last year researchers at the Mayo Clinic created pigs with human blood flowing through their bodies.

And at Stanford University in California an experiment might be done later this year to create mice with human brains.

Scientists feel that, the more humanlike the animal, the better research model it makes for testing drugs or possibly growing "spare parts," such as livers, to transplant into humans.

Watching how human cells mature and interact in a living creature may also lead to the discoveries of new medical treatments.

But creating human-animal chimeras—named after a monster in Greek mythology that had a lion's head, goat's body, and serpent's tail—has raised troubling questions: What new subhuman combination should be produced and for what purpose? At what point would it be considered human? And what rights, if any, should it have?

There are currently no U.S. federal laws that address these issues.

Ethical Guidelines

The National Academy of Sciences, which advises the U.S. government, has been studying the issue. In March it plans to present voluntary ethical guidelines for researchers.

A chimera is a mixture of two or more species in one body. Not all are considered troubling, though.

For example, faulty human heart valves are routinely replaced with ones taken from cows and pigs. The surgery—which makes the recipient a human-animal chimera—is widely accepted. And for years scientists have added human genes to bacteria and farm animals.

What's caused the uproar is the mixing of human stem cells with embryonic animals to create new species.

Biotechnology activist Jeremy Rifkin is opposed to crossing species boundaries, because he believes animals have the right to exist without being tampered with or crossed with another species.

He concedes that these studies would lead to some medical breakthroughs. Still, they should not be done.

"There are other ways to advance medicine and human health besides going out into the strange, brave new world of chimeric animals," Rifkin said, adding that sophisticated computer models can substitute for experimentation on live animals.

"One doesn't have to be religious or into animal rights to think this doesn't make sense," he continued. "It's the scientists who want to do this. They've now gone over the edge into the pathological domain."

David Magnus, director of the Stanford Center for Biomedical Ethics at Stanford University, believes the real worry is whether or not chimeras will be put to uses that are problematic, risky, or dangerous.

Human Born to Mice Parents?

For example, an experiment that would raise concerns, he said, is genetically engineering mice to produce human sperm and eggs, then doing in vitro fertilization to produce a child whose parents are a pair of mice.

"Most people would find that problematic," Magnus said, "but those uses are bizarre and not, to the best of my knowledge, anything that anybody is remotely contemplating. Most uses of chimeras are actually much more relevant to practical concerns."

Last year Canada passed the Assisted Human Reproduction Act, which bans chimeras. Specifically, it prohibits transferring a nonhuman cell into a human embryo and putting human cells into a nonhuman embryo.

Cynthia Cohen is a member of Canada's Stem Cell Oversight Committee, which oversees research protocols to ensure they are in accordance with the new guidelines.

She believes a ban should also be put into place in the U.S.

Creating chimeras, she said, by mixing human and animal gametes (sperms and eggs) or transferring reproductive cells, diminishes human dignity.

"It would deny that there is something distinctive and valuable about human beings that ought to be honored and protected," said Cohen, who is also the senior research fellow at Georgetown University's Kennedy Institute of Ethics in Washington, D.C.

But, she noted, the wording on such a ban needs to be developed carefully. It shouldn't outlaw ethical and legitimate experiments—such as transferring a limited number of adult human stem cells into animal embryos in order to learn how they proliferate and grow during the prenatal period.

Irv Weissman, director of Stanford University's Institute of Cancer/Stem Cell Biology and Medicine in California, is against a ban in the United States.

"Anybody who puts their own moral guidance in the way of this biomedical science, where they want to impose their will—not just be part of an argument—if that leads to a ban or moratorium. … they are stopping research that would save human lives," he said.

Mice With Human Brains

Weissman has already created mice with brains that are about one percent human.

Later this year he may conduct another experiment where the mice have 100 percent human brains. This would be done, he said, by injecting human neurons into the brains of embryonic mice.

Before being born, the mice would be killed and dissected to see if the architecture of a human brain had formed. If it did, he'd look for traces of human cognitive behavior.

Weissman said he's not a mad scientist trying to create a human in an animal body. He hopes the experiment leads to a better understanding of how the brain works, which would be useful in treating diseases like Alzheimer's or Parkinson's disease.

The test has not yet begun. Weissman is waiting to read the National Academy's report, due out in March.

William Cheshire, associate professor of neurology at the Mayo Clinic's Jacksonville, Florida, branch, feels that combining human and animal neurons is problematic.

"This is unexplored biologic territory," he said. "Whatever moral threshold of human neural development we might choose to set as the limit for such an experiment, there would be a considerable risk of exceeding that limit before it could be recognized."

Cheshire supports research that combines human and animal cells to study cellular function. As an undergraduate he participated in research that fused human and mouse cells.

But where he draws the ethical line is on research that would destroy a human embryo to obtain cells, or research that would create an organism that is partly human and partly animal.

"We must be cautious not to violate the integrity of humanity or of animal life over which we have a stewardship responsibility," said Cheshire, a member of Christian Medical and Dental Associations. "Research projects that create human-animal chimeras risk disturbing fragile ecosystems, endanger health, and affront species integrity."

Han et al.,

Forebrain Engraftment by Human Glial Progenitor Cells Enhances Synaptic Plasticity and Learning in Adult Mice,

CELL STEM CELL, vol. 12, pp. 342-353 (Mar. 7, 2013)

Cell Stem Cell
Article

Forebrain Engraftment by Human Glial Progenitor Cells Enhances Synaptic Plasticity and Learning in Adult Mice

Xiaoning Han,[1,2] Michael Chen,[1,2] Fushun Wang,[1,2] Martha Windrem,[1,3] Su Wang,[1,3] Steven Shanz,[1,3] Qiwu Xu,[1,2] Nancy Ann Oberheim,[1,2] Lane Bekar,[1,2] Sarah Betstadt,[4] Alcino J. Silva,[5] Takahiro Takano,[1,2] Steven A. Goldman,[1,2,3,*] and Maiken Nedergaard[1,2,3,*]

[1]Center for Translational Neuromedicine
[2]Department of Neurosurgery
[3]Department of Neurology
[4]Department of Obstetrics and Gynecology
University of Rochester Medical Center, Rochester, NY 14642, USA
[5]Departments of Neurobiology, Psychiatry and Psychology, Integrative Center for Learning and Memory, University of California, Los Angeles, David Geffen School of Medicine, Los Angeles, CA 90095, USA
*Correspondence: steven_goldman@urmc.rochester.edu (S.A.G.), nedergaard@urmc.rochester.edu (M.N.)
http://dx.doi.org/10.1016/j.stem.2012.12.015

SUMMARY

Human astrocytes are larger and more complex than those of infraprimate mammals, suggesting that their role in neural processing has expanded with evolution. To assess the cell-autonomous and species-selective properties of human glia, we engrafted human glial progenitor cells (GPCs) into neonatal immunodeficient mice. Upon maturation, the recipient brains exhibited large numbers and high proportions of both human glial progenitors and astrocytes. The engrafted human glia were gap-junction-coupled to host astroglia, yet retained the size and pleomorphism of hominid astroglia, and propagated Ca^{2+} signals 3-fold faster than their hosts. Long-term potentiation (LTP) was sharply enhanced in the human glial chimeric mice, as was their learning, as assessed by Barnes maze navigation, object-location memory, and both contextual and tone fear conditioning. Mice allografted with murine GPCs showed no enhancement of either LTP or learning. These findings indicate that human glia differentially enhance both activity-dependent plasticity and learning in mice.

INTRODUCTION

The unique processing capabilities of the human brain reflect a number of evolutionary adaptations by its cellular constituents (Fields, 2004). One especially distinct feature of the adult human brain's cellular composition is the size and complexity of its astrocytic cohort. Human astrocytes are both morphologically and functionally distinct from those of infraprimate mammals, in that human astroglia are larger and exhibit far greater architectural complexity and cellular pleomorphism, as well as more rapid syncytial calcium signaling, than their murine counterparts (Colombo, 1996; Oberheim et al., 2009). These phylogenetic differences are of particular interest, since astrocytes can both coordinate and modulate neural signal transmission (Rusakov et al., 2011; Verkhratsky et al., 1998). These observations promise to fundamentally transform our view of astrocytes, since current concepts of the role of astrocytes in neural network performance are based almost entirely on studies of astrocytic physiology in the rodent brain (Oberheim et al., 2006).

In this study, we have used a human glial chimeric mouse brain to ask whether the structural complexity and unique functional properties of human astrocytes influence activity-dependent plasticity in an otherwise stable neural network. In particular, we have tested the hypothesis that human astrocytes might enhance synaptic plasticity and learning relative to their murine counterparts.

RESULTS

Human Glial Progenitors Exhibit Cell-Autonomous Astrocytic Differentiation in Mouse Brain

To study human astrocytes in the live adult brain, we generated chimeric mice in which human glial progenitor cells (GPCs)—isolated by being sorted on the basis of an $A2B5^+$/PSA-$NCAM^-$ phenotype, and then being expanded via a protocol that promoted differentiation into hGFAP- and A2B5-expressing astrocytes (Figure S1A available online)—were xenografted into neonatal immune-deficient mice; these matured to become adults chimeric for both mouse and human astroglia (Windrem et al., 2004, 2008) (Figure 1A). The human GPCs were labeled ex vivo, prior to implantation, with VSVg-pseudotyped lentiviral-CMV-EGFP; in antecedent pilot experiments, we had determined that this vector sustained the expression of EGFP by astroglia for at least 1 year in vivo (Figure 1A). The neonatally implanted mice were sacrificed at time points ranging from 0.5 to 20 months of age, and their brains were assessed both histologically and electrophysiologically. Human donor cells were first identified based on their expression of human nuclear antigen (hNuclei). The $hNuclei^+$ cells were found to distribute relatively evenly throughout the forebrain, infiltrating both hippocampus

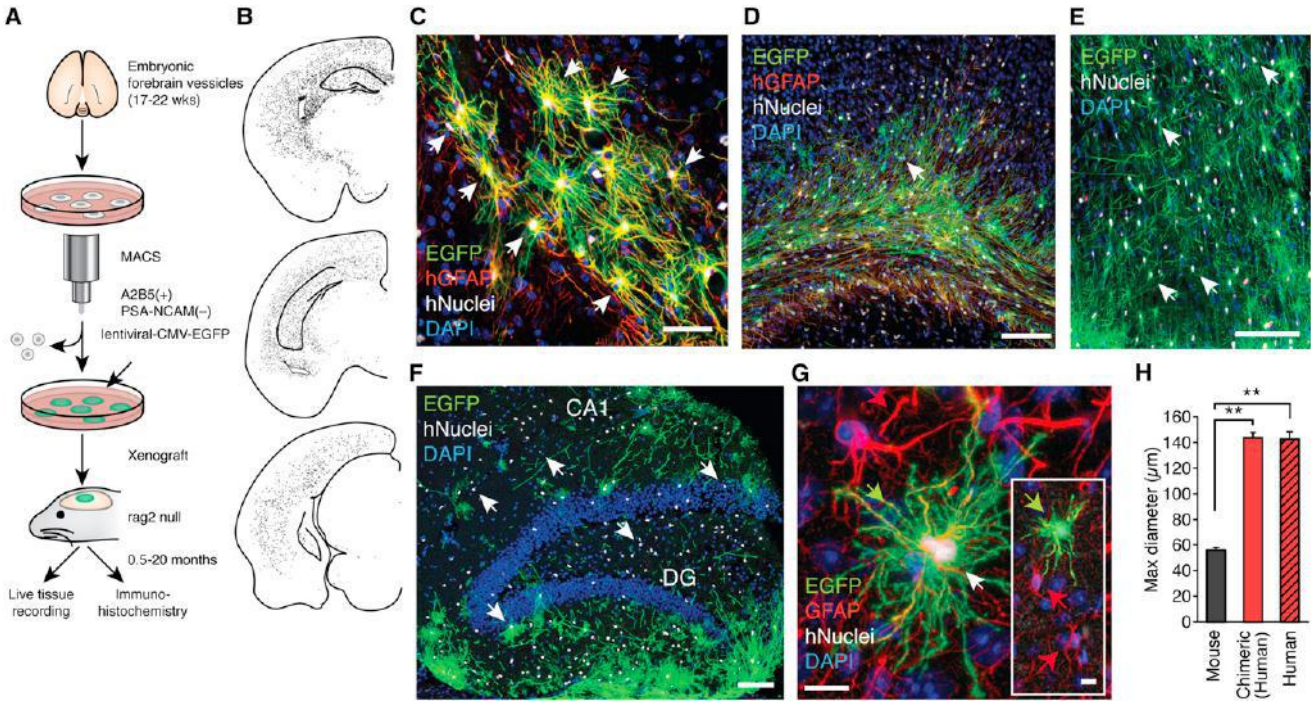


Figure 1. Human Astrocytes Replace Host Glia in Mice Engrafted with Human Glial Progenitors

(A) Schematic outlining the procedure for magnetic cell sort-based isolation (MACS) of human glial progenitors, tagging with EGFP, and xenografting at P1. The chimeric mice brains were analyzed in 0.5- to 20-month-old chimeric mice.

(B) Representative dot map showing the distribution of human nuclear antigen $(hNuclei)^+$ cells in three coronal sections from a 10-month-old human chimeric mouse.

(C) The complex fine structure of human astrocytes in chimeric brain replicates the classical star-shaped appearance of human astrocytes labeled with hGFAP in situ. Most cells in the field are $EGFP^+/hNuclei^+/hGFAP^+$ (hGFAP, red). Arrows in (C) through (F) show representative examples of human cells (hNuclei, white).

(D) At 5 months, $EGFP^+$ cells typically infiltrated corpus callosum and cortical layers V and VI. All $EGFP^+$ cells labeled with an antibody directed against human nuclear antigen (hNuclei) and most of the human cells were also labeled with an antibody directed against human GFAP (hGFAP, red).

(E) At 11 months, many areas of cortex were infiltrated by evenly distributed $EGFP^+/hNuclei^+$ cells.

(F) The hippocampus was also populated with $EGFP^+/hNuclei^+$ cells in a 14-month-old animal, with the highest density in the dentate.

(G) Human $EGFP^+/hNuclei^+/GFAP^+$ cells (green arrows) were significantly larger than host murine astrocytes (red arrow). The anti-GFAP antibody cross-reacted with both human and mouse GFAP (red). Inset shows same field in lower magnification.

(H) Histogram comparing the diameter of mouse cortical astrocytes to human cortical astrocytes in situ (freshly resected surgical samples) and xenografted human astrocytes in cortex of chimeric mouse brain. The maximal diameter of mouse and human astrocytes (in situ and in chimeric mice) was determined in sections stained with an anti-GFAP antibody that labels both human and mouse GFAP. ($n = 50–65$; $^{**}p < 0.01$, Bonferroni t test.)

EGFP, green; hNuclei, white and white arrow; DAPI, blue (B–F). Scale bars: 50 μm (C); 100 μm (D–F); and 10 μm (G). Data graphed as means ± SEM. See also Figure S1.

and cortex (Figure 1B). Human astrocytes were specifically identified by their intricate $EGFP^+$ fluorescent processes and, in fixed tissue, by their coexpression of human glial fibrillary acidic protein (hGFAP) and hNuclei (Figure 1C). By 4–5 months of age, mice engrafted with human GPCs exhibited substantial addition of human astrocytes to both the hippocampus and deep neocortical layers; by 12–20 months, human astrocytes further populated large regions of the amygdala, thalamus, neostriatum, and cortex (Figures 1D–1F). The human astrocytes appeared to develop and mature in a cell-autonomous fashion, maintaining their larger size and more complex structure relative to murine astrocytes (Figures 1G and 1H).

Human astrocytes, defined as $EGFP^+/hGFAP^+/hNuclei^+$, regularly extended processes that terminated in end-feet contiguously arrayed along blood vessel walls (Figures 2A and 2B). Their long processes were often tortuous and resembled the processes of interlaminar astroglia, a phenotype previously described only in adult human and ape brain (Oberheim et al., 2006) (Figure 2C); these cells are characterized by long, unbranched processes that traverse multiple cortical laminae (Colombo, 2001). Many of the engrafted human astrocytes in chimeric mice extended processes that spanned >0.5 mm (Figure 2D). A large number of mitochondria were present in the long processes (Figure 2E). Other engrafted human cells exhibited the long, varicosity-studded processes of varicose projection astrocytes, a second class of hominid astrocytes (Oberheim et al., 2009).

Of note, $rag2^{-/-}$ immunodeficient mice on a C3h background were generally used as recipients for these experiments, although $rag1^{-/-}$ immunodeficient mice (on a C57/Bl6 background) were used for vision-dependent behavioral tests, since the C3h background of the rag2 mice is a visually impaired strain; we observed no difference in xenograft acceptance, cell dispersal, or differentiation patterns between these two hosts.

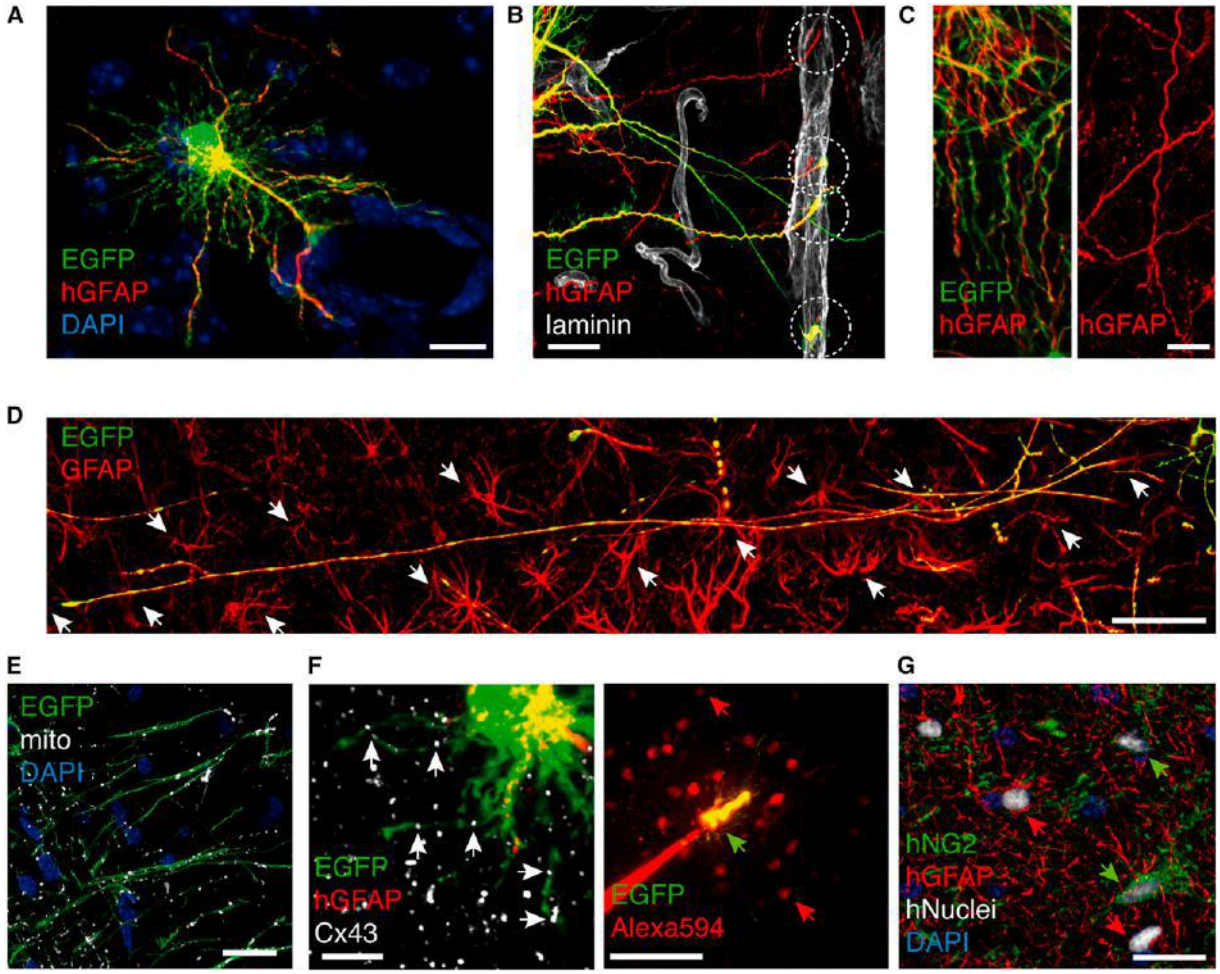


Figure 2. Human Astrocytes Retain Hominid-Specific Morphologies in Chimeric Mice

Human protoplasmic astrocytes matured in a cell-autonomous fashion in the chimeric mouse brain environment and retained the long GFAP$^+$, mitochondrial-enriched processes of native human astroglia.

(A) An EGFP$^+$/hGFAP$^+$ astrocyte makes contact with the vasculature in a 1-month-old chimeric mouse.

(B) Long, unbranched EGFP$^+$ and hGFAP$^+$ astrocytic processes terminated (dashed circle) on the vasculature (laminin; white) 16 days after implantation.

(C) The tortuous shape of EGFP$^+$/hGFAP$^+$ processes in chimeric brains replicate the appearance of GFAP$^+$ processes of interlaminar astroglia in intact human tissue.

(D) An example of an EGFP$^+$/GFAP$^+$ process that spans >600 μm and penetrates the domains of at least 14 host murine astrocytes (white arrow) (GFAP, red).

(E) Long EGFP$^+$ processes contain a large number of mitochondria (white) in an 11-month-old chimeric mouse.

(F) An EGFP$^+$/hGFAP$^+$ human astrocyte expresses Cx43 (white) gap junction plaques (left panel). An EGFP$^+$ cell (green arrow) loaded with a small gap junction permeable tracer, Alexa 594 (MW 760) in a cortical slice (P15), is also shown. Alexa 594 (red) diffused into multiple neighboring EGFP$^-$ cells (red arrows).

(G) Coexistence of hGFAP$^+$ (red)/hNuclei+ (white) cells (red arrows) and hNG2$^+$ (green)/hNuclei$^+$ cells (green arrows) in the dentate of a 12-month-old chimeric mouse.

EGFP, green (A–F); hGFAP, red (A–C). Scale bars: 10 μm (A and C); 20 μm (B, E, and G); 50 μm (D and F, right panel); and 5 μm (F, left panel). See also Figure S2.

In that regard, we found no evidence of microglial activation in the xenografted mice, whether in rag1 null or rag2 null hosts, reflecting both their neonatal engraftment and immunodeficient backgrounds (Figures S1B–S1F).

Human Astrocytes Coupled Structurally and Functionally with Mouse Astrocytes

Their cell-autonomous maturation and morphologies notwithstanding, the engrafted human cells rapidly integrated with murine host cells. The gap junction tracer Alexa 594 (MW 760), once injected into EGFP$^+$ human cells, spread rapidly into multiple neighboring EGFP$^-$ host cells, suggesting the competence of interspecies gap junctions linking human and mouse astroglia, likely derived from the apposition of human and mouse Cx43 hemichannels (Figure 2F). A large number of hNuclei$^+$ cells failed to express GFAP but did express a human-specific isoform of the chondroitin sulfate proteoglycan NG2 (Figure 2G), a prototypic marker of parenchymal glial progenitor cells (Mangin and Gallo, 2011; Robel et al., 2011). Of note, hGFAP$^+$ and hNG2$^+$ human cells often coexisted in close proximity, although their relative ratios exhibited considerable variation across regions as well as between individual

mice (Figure 2G). Transferrin immunostaining failed to detect any human oligodendroglia, consistent with our prior assessment of glial progenitor cell fate upon transplantation to normally myelinated brain (Windrem et al., 2009) (Figures S2A and S2B): whereas a large proportion of engrafted human GPCs differentiate into oligodendrocytes in hypomyelinated shiverer mice, essentially no human oligodendrocytes were found in similarly engrafted wild-type mice (Windrem et al., 2009).

Human GPCs and Astrocytes Exhibited Distinct Physiological Phenotypes in Mouse Brain

To evaluate the electrophysiological properties of human astrocytes engrafted in mice, acute hippocampal slices were prepared from chimeric mice ranging from 4 to 10 months of age (6.5 ± 0.4 months old, mean ± SD). Donor astrocytes could be readily identified by their EGFP fluorescence and by their large, symmetric, highly branched astrocytic morphologies. The tagged donor cells were filled with Alexa 594 or the Ca^{2+} indicator rhod2 during whole-cell recordings, and their phenotype was verified by immunolabeling for GFAP (Figure 3A). $EGFP^+$ human astrocytes exhibited a higher input resistance than that of host murine astrocytes (51.6 ± 2.5 MΩ, n = 37, versus 29.2 ± 3.2 MΩ, n = 17, respectively, means ± SEM; $p < 0.05$, Steel-Dwass test). In contrast, the resting membrane potential of human astrocytes (−69.2 ± 1.5 mV, n = 37) was not significantly different from that of untagged host astrocytes (−73.9 ± 1.7 mV, n = 17, $p > 0.05$) (Figures 3B–3D). Whereas all large and symmetric $EGFP^+$ donor cells exhibited passive membrane currents and linear current to voltage (I/V) curves, another population of smaller $EGFP^+$ human cells with compact, asymmetrically branched morphologies manifested a much higher input resistance (147.8 ± 11.7 MΩ, n = 14). These donor cells manifested voltage-gated currents and depolarization-triggered outward currents with delayed activation (Figure 3B) and expressed a human epitope of chondroitin sulfate proteoglycan NG2, identifying them as persistent glial progenitors (Figure 2G) (Kang et al., 2010; Robel et al., 2011). Together, these histological and electrophysiological analyses supported the notion that a large proportion of engrafted human cells differentiated into protoplasmic astrocytes, forming a functional syncytium with their murine host, and that these were accompanied by large numbers of coengrafted $NG2^+$ human glial progenitors.

Human Astrocytes Propagate Calcium Waves More Quickly than Do Murine Astroglia

Astrocytes are electrically nonexcitable and are incapable of electrochemical communication. Instead, the principle mechanism of astrocytic signaling involves transient elevations of cytosolic Ca^{2+} (Cotrina and Nedergaard, 2005). In light of the larger and more complex architecture of human astrocytes, we next asked whether propagation of intracellular Ca^{2+} signals in human astrocytes differs from that of rodents. To compare intracellular Ca^{2+} wave propagation between human and mouse astrocytes, we initiated localized Ca^{2+} increases by photolysis of caged Ca^{2+} (Parpura and Verkhratsky, 2012; Rusakov et al., 2011). Photolysis of caged Ca^2 loaded specifically into astrocytes was used to avoid potentially confounding alterations in local synaptic activity. Intracellular Ca^{2+} waves were evoked when we directed a UV beam at long processes of astrocytes filled with rhod2 and NP-EGTA by a patch pipette. The subsequent spread of Ca^{2+} signals was visualized using two-photon excitation (Figure 3E). Line scanning with high temporal resolution (2–4 ms) showed that intracellular Ca^{2+} wave propagation was significantly faster in human astrocytes than in murine cells; intracellular Ca^{2+} increases propagated with a velocity of 15.8 ± 0.7 μm/s among human glia compared to 5.7 ± 0.4 μm/s in resident murine astrocytes (n = 22–34, 6.5 ± 0.4 versus 7.0 ± 0.5 months old, mean ± SEM, $p < 0.05$, Steel-Dwass test) (Figures 3F–3H). To determine whether the faster intracellular Ca^{2+} waves in human astrocytes were an artifact of xenograft, we also assessed intracellular Ca^{2+} wave spread in slices of fresh human brain tissue obtained at surgical resection for distant lesions (mean age of patients: 30.6 ± 8.8 years, n = 3). Human astrocytes in these surgical resections similarly propagated intracellular Ca^{2+} waves much more rapidly than did murine astrocytes (n = 10) (Figure 3H). Together, these experiments demonstrated that intracellular Ca^{2+} signals propagate at least 3-fold faster within human astrocytes than in their rodent counterparts, and do so in human glial chimeric mice just as in human brain tissue. Of note, we were unable to evaluate intercellular Ca^{2+} wave propagation, as only slices prepared from young mice pups load well with esterified (AM) Ca^{2+} indicators (Dawitz et al., 2011).

Human Astrocytes Accentuate Excitatory Synaptic Transmission in the Murine Hippocampus

A principal function of astrocytes is to monitor local synaptic activity by their expression of metabotropic neurotransmitter receptors for both glutamate and GABA (Parpura and Verkhratsky, 2012; Rusakov et al., 2011). These receptors activate intracellular signaling pathways, mediated primarily by increases in cytosolic Ca^{2+}, which are linked to synaptic plasticity (Parpura and Zorec, 2010). To assess the selective impact of human astrocytes on neural transmission within the host murine neural network, we compared synaptic activity in hippocampal slices prepared from human glial chimeric mice to that of both their unengrafted and allografted littermate controls. We focused on the hippocampal dentate granule layer because of the many electrophysiological and behavioral tests by which hippocampal function, learning, and LTP could be assessed (Lee and Silva, 2009). In addition, human cells typically densely engrafted this area; these included an admixture of $GFAP^+$/$hNuclei^+$ and $NG2^+$/$hNuclei^+$ cells (Figures 1B, 1F, and 2G). Stimulation of the medial perforant path (Colino and Malenka, 1993) consistently evoked a significantly steeper slope of field excitatory postsynaptic potentials (fEPSP) in the humanized chimeric mice than that in either their uninjected littermates or mouse GPC allografted controls (n = 3–40, F = 3.15, by two-way ANOVA, $p = 0.044$) (Figure 4A). The allograft controls comprised a set of mice neonatally engrafted with murine GPCs derived from EGFP transgenic mice, and they otherwise underwent the same isolation and engraftment protocols as those using human GPCs. The steeper slope of the fEPSPs in the humanized chimeras compared to that of the uninjected controls was still evident after normalization to the fiber volley amplitudes, a measure thought to reflect the number of stimulated axons (Figure S3A). Thus, slices with human glia exhibited a significant enhancement in their basal level of excitatory synaptic transmission over a wide range of stimulation intensities.

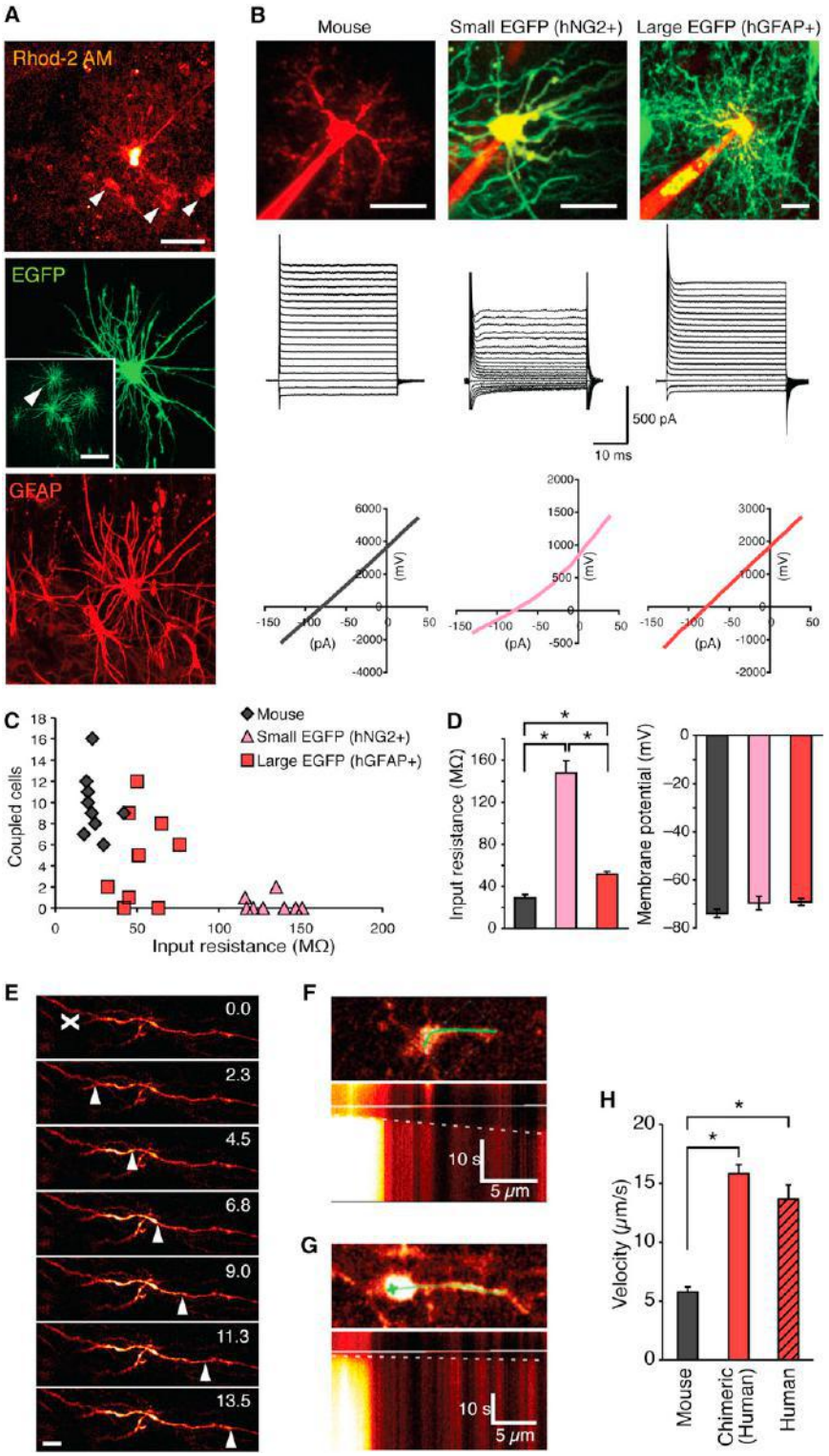


Figure 3. Functional Properties Indicate High-Density Host Engraftment by Both Human Glial Progenitors and Astrocytes

(A) Large and symmetric EGFP$^+$ cell (green) in an acute cortical slice prepared from a mouse engrafted with human EGFP$^+$ glial progenitors 4 months earlier. Inset: lower magnification of the same field. The EGFP$^+$ cell was loaded with rhod2 (red) by a patch pipette. Rhod2 diffused into several neighboring EGFP$^-$ cells (white arrows, top panel). Cell identity was verified when we immunolabeled against GFAP (red, below panel). Neighboring cells were GFAP$^+$ and their shape was characteristic of mouse astrocytes, indicating that the human EGFP$^+$/GFAP$^+$ astrocytes were coupled by functional gap junctions to host GFAP$^+$ astrocytes.

(B) I/V curves from host mouse astrocytes (n = 17); smaller, less complex EGFP$^+$ human cells, presumably glial progenitor cells (n = 14); and large and symmetric human EGFP$^+$ cells, presumably astrocytes (n = 37).

(C and D) Comparison of the input resistance and gap-junction-coupled cells detected as the number of neighboring cells labeled with Alexa 594. Mouse and large EGFP$^+$ cells (presumed human astrocytes) both manifested low input resistance and were extensively coupled by gap junctions. In contrast, small EGFP$^+$ cells—presumed human GPCs—exhibited high input resistance and were not gap junction coupled. (n = 14–37, *p < 0.05, Steel-Dwass test.) Membrane potentials were not significantly different.

(E) Photolysis of caged Ca^{2+} in an EGFP$^+$ astrocytic process. White "X" shows initiated point; white arrowhead shows Ca^{2+} propagation.

(F) Top: line scan position across the length of a mouse astrocyte filled with NP-EGTA and rhod2. Bottom: line scan image of an intra-astrocytic Ca^{2+} wave initiated by photolysis of the cell body. White dashed line indicates the velocity of the intracellular Ca^{2+} wave.

(G) Line scan image of a human astrocyte in a chimeric mouse.

(H) Comparison of velocities of intracellular Ca^{2+} waves in host murine and engrafted human EGFP$^+$ astrocytes and in human astrocytes in freshly resected surgical tissue. (n = 8–35, *p < 0.05, Steel-Dwass test.)

Scale bars: 30 μm (A); 100 μm (A, insert); 20 μm (B); and 10 μm (E). Data graphed as means ± SEM.

Human Astrocytes Enhance LTP in the Adult Murine Hippocampus

We next asked if human astrocytes might affect synaptic plasticity by assessing the effect of human glia on long-term potentiation (LTP). Two trains of high-frequency stimulation (HFS) potentiated the fEPSP slope to 151.2% ± 8.1% of baseline in chimeric mice, compared with 138.6% ± 7.6% in control littermates (n = 7 mice in both groups, 13.8 ± 1.1 versus 12.6 ± 0.4 months old, respectively, ages provided as mean ± SEM) (Figure 4B). The enhancement of fEPSP slope persisted at 60 min in humanized chimeric mice (113.6% ± 3.8%, p < 0.05), whereas fEPSP slope in unengrafted controls fell to 103.2% ± 3.9% (not significantly

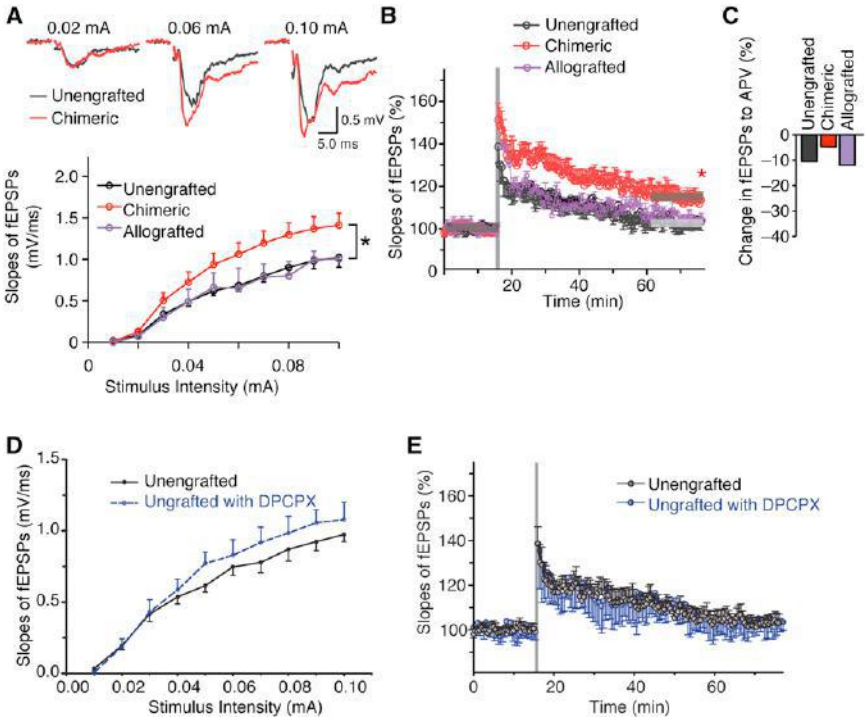


Figure 4. Strengthening of Excitatory Transmission and Synaptic Plasticity in Murine Brain by Engrafting of Human Glial Cells

(A) Comparison of field EPSPs (fEPSPs) in humanized chimeric mice and their unengrafted littermate and mouse GPC allografted controls. The slopes of fEPSP were significantly increased in human chimeric mice. (n = 3–40, F = 3.15, by two-way ANOVA with Bonferroni post hoc t test, *p < 0.05).

(B) Induction of LTP by two trains of high-frequency stimulation (each train consisted of 100 pulses at 100 Hz, with 30 s between bursts) in human chimeric mice, but not in unengrafted littermates and allografted mice. (n = 7 mice each group, *p < 0.05, t test compared between before and 60 min after the stimulation for each group.)

(C) Relative decreased percentage of fEPSP by addition of NMDA receptor antagonist APV (50 μM) in each group (n = 15–27).

(D) The adenosine A1 receptor antagonist DPCPX failed to increase the fEPSP slope in unengrafted rag2 controls (100 nM DPCPX, n = 8, p > 0.05, Bonferroni test).

(E) The adenosine A1 receptor antagonist DPCPX did not decrease the threshold for induction of LTP in unengrafted controls; the fEPSP slope returned to 101.9% ± 3.6% by 60 min after HFS, similar to the rate of extinction in untreated slices (n = 8, t test).

Data graphed as means ± SEM. See also Figure S3.

different from the fEPSP slope prior to HFS, p = 0.169). Mouse allografted controls exhibited an initial increase to 138.5% ± 2.3%, which fell to 103.8% ± 1.3% at 60 min (not significantly different from the fEPSP slope prior to HFS, n = 7, 14.0 ± 0.1 months old, p = 0.29, t test) (Figure 4B). Thus, the observed enhancement of LTP was a specific feature of human glial chimerization, and was not attributable to cell engraftment per se.

The enhancement of LTP can result from both presynaptic and postsynaptic mechanisms. An analysis of paired-pulse facilitation before and after HFS in chimeric mice suggested that postsynaptic mechanisms most likely underlie the enhancement of fEPSP slope in humanized chimeric mice (Figures S3B and S3C). To evaluate the relative contribution of AMPA- and NMDA-receptor-mediated currents to the enhancement in LTP in the chimeric mice, we analyzed the effect of NMDA receptor blockade using the NMDA receptor antagonist APV. We found that the NMDA receptor component accounted for only 4.7%–12% of fEPSP, with no significant differences across the groups analyzed, indicating that NMDA NR1 expression was not increased in the human glial chimeras. These findings suggest that NMDA receptor activation played a minor role, if any, in the enhancement of synaptic plasticity in the chimeric mice (n = 15–27) (Figure 4C). Since NMDA receptors have a higher affinity for glutamate than do AMPA receptors (Malinow and Malenka, 2002), these observations also suggest that the potentiation of fEPSPs in human glial chimeric mice was not the result of increased synaptic release of glutamate; this is consistent with the lack of enhancement of paired-pulse suppression in the chimeric mice (Figures S3B and S3C).

Neither Adenosine nor D-Serine Accounted for the Enhancement of LTP by Human Glia

Several mechanisms exist by which astrocytes can modulate excitatory transmission. Astrocytes release ATP, which, after degradation to adenosine by extracellular ectonucleotidases, can suppress both basal synaptic transmission and activity-dependent increases in synaptic strength (Pascual et al., 2005; Zhang et al., 2003). However, it seems unlikely that adenosine contributed to the enhanced synaptic strength observed in the xenografted mice. The A1 receptor antagonist 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) (Grover and Teyler, 1993; Wu and Saggau, 1994) did not decrease the threshold for induction of LTP in control mice; in slices exposed to 100 nM DPCPX, the fEPSP slope returned to 101.9% ± 3.6% 60 min after HFS, similar to untreated slices (Figures 4D and 4E). Thus, it is unlikely that the reduced threshold for LTP in chimeric mice was a consequence of altered adenosine concentrations.

Astrocytes can also modulate excitatory transmission via their release of D-serine (Panatier et al., 2006; Yang et al., 2003). D-serine acts as an endogenous coagonist of NMDA receptors and facilitates NMDA receptor activation, thereby potentiating the insertion of additional AMPA receptors into the postsynaptic membrane (Panatier et al., 2006; Yang et al., 2003). We tested the effect of adding D-serine to the bath of slices prepared from control mice. D-serine had no effects on the fEPSP slopes in accordance with previous reports (p = 0.216, n = 6) (Panatier et al., 2006; Yang et al., 2003). Moreover, neither D-serine nor immunolabeling for its synthetic enzyme, serine racemase, differed between human glial chimeric and uninjected control mice (Figures S3D and S3E). These observations suggest that the lower threshold for induction of LTP in human glial chimerics

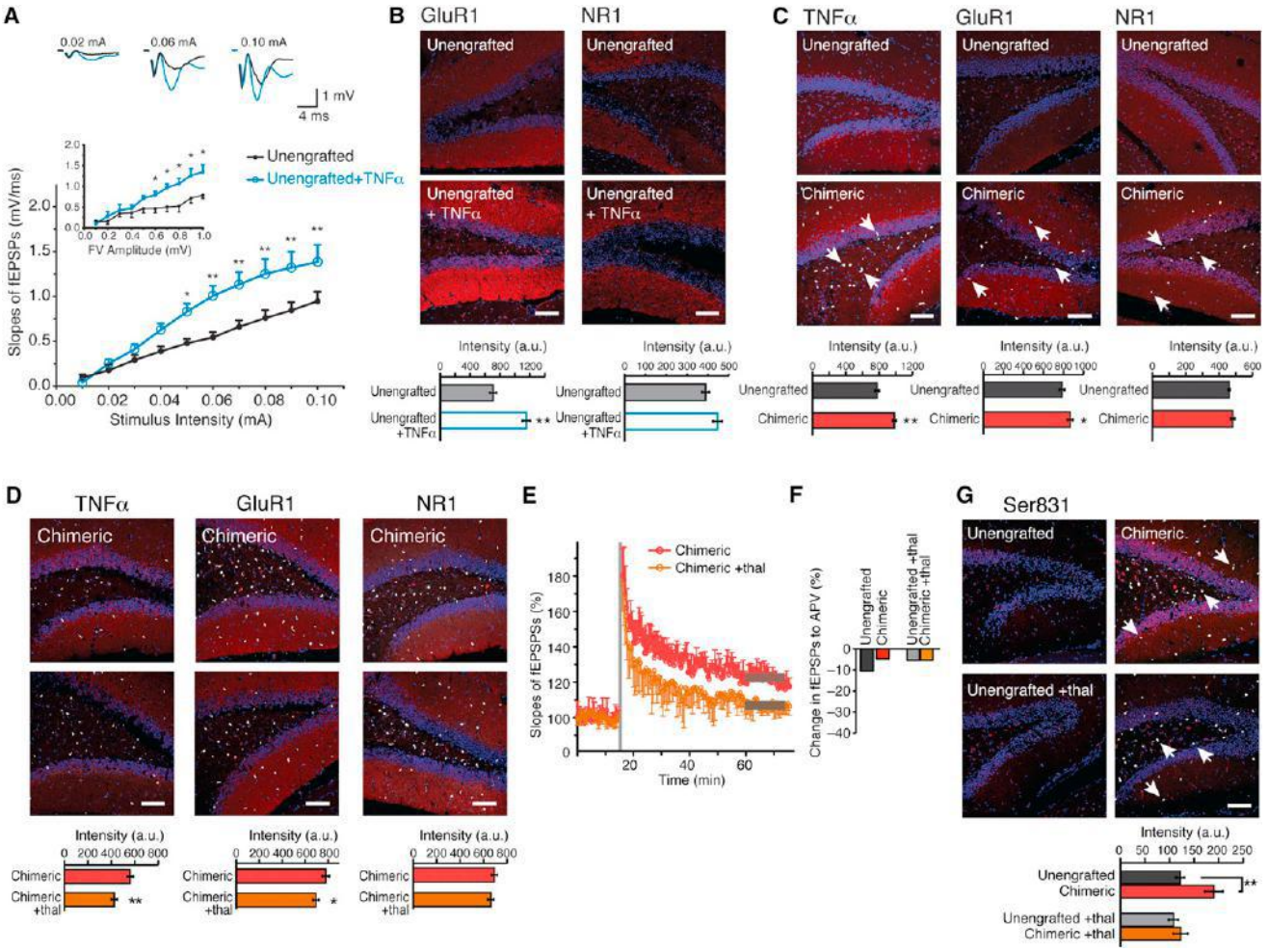


Figure 5. Astrocytic TNFα Contributes to LTP Facilitation in Chimeric Mice, which Is Attenuated by Thalidomide

(A) Hippocampal slices prepared from littermate control immunodeficient mice exhibited a potentiation of fEPSP in response to TNFα (n = 6, 12–16 months, *p < 0.05, **p < 0.01, Bonferroni post hoc t test). Inset: fEPSP slopes plotted as a function of fiber volley amplitude.

(B) Hippocampal slices exposed to TNFα (600 nM; 2–4 hr) exhibited an increase in the intensity of the GluR1 subunit of AMPA receptors as seen via immunolabeling, but not in that of the NR1 subunit of NMDA receptors (n = 5, 9–11 months, **p < 0.01, t test).

(C) Human chimeric mice exhibited a higher intensity of immunolabeling for TNFα and GluR1, but not for NR1 (n = 7, 7–20 months, *p < 0.05, **p < 0.01, t test). (hNuclei, white; representative human cells, white arrows).

(D) Thalidomide also decreased the immunolabeling of TNFα and GluR1, but not that of NR1, in chimeric mice (hNuclei, white; n = 6, 12–16 months, *p < 0.05, **p < 0.01, t test).

(E) The facilitation of LTP in chimeric mice was impaired by thalidomide (n = 6, 12.6 ± 0.3 versus 12.5 ± 0.5 months old, respectively, means ± SEM, p < 0.05, t test).

(F) Thalidomide did not change the contribution of NMDA receptor activation to fEPSP. Recordings of fEPSPs were obtained before and after addition of the NMDA receptor antagonist APV (50 μM), and the difference was calculated (n = 4).

(G) Phosphorylation of the Ser831 site of GluR1 was increased in chimeric mice compared with unengrafted littermate controls. Thalidomide attenuated the increase in phosphorylation of the Ser831 site of GluR1, but had no effect in unengrafted littermate controls, white arrows shows $hNuclei^{+}$ cells. (n = 6, 9–16 months, *p < 0.05, t test).

Scale bars: 100 μm (B, C, D, and G). All data are graphed as means ± SEM. See also Figure S4.

was not a consequence of altered adenosine tone or increased glial release of D-serine.

Human Glial TNFα Potentiates Synaptic Transmission via an Increase in GluR1 Receptors

Release of the cytokine TNFα comprises an alternative mechanism by which glia might modulate LTP. Cultured astrocytes constitutively release TNFα, which induces the addition of AMPA receptors to neuronal membranes, thereby enhancing excitatory synaptic transmission (Beattie et al., 2002; Stellwagen and Malenka, 2006). To assess the involvement of TNFα in the strengthening of excitatory transmission in the human glial chimeric mice, we first confirmed that TNFα increased both AMPA receptor current (Figure 5A) and AMPA GluR1 immunolabeling in hippocampal slices (Figure 5B). In contrast, TNFα did not affect expression of the NMDA receptor NR1 subunit in the same slices (Figure 5B). On that basis, we next asked whether chimeric mice expressed human TNFα. Using qPCR

we found that human-specific sequence encoding TNFα was indeed highly expressed in the chimeras, yet undetectable in unengrafted mice (Figure S4A). Immunolabeling confirmed that the human glial chimeras exhibited significant increases in both TNFα and GluR1, but not in NR1 (Figure 5C). We thus asked whether the inhibition of TNFα production might suppress excitatory hippocampal transmission in chimeric mice, and if so, whether TNFα inhibition might abrogate the effects of human glial chimerization on LTP.

Previous studies have analyzed the effect of TNFα on excitatory transmission in vitro by adding soluble TNFR1 receptors to scavenge free TNFα (Beattie et al., 2002; Stellwagen and Malenka, 2006). Since soluble TNFR1 would not be expected to be an efficient inhibitor in vivo, we instead administered thalidomide, a potent, BBB permeable inhibitor of TNFα production (Ryu and McLarnon, 2008). Human glial chimeric mice treated with thalidomide exhibited a significant suppression of fEPSP slopes compared to those receiving vehicle (0.5% carboxymethylcellulose) (1.41 ± 0.15 mV/ms versus 1.05 ± 0.24 mV/ms at 0.1 mA, means ± SEM, $p < 0.05$, $n = 12$). In contrast, excitatory transmission in unengrafted littermates was unaffected by thalidomide (1.02 ± 0.12 mV/ms versus 0.97 ± 0.20 mV/ms at 0.1 mA, $p = 0.32$, $n = 12$). These observations suggested that thalidomide selectively targeted the potentiation of excitatory transmission mediated by human glial TNFα. Accordingly, thalidomide also reduced the expression of both TNFα and GluR1 in the human glial chimeras, but not that of NR1 (Figure 5D). Importantly, thalidomide also prevented the facilitation of LTP in the human glial chimeras: two trains of HFS failed to trigger LTP in slices taken from chimeras pretreated with thalidomide (106.3% ± 3.9%, $n = 6$, 12.6 ± 0.3 months of age), whereas the activity-dependent potentiation of fEPSPs persisted in vehicle-treated human glial chimeras (117.6% ± 4.8%, $n = 6$, 12.5 ± 0.5 months, $p < 0.05$, t test) (Figure 5E). Thalidomide did not alter the number of NMDA receptors activated in response to medial perforant-path fiber stimulation in either chimeric or unengrafted controls, suggesting that thalidomide specifically suppressed the number of functional AMPA receptors consistent with prior publications showing that TNFα drives membrane insertion of AMPA receptors (Figure 5F) (Beattie et al., 2002; Stellwagen and Malenka, 2006). Thus, TNFα released by human glial cells (Figure S4A, Figure 5C) enhanced host neuronal fEPSPs by increasing the number of functional postsynaptic GluR1 AMPA receptors (Figure 5C), and conversely, thalidomide suppressed plasma membrane insertion of AMPA receptors, but not NMDA receptors, by inhibiting TNFα production (Figure 5D).

TNFα regulates a number of cellular processes through protein kinase C (PKC)-mediated phosphorylation (Faurschou and Gniadecki, 2008), which is thus disrupted by thalidomide. Since phosphorylation of GluR1, at sites critical for its synaptic delivery, is both necessary and sufficient for lowering the threshold for inducing LTP (Hu et al., 2007), we thus next asked if the phosphorylation state of the GluR1 subunit differed between human glial chimeric mice and their littermate controls. We focused on two phosphorylation sites, Ser845 (PKA site) and Ser831 (PKC/CaMKII site), each of which is critical for the synaptic insertion of GluR1 (Hu et al., 2007), and assessed the effects upon each of human glial chimerization and of thalidomide. Quantitative immunohistochemistry revealed that human glial chimeric mice exhibited a significant increase in Ser831 phosphorylation, the PKC-sensitive site ($n = 6$, $p = 0.008$, t test); this was significantly attenuated in human glial chimeras receiving thalidomide, but not in their unengrafted control littermates ($n = 9–10$; $p > 0.4$, t test) (Figure 5G). In contrast, phosphorylation of the Ser845 PKA site was unaffected either by the engraftment of human glia or by thalidomide ($n = 6$, $p > 0.05$, t test) (Figures S4B and S4C). Together, these results suggested that human glia facilitate synaptic insertion of the GluR1 subunit in host murine neurons through a TNFα-dependent, PKC/CaMKII-mediated pathway, which lowers the threshold for induction of LTP in human glial chimeric mice.

Enhanced Learning in Humanized Chimeric Mice

Stable, long-lasting changes in synaptic function, such as those revealed by our LTP studies, are thought to be involved in learning and memory (Lee and Silva, 2009). Since LTP was markedly enhanced in human glial chimeric mice, we next asked if these mice also exhibited improved learning. We first assessed whether auditory fear conditioning (AFC)—a task in which the mice learn to fear an innocuous tone by pairing it with foot shock (Zhou et al., 2009), and which does not require visual input (*rag2*$^{-/-}$ mice are blind)—was potentiated in the human glial chimeras. To this end, we compared the rate of acquisition of AFC in xenografted human glial chimeras to that of both allografted murine glial chimeras and unengrafted littermate controls (Figure 6A). The allografted mice—which were also generated in immunodeficient rag2 null hosts—received neonatal grafts of A2B5$^+$ cells isolated from transgenic mice with constitutive EGFP expression, which allowed us to readily identify murine donor cells. After just a single pairing of the tone with foot shock, the human glial chimeric mice exhibited a significant enhancement in learning of the tone foot shock association: they showed greater fear to the tone as measured by scoring freezing behavior (the cessation of all movement except for respiration) than did either allografted chimeras or unengrafted controls ($n = 5–20$, 9.6 ± 1.0 months old, $F = 18.9$, two-way repeated-measures ANOVA, $p < 0.001$). Moreover, after 3 continuous days of training, humanized chimeric mice also showed enhanced AFC during the 3 remaining days of testing, as manifested by their higher levels of freezing in response to the conditioned tone ($p < 0.01$, post hoc Bonferroni test). In contrast, neither murine glial chimeric mice nor unengrafted controls manifested any increase in freezing behavior during the same period, despite having been subjected to an identical fear conditioning paradigm ($p > 0.05$; Bonferroni test) (Figure 6A). Of note, no differences were observed between the human glial chimeras and their controls in the reaction to foot shock ($n = 5$, 9.6 ± 1.0 months old, $F = 0.08$ by two-way ANOVA, $p > 0.5$) (Figure S5A), suggesting that their respective nociceptive thresholds were analogous.

To specifically assess hippocampus-dependent learning, we next prepared chimeric mice using rag1 immunodeficient mice (maintained on a C57/Bl6 background), which differ from their rag2 null counterparts (on a C3h background) by having normal vision. We first compared the net engraftment of human GPCs, as well as their relative differentiation into hNG2$^+$ GPCs or GFAP$^+$ astroglia, in human glial chimeras established in rag1 null and rag2 null mice. We focused on hippocampal learning, as this region was used for our analysis of LTP (Lee and Silva,

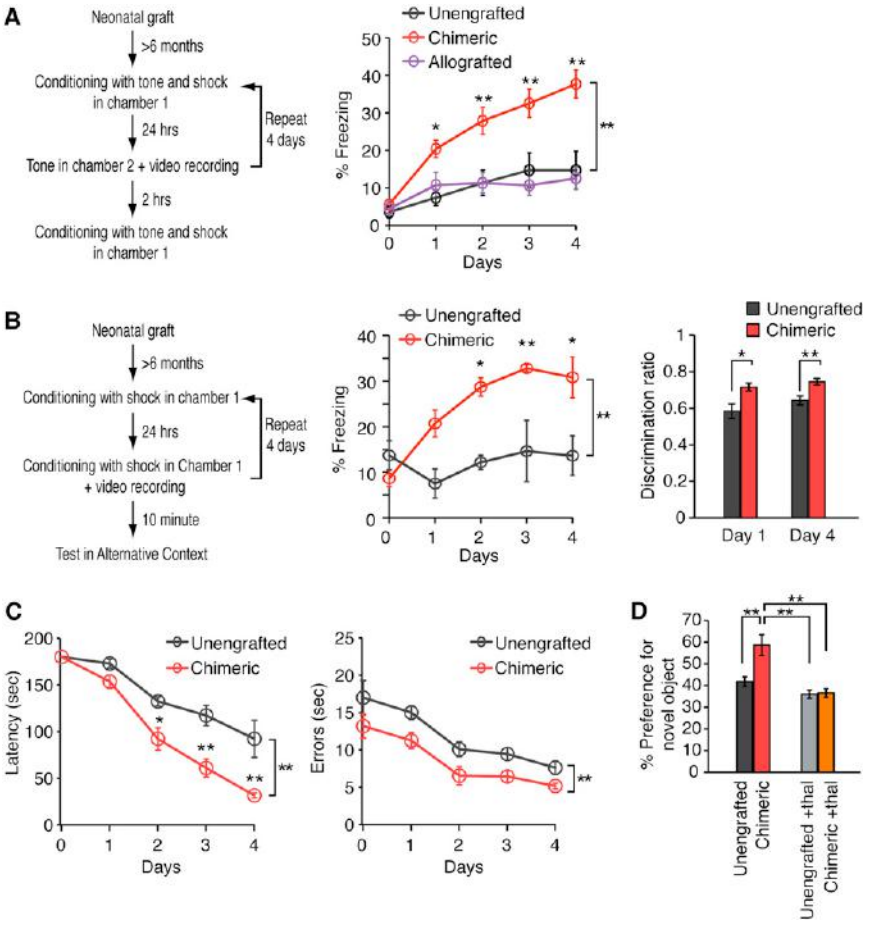


Figure 6. Humanized Chimeric Mice Learn Faster than Controls

(A) Auditory fear conditioning assessed in a cohort of human chimeric, mouse chimeric, and unengrafted control rag2 null mice. Chimeric mice exhibit prolonged freezing behavior in test chamber 2 during exposure to the tonal conditioned stimulus when compared to unengrafted mice and allografted mice (n = 5–20, *p < 0.05, **p < 0.01, two-way repeated-measures ANOVA with Bonferroni test, means ± SEM). This difference persisted throughout all 4 days.

(B) Contextual fear conditioning in human glial chimeric mice and littermate control rag1 null mice. Freezing behavior was quantified for chimeric and unengrafted littermate controls during the 2 min acclimatization period (n = 6, *p < 0.05, **p < 0.01, two-way repeated-measures ANOVA with Bonferroni test). In addition the mean discrimination ratio for each day was obtained from freezing scores in the training chamber and the alternative chamber (freezing in training chamber/total freezing time). Chimeric mice demonstrated significantly greater abilities to discriminate the chambers (n = 8–13, *p < 0.05, **p < 0.01, two-way repeated-measures ANOVA with Bonferroni test).

(C) Barnes maze testing in chimeric and unengrafted rag1 null littermate controls. Chimeric mice demonstrated a significant learning advantage, as reflected in a shorter latency and fewer errors in solving the maze (n = 6, *p < 0.05, **p < 0.01, two-way repeated-measures ANOVA with Bonferroni test).

(D) Object-Location Memory Task (OLT) in chimeric mice and their unengrafted rag1 null littermate controls demonstrated a learning advantage in chimeric mice via enhanced recognition of the novel displaced object. Thalidomide eliminated the learning advantage of chimeric mice, suggesting that the learning enhancement was TNFα mediated (n = 7, **p < 0.01, one-way ANOVA with Bonferroni test).

All data are plotted as means ± SEM. See also Figure S5.

2009; Manns and Eichenbaum, 2009). We found that both the engraftment and differentiation of human GPCs in rag1 and rag2 immunodeficient mice were indistinguishable from one another (Figures S5B and S5C). On that basis, we next assessed the effect of chimerization of rag1 null immunodeficient mice on contextual fear conditioning (CFC), a hippocampal-dependent task in which mice learn to fear a context in which they receive a foot shock (Fanselow and Poulos, 2005). The human glial-chimeric mice exhibited enhanced performance in CFC throughout all 4 days of training (Figure 6B). By just the second day, the human glial chimeric mice exhibited substantially more rapid and robust CFC than their nonchimeric littermate controls (n = 6, 6.9 ± 0.1 months of age, F = 14.8 by two-way repeated-measures ANOVA, p = 0.003), and continued to display enhanced CFC during the subsequent 2 days of CFC training (Figure 6B). To exclude the possibility that a generalized increase in freezing behavior could explain the observed differences, we also examined the context specificity of freezing responses. In these experiments, the mice were placed in a second chamber with a different floor and odor. Chimeric mice exhibited superior discrimination between the two contexts, suggesting stronger contextual learning, as opposed to a nonspecific higher level of fear (n = 8–13, 7.6 ± 0.1 months, p < 0.05, t test) (Figure 6B). No differences were observed in the reaction times to foot shock between the human glial chimeras and their rag1 null immunodeficient controls (n = 5, 9.6 ± 0.95 months, F = 0.08 by two-way ANOVA, p > 0.5) (Figure S5A). Moreover, neither thermal nor mechanical sensitivity were affected by chimerization of either rag1 or rag2 mice (Figures S5D and S5E), suggesting that their respective nociceptive thresholds were analogous.

To better assess the scope of performance enhancement in the human glial chimeras, we next assessed their performance in the Barnes maze, another hippocampal-dependent learning task. In this spatial learning task mice learn the location of a hole that leads to an escape/drop box. By just the second day of serial daily testing, the human glial chimeras made fewer errors and displayed a significantly shorter latency in finding the drop box compared to their littermate controls (n = 6, 7.4 ± 0.1 months, F = 13.4 by two-way repeated-measures ANOVA, p = 0.004) (Figure 6C). These differences persisted throughout the four-trial testing period (n = 6, 7.4 ± 0.1 months, F = 11.4, p = 0.007). With additional training, the unengrafted control mice were capable of completing the task, indicating

that they could master the task if given sufficient training (Figure S5F).

Next, we tested the mice in the Object-Location Memory Task (OLT), another hippocampal-dependent task (Manns and Eichenbaum, 2009). OLT tests the ability of the animal to recognize a familiar object in a novel location. Chimeric mice exhibited a substantially greater preference for objects in novel locations than their controls (58.6% ± 4.8% versus 41.8% ± 2.3%, means ± SEM, n = 7, 7.2 ± 0.1 months, p = 0.008, t test) (Figure 6D). Thalidomide treatment did not affect appreciably the performance of the unengrafted littermate controls on the OLT, but reduced the performance of the human glial chimeric mice to the levels of controls (n = 7, 7.8 ± 0.1 months, p = 0.82, t test) (Figure 6D). Thus, thalidomide selectively abrogated the chimerization-associated performance enhancement of the human glial chimeras.

Together, these results indicate that relative to either unengrafted mice or mice allografted with A2B5$^+$-sorted, EGFP$^+$ murine GPCs, human glial chimeric mice exhibit enhanced performance in four different learning tasks: AFC, CFC, Barnes maze, and novel object location. Moreover, the analysis of AFC indicates that alloengraftment by mouse GPCs did not affect the learning of the recipient mice, supporting the notion that the improved learning in the humanized chimeras resulted from the presence of human glia, rather than from cell engraftment per se. As an additional control, we also noted that social interactions did not differ between human chimeras generated by engraftment in rag1 mice and their littermate controls (n = 5, 6.9 ± 0.1 months, p > 0.05, t test) (Figures S5G and S5H), indicating that chimerization did not seen to affect their interactions with other mice.

DISCUSSION

Prior studies have documented that astrocytes regulate synaptic transmission and actively participate in the synaptic efficiency of neural circuits in the rodent CNS (Fields, 2004; Nedergaard and Verkhratsky, 2012; Parpura and Verkhratsky, 2012; Rusakov et al., 2011). A parallel, hitherto nonoverlapping line of work has shown that human astrocytes are larger and far more structurally complex than those of rodents (Colombo, 1996; Oberheim et al., 2009); this has led to the hypothesis that astrocytic evolution has been critical to the increased scope and capacity of central neural processing that have attended hominid evolution (Colombo, 1996; Oberheim et al., 2006, 2012). In support of this hypothesis, genomic studies have revealed that the greatest differences in brain gene expression between humans and mice are in glial transcripts (Miller et al., 2010).

In this study, we created human glial chimeric mice, in which immunodeficient but otherwise normal mice were engrafted neonatally with large numbers of human glial progenitors, resulting in the widespread integration of human glia into the mouse brain. By the time these mice reached adulthood, a large proportion of their forebrain glia were replaced by human cells. The chimerization was slowly progressive, so that extensive infiltration of cortex and hippocampus by human cells was evident by 4–12 months (Figure 1). The xenografted human cells remained as NG2-defined glial progenitor cells or differentiated as hGFAP$^+$ astrocytes; remarkably, the latter maintained their characteristic, large, and complex hominid-selective morphologies (Figure 2). In addition, some assumed the characteristic long-distance fiber extensions of interlaminar astrocytes, a domain-traversing astrocytic phenotype specific to the hominid brain (Colombo, 2001; Colombo et al., 1995; Oberheim et al., 2006). Electrophysiological analysis validated that most EGFP$^+$/hGFAP$^+$/hNuclei$^+$ human glia were protoplasmic astrocytes, based on their low input resistance, passive membrane properties, extensive gap junction coupling, and Ca^{2+} wave propagation (Figure 3).

The striking population of the recipient mouse brains by human glia raised the possibility that the engrafted human cells might significantly modulate information processing within the host murine neural networks. Indeed, the basal level of excitatory synaptic transmission was increased over a wide range of stimulation intensities. The presence of human glia also enhanced LTP in human glial chimeric hippocampal slices relative to mice that had received conspecific murine glial progenitors or vehicle injection (Figure 4). Our analysis showed that TNFα was significantly elevated in the human glial chimeric brains, consistent with the potentiation of AMPA-receptor-mediated currents (Beattie et al., 2002; Stellwagen and Malenka, 2006). Additional analysis suggested that TNFα may have directly facilitated insertion of the GluR1 subunit into the plasma membrane (Hu et al., 2007), perhaps via its increased phosphorylation at Ser831. TNFα might also potentiate astrocytic glutamate release (Ni and Parpura, 2009; Parpura and Zorec, 2010), which in turn could increase GluR1 subunit phosphorylation by NMDA-receptor-mediated activation of PKC (Figure 5) (Malinow and Malenka, 2002). Both of these pathways might have contributed to the enhancement of hippocampal LTP that we observed in the human glial chimeras, although we found no evidence of enhancement of NMDA receptor activation after engraftment (Figure 4C). Importantly, we found that thalidomide, a BBB-permeable inhibitor of TNFα, both diminished the enhancement of postsynaptic AMPA receptor current and reduced LTP in chimeric mice, yet had no such effects in unengrafted littermate controls. Behavioral analyses then revealed that human glial chimeric mice exhibited improved learning and memory in four different tasks, including AFC, CFC, the Barnes Maze, and OLT (Figure 6). As with the chimerization-associated enhancement in LTP, the enhanced learning of chimeric mice in the object location recognition assay was eliminated by thalidomide treatment (Figure 6D). Engraftment by neonatally delivered mouse GPCs did not enhance LTP, AFC, or Barnes maze performance, strongly suggesting that the potentiation of synaptic plasticity and learning afforded by glial progenitor cell chimerization was specific to human glia, and not a product of cell engraftment per se (Figures 4B and 6A).

Together, these studies demonstrate that human astrocytes generated within the mouse brain maintain their complex phenotype in a cell-autonomous fashion; they assume morphologies and Ca^{2+} wave characteristics typical of the human brain, but, to our knowledge, hitherto never observed in experimental animals. These observations strongly support the notion that the evolution of human neural processing, and hence the species-specific aspects of human cognition, in part may reflect the course of astrocytic evolution (Oberheim et al., 2006). As such, these human glial chimeric mice may present a useful

experimental model by which human glial cells, and both normative and pathological species-specific aspects of human glial biology, may now be effectively studied in the live adult brain.

EXPERIMENTAL PROCEDURES

Isolation of Human and Murine Glial Progenitor Cells

Fetal glial cell progenitors were extracted from 17- to 22-week-old human fetuses obtained at abortion. The forebrain ventricular and subventricular zones were dissected free on ice and then were dissociated using papain/DNAase as described (Windrem et al., 2004). All samples were obtained with consent under approved protocols of the University of Rochester Research Subjects Review Board. Human glial progenitor cells were isolated by magnetic activated cell sorting, as described in the Supplemental Experimental Procedures. In addition, murine A2B5$^+$ cells were identically prepared from newborn Tg(CAG-EGFP)B5Nagy/J pups (Jackson Laboratory).

Transfection and Differentiation

Human A2B5$^+$/PSA-NCAM$^-$ cells were transfected to express enhanced EGFP, and were maintained as described in the Supplemental Experimental Procedures.

Transplantation

Human glial chimeras were prepared as described, using either *rag1*$^{-/-}$ or *rag2*$^{-/-}$ immunodeficient mice (as described in Windrem et al., 2008, though using mice wild-type for myelin); see Supplemental Experimental Procedures for additional detail. All experiments were approved by the University of Rochester's Research Animal Care and Use Committee.

Quantitative Immunohistochemistry

Chimeric mice and littermate controls (ranging from 2 weeks to 20 months, depending upon experimental endpoint) were perfusion-fixed, processed histologically, and analyzed as described in the Supplemental Experimental Procedures.

Electrophysiological Characterization of Human Glia in Chimeric Mice

Patch-clamp assessment of engrafted human glia was performed in slice preparations under two-photon microscopy, as detailed in the Supplemental Experimental Procedures.

LTP

Slice preparations of both chimeric mice and their littermate controls (with an age range of 7–20 months) were used for recordings of fEPSPs and analysis of activity-dependent changes in hippocampal synaptic strength, as outlined in the Supplemental Experimental Procedures.

Ca^{2+} Imaging and Photolysis of Caged Ca^{2+}

Chimeric mice (with an age range of 4–10 months) were used for imaging intracellular Ca^{2+} in xenografted human glia. In addition, as positive controls, surgical resections of human cortex (n = 3 patients, 30.6 ± 8.8 years old) were obtained with patient consent and the approval of the University of Rochester Research Subjects Review Board; all samples were prepared for physiological assessment and analyzed as described in the Supplemental Experimental Procedures.

Detection of Human TNFα in Human Glial Chimeras

RNA isolation, PCR primer design, reverse transcription, and PCR reaction conditions and analysis were all as described in the Supplemental Experimental Procedures.

Learning Tasks and Behavioral Assessment

AFC, CFC, Barnes maze navigation, object location memory, Crawley's social interaction tasks, and both thermal and mechanical sensitivity thresholds were assessed in human glial chimeric and control mice; the latter included allografted and/or unengrafted negative controls. All tests and analyses were performed as outlined in the Supplemental Experimental Procedures.

Statistics

All data are presented and graphed as means ± SEM. The Steel-Dwass test was used to assess the relative diameters of cells, input resistances, and Ca^{2+} velocities, all variables for which normality of the data could not be assumed. For other electrophysiological data, either Student's t test for two groups or two-way ANOVA with Bonferroni post hoc t tests were used. For behavioral data, Student's t test or two-way repeated-measures ANOVA with Bonferroni post hoc test were used. Normality of the data was assessed by the Shapiro-Wilk test. $p < 0.05$ was considered significant.

SUPPLEMENTAL INFORMATION

Supplemental Information for this article includes five figures and Supplemental Experimental Procedures and can be found with this article online at http://dx.doi.org/10.1016/j.stem.2012.12.015.

ACKNOWLEDGMENTS

This work was supported by the G. Harold and Leila Y. Mathers Charitable Foundation, the Dr. Miriam and Sheldon G. Adelson Medical Research Foundation, the National Multiple Sclerosis Society, NIMH, and NINDS. We thank R. Malenka for valuable comments on the manuscript. Drs. Goldman and Windrem have a patent on the generation and use of glial chimeric mice, entitled, "Non-human animals with human glial chimeric brains," US patent no. 7,524,491. They receive no income or financial consideration from this patent.

Received: December 28, 2010
Revised: November 21, 2012
Accepted: December 19, 2012
Published: March 7, 2013

REFERENCES

Beattie, E.C., Stellwagen, D., Morishita, W., Bresnahan, J.C., Ha, B.K., Von Zastrow, M., Beattie, M.S., and Malenka, R.C. (2002). Control of synaptic strength by glial TNFalpha. Science *295*, 2282–2285.

Colino, A., and Malenka, R.C. (1993). Mechanisms underlying induction of long-term potentiation in rat medial and lateral perforant paths in vitro. J. Neurophysiol. *69*, 1150–1159.

Colombo, J.A. (1996). Interlaminar astroglial processes in the cerebral cortex of adult monkeys but not of adult rats. Acta Anat. (Basel) *155*, 57–62.

Colombo, A.J. (2001). A columnar-supporting mode of astroglial architecture in the cerebral cortex of adult primates? Neurobiology (Bp.) *9*, 1–16.

Colombo, J.A., Yáñez, A., Puissant, V., and Lipina, S. (1995). Long, interlaminar astroglial cell processes in the cortex of adult monkeys. J. Neurosci. Res. *40*, 551–556.

Cotrina, M., and Nedergaard, M. (2005). Intracellular Calcium Control Mechanisms in Glia, Second Edition (New York: Oxford University Press).

Dawitz, J., Kroon, T., Hjorth, J.J., and Meredith, R.M. (2011). Functional calcium imaging in developing cortical networks. J. Vis. Exp. *56*, pii: 3550.

Fanselow, M.S., and Poulos, A.M. (2005). The neuroscience of mammalian associative learning. Annu. Rev. Psychol. *56*, 207–234.

Faurschou, A., and Gniadecki, R. (2008). TNF-alpha stimulates Akt by a distinct aPKC-dependent pathway in premalignant keratinocytes. Exp. Dermatol. *17*, 992–997.

Fields, R.D. (2004). The other half of the brain. Sci. Am. *290*, 54–61.

Grover, L.M., and Teyler, T.J. (1993). Role of adenosine in heterosynaptic, posttetanic depression in area CA1 of hippocampus. Neurosci. Lett. *154*, 39–42.

Hu, H., Real, E., Takamiya, K., Kang, M.G., Ledoux, J., Huganir, R.L., and Malinow, R. (2007). Emotion enhances learning via norepinephrine regulation of AMPA-receptor trafficking. Cell *131*, 160–173.

Kang, S.H., Fukaya, M., Yang, J.K., Rothstein, J.D., and Bergles, D.E. (2010). NG2+ CNS glial progenitors remain committed to the oligodendrocyte lineage in postnatal life and following neurodegeneration. Neuron *68*, 668–681.

Lee, Y.-S., and Silva, A.J. (2009). The molecular and cellular biology of enhanced cognition. Nat. Rev. Neurosci. *10*, 126–140.

Malinow, R., and Malenka, R.C. (2002). AMPA receptor trafficking and synaptic plasticity. Annu. Rev. Neurosci. *25*, 103–126.

Mangin, J.M., and Gallo, V. (2011). The curious case of NG2 cells: transient trend or game changer? ASN Neuro *3*, e00052.

Manns, J.R., and Eichenbaum, H. (2009). A cognitive map for object memory in the hippocampus. Learn. Mem. *16*, 616–624.

Miller, J.A., Horvath, S., and Geschwind, D.H. (2010). Divergence of human and mouse brain transcriptome highlights Alzheimer disease pathways. Proc. Natl. Acad. Sci. USA *107*, 12698–12703.

Nedergaard, M., and Verkhratsky, A. (2012). Artifact versus reality—how astrocytes contribute to synaptic events. Glia *60*, 1013–1023.

Ni, Y., and Parpura, V. (2009). Dual regulation of Ca2+-dependent glutamate release from astrocytes: vesicular glutamate transporters and cytosolic glutamate levels. Glia *57*, 1296–1305.

Oberheim, N.A., Wang, X., Goldman, S., and Nedergaard, M. (2006). Astrocytic complexity distinguishes the human brain. Trends Neurosci. *29*, 547–553.

Oberheim, N.A., Takano, T., Han, X., He, W., Lin, J.H., Wang, F., Xu, Q., Wyatt, J.D., Pilcher, W., Ojemann, J.G., et al. (2009). Uniquely hominid features of adult human astrocytes. J. Neurosci. *29*, 3276–3287.

Oberheim, N.A., Goldman, S.A., and Nedergaard, M. (2012). Heterogeneity of astrocytic form and function. Methods Mol. Biol. *814*, 23–45.

Panatier, A., Theodosis, D.T., Mothet, J.P., Touquet, B., Pollegioni, L., Poulain, D.A., and Oliet, S.H. (2006). Glia-derived D-serine controls NMDA receptor activity and synaptic memory. Cell *125*, 775–784.

Parpura, V., and Verkhratsky, A. (2012). The astrocyte excitability brief: From receptors to gliotransmission. Neurochem. Int. *61*, 610–621.

Parpura, V., and Zorec, R. (2010). Gliotransmission: Exocytotic release from astrocytes. Brain Res. Brain Res. Rev. *63*, 83–92.

Pascual, O., Casper, K.B., Kubera, C., Zhang, J., Revilla-Sanchez, R., Sul, J.Y., Takano, H., Moss, S.J., McCarthy, K., and Haydon, P.G. (2005). Astrocytic purinergic signaling coordinates synaptic networks. Science *310*, 113–116.

Robel, S., Berninger, B., and Götz, M. (2011). The stem cell potential of glia: lessons from reactive gliosis. Nat. Rev. Neurosci. *12*, 88–104.

Rusakov, D.A., Zheng, K., and Henneberger, C. (2011). Astrocytes as regulators of synaptic function: a quest for the Ca2+ master key. Neuroscientist *17*, 513–523.

Ryu, J.K., and McLarnon, J.G. (2008). Thalidomide inhibition of perturbed vasculature and glial-derived tumor necrosis factor-alpha in an animal model of inflamed Alzheimer's disease brain. Neurobiol. Dis. *29*, 254–266.

Stellwagen, D., and Malenka, R.C. (2006). Synaptic scaling mediated by glial TNF-alpha. Nature *440*, 1054–1059.

Verkhratsky, A., Orkand, R.K., and Kettenmann, H. (1998). Glial calcium: homeostasis and signaling function. Physiol. Rev. *78*, 99–141.

Windrem, M.S., Moyle, C., Chandler-Militello, D., Han, X., Nedergaard, M., Wang, S., and Goldman, S. (2009). Neonatally-implanted human glial progenitor cells out-compete endogenous glial progenitor cells to generate fully humanized glial-chimeric mouse brains. Program No 12621/A48 Neuroscience Meeting Chicago, IL: Society for Neuroscience Online.

Windrem, M.S., Nunes, M.C., Rashbaum, W.K., Schwartz, T.H., Goodman, R.A., McKhann, G., 2nd, Roy, N.S., and Goldman, S.A. (2004). Fetal and adult human oligodendrocyte progenitor cell isolates myelinate the congenitally dysmyelinated brain. Nat. Med. *10*, 93–97.

Windrem, M.S., Schanz, S.J., Guo, M., Tian, G.F., Washco, V., Stanwood, N., Rasband, M., Roy, N.S., Nedergaard, M., Havton, L.A., et al. (2008). Neonatal chimerization with human glial progenitor cells can both remyelinate and rescue the otherwise lethally hypomyelinated shiverer mouse. Cell Stem Cell *2*, 553–565.

Wu, L.G., and Saggau, P. (1994). Adenosine inhibits evoked synaptic transmission primarily by reducing presynaptic calcium influx in area CA1 of hippocampus. Neuron *12*, 1139–1148.

Yang, Y., Ge, W., Chen, Y., Zhang, Z., Shen, W., Wu, C., Poo, M., and Duan, S. (2003). Contribution of astrocytes to hippocampal long-term potentiation through release of D-serine. Proc. Natl. Acad. Sci. USA *100*, 15194–15199.

Zhang, J.M., Wang, H.K., Ye, C.Q., Ge, W., Chen, Y., Jiang, Z.L., Wu, C.P., Poo, M.M., and Duan, S. (2003). ATP released by astrocytes mediates glutamatergic activity-dependent heterosynaptic suppression. Neuron *40*, 971–982.

Zhou, Y., Won, J., Karlsson, M.G., Zhou, M., Rogerson, T., Balaji, J., Neve, R., Poirazi, P., and Silva, A.J. (2009). CREB regulates excitability and the allocation of memory to subsets of neurons in the amygdala. Nat. Neurosci. *12*, 1438–1443.

Blurton-Jones et al.,

Presentation,

Restoration of memory in mouse models of Alzheimer's disease and neuronal loss;

a new paradigm using human neural stem cell transplantation (July 17, 2012)

P3-341 EFFECTS OF TETRAHYDROXYSTILBENE GLUCOSIDE ON SYNAPSES AND ALPHA-SYNUCLEIN IN BRAINS OF AGED MICE

Lin Li[1], Funling Sun[2], Ruyi Zhang[2], Yu-qin Wang[2], *[1]Department of Pharmacology, Xuanwu Hospital of Capital Medical University, Beijing, China; [2]Xuanwu Hospital, Beijing, China.*

Background: Synaptic dysfunction and alpha-synuclein play important roles in the progression of neurodegenerative diseases such as Alzheimer's disease (AD), Parkinson's disease dementia (PDD) and dementia with Lewy bodies (DLB). 2,3,5,4'-Tetrahydroxystilbene glucoside (TSG) is a main component extracted from Polygonum multiflorum. The aim of this study was to investigate the effects of TSG on synapses and its possible mechanisms in the brain of aged mice for the treatment of age-related neurodegenerative diseases. **Methods:** TSG (50, 100 and 200mg/kg) or vehicle was intragastrically administered daily to 18-month-old C57BL mice for 3 months. The learning and memory ability was detected by Morris water maze test and step-through task. The movement ability was measured by the rotorod, pole test and locomotor activity tests. The synaptic ultrastructure was detected by electron microscopy. The expression of synaptic proteins and phosphorylated CaMKII was measured by immunoblotting. **Results:** (1) TSG decreased the escape latency in Morris water maze test, and extended latency and reduced error times in step-through task, dementrating that TSG improved the learning and memory ability in aged mice (21 months old). (2) TSG extended the time on rotorod, and shortened the turn-time and the down-time when climbing down from the pole, indicating that TSG improved movement function in aged mice. (3) TSG effectively protected synaptic ultrastructure of the hippocampal CA1 area, increased postsynaptic density length in striatum, increased the number of synaptic appositional zone area in hippocampus and striatum, and protected the mitochondrial ultrastructure in aged mice. (4) TSG enhanced the expression of phosphorylated CaMKII, synaptophysin, phosphorylated synapsin I and PSD-95, and reduced the expression of synaptotagmin I in the hippocampus, cortex and striatum of aged mice, consequently improved the synaptic plasticity. (5) TSG inhibited the overexpression and aggregation of alpha-synuclein in the hippocampus, cortex and striatum of aged mice, thus improved the abnormity of alpha-synuclein. **Conclusions:** TSG improved both learning-memory ability and movement ability in aged mice, through protecting synaptic structure and functions and inhibiting the overexpression and aggregation of alpha-synuclein. The results suggest that TSG may have a promising prospect in treatment of neurodegenerative diseases such as AD, DLB and PDD.

P3-342 INTRANASAL DELIVERY OF BIOACTIVE POLYPHENOL METABOLITES TO PREVENT AND/OR TREAT ALZHEIMER'S DISEASE AND OTHER FORMS OF DEMENTIA

Lindsay Knable[1], Prashant Vempati[1], Daniel Freire[1], Mario Ferruzzi[2], Giulio Pasinetti[1], *[1]Mount Sinai School of Medicine, New York, New York, United States; [2]Purdue University, West Lafayette, Indiana, United States.*

Background: While polyphenolic compounds have many health benefits, the potential development of polyphenols for the prevention/treatment of neurological disorders is largely hindered by their complexity and limited knowledge regarding their bioavailability, metabolism and bioactivity in the brain. We recently demonstrated that dietary supplementation with a grape-derived polyphenolic preparation, namely a monomeric-enriched catechin and epicatechin fraction (Mo), significantly improves cognitive function in a mouse model of Alzheimer's disease (AD). We also found that Mo treatment resulted in the accumulation of proanthocyanidin metabolites in the brain at a concentration of ~400 nM. One of the metabolites identified in the brain following Mo treatment, Metaphenol-A1, was shown to promote basal synaptic transmission and long-term potentiation (LTP) at physiologically relevant concentrations in hippocampal slices through mechanisms associated with cAMP-response-element-binding-protein signaling. **Methods:** C57BL/6 mice were treated with Metaphenol-A1 (7.5 μM) by intranasal route. Brain sections were then harvested at 5, 10, 15, and 60 minutes. Pharmacokinetics and neuronal molecular changes were assessed in the samples collected. The same delivery approach will be applied to the Tg2576 mouse model of AD to assess its effect on cognitive function. **Results:** We are currently evaluating the pharmacokinetics and brain bioavailability of Metaphenol-A1 delivered via a novel, non-invasive intranasal delivery apparatus. We will also asses the effects of Metaphenol-A1 delivered in this manner on LTP and cognitive function in AD mice. **Conclusions:** Our study will provide insights into developing a novel, safe approach to directly deliver a bioactive therapeutic agent to the central nervous system for AD prevention/treatment.

P3-344 CATHEPSIN B GENE DELETION AND CYSTEINE PROTEASE INHIBITION ARE EFFECTIVE IN A TRAUMATIC BRAIN INJURY MODEL

Mark Kindy[1], Jin Yu[1], Michael Pierschbacher[2], Nancy Sipes[2], Greg Hook[2], *[1]MUSC, Charleston, South Carolina, United States; [2]ALSP, San Diego, California, United States.*

Background: Data suggest that the there are common pathological mechanisms in neurodegeneration caused by Alzheimer's disease (AD) and by traumatic brain injury (TBI). For example, both AD and TBI result in elevated brain activity of the cysteine potease, cathepsin B (CatB). To explore further the commonality of CatB activity in AD and TBI pathology, we extended our previous studies, which showed that CatB is an AD drug target and that cysteine protease inhibitors are effective AD treatments, by studying the effects of deleting the CatB gene or treatment with a cysteine protease inhibitor in a TBI animal model on behavior and pathology. **Methods:** An open skull, single traumatic injury model of TBI was used. Briefly, mice were anesthetized, the skin retracted, the skullcap removed without dura disruption and the cortex injured using the controlled cortical impact device. The skullcap was then replaced and the skin sutured together. CatB gene knockout mice were generated and treated and compared to sufficient CatB treated and sham animals. Treated animals received one dose of the cysteine protease inhibitor, E64d, by gavage immediately post treatment and compared to vehicle treated and sham animals. Motor skills were assessed over seven days using a Rotor-Rod system after which the animals were sacrificed and the CA3 hippocampal neuron density and lesion volume determined by histology and brain CatB activity determined using a fluorometric activity assay. **Results:** The CatB gene knockout and E64d treated animals had significantly improved motor skills, increased neuron densities and reduced lesion volumes relative to CatB sufficient and vehicle treated animals, respectively. Brain CatB activity was significantly increased relative to sham animals in the CatB sufficient and vehicle treated animals and abolished in CatB deficient and significantly reduced in E64d treated animals. **Conclusions:** The results validate CatB as a drug target for TBI and demonstrate that a cysteine protease inhibitor is an effective TBI treatment. The data support the hypothesis that increased CatB activity is a common pathology in both AD and TBI.

P3-345 RESTORATION OF MEMORY IN MOUSE MODELS OF ALZHEIMER'S DISEASE AND NEURONAL LOSS: A NEW PARADIGM USING HUMAN NEURAL STEM CELL THERAPY

Mathew Blurton-Jones[1], Rahasson Ager[1], Joy Nerhus[1], Andy Agazaryan[1], Stephen Huhn[2], Alexandra Capela[2], Frank LaFerla[3], *[1]University of*

California, Irvine, Irvine, California, United States; [2]StemCells, Inc., Newark, California, United States; [3]UC Irvine, Irvine, California, United States.

Background: Alzheimer's disease (AD) is the leading cause of age-related dementia, yet currently approved therapies are largely palliative. There is therefore a critical need to identify and test novel approaches to treat this disorder. We previously showed that murine neural stem cell (mNSC) transplantation improves cognition and enhances synaptic connectivity in aged 3xTg-AD mice. As most transgenic AD models exhibit little neuronal loss, we also assessed mNSC transplantation in an inducible model of hippocampal ablation (CaM/Tet-DT A mice). Despite extensive death of CA1 neurons, mNSCs increased synaptic density and improved cognition in this model. In order to translate this NSC approach into potential clinical investigation, we have initiated a study testing the efficacy of human central nervous system stem cells (hCNS-SCns) in both the above transgenic models. This cell transplantation approach is currently being tested in other CNS indications and has an established human safety profile from two Phase I studies involving transplantation into the brain. **Methods:** Immunosuppressed 3xTg-AD and CaM/Tet-DT A mice received hippocampal transplants of hCNS-SCns. One-month after transplantation, mice were tested on a battery of behavioral tasks followed by histological and biochemical analysis. **Results:** We have found that hippocampal transplantation of hCNS-SCns improves cognition in both 3xTg-AD and Cam/Tet-DT A mouse models. Assessments in Morris water maze, context-dependent object recognition, and place recognition revealed significant improvements in hCNS-SCns versus vehicle-injected mice. Evidence of increased presynaptic terminals in hCNS-SCns-injected mice was also noted. **Conclusions:** Taken together, our data reveal that hCNS-SCns can improve cognition and enhance synaptic connectivity in two complimentary models of neurodegeneration. Our studies suggest that human neural stem cell transplantation holds considerable therapeutic promise for AD.

P3-346 INTENSIVE COGNITIVE STIMULATION INCREASES FUNCTION IN THE TEMPORAL CORTEX AND PRECUNEUS IN MILD COGNITIVE IMPAIRMENT

Matteo De Marco[1], Francesca Meneghello[2], Annalena Venneri[1], *[1]University of Sheffield, Sheffield, United Kingdom; [2]IRCCS Fondazione Ospedale San Camillo, Venezia, Italy.*

Background: Effective treatment strategies (including non-pharmacological strategies) are an urgent need to prevent and counteract the symptoms of Alzheimer's Disease (AD). Cognitive stimulation techniques might slow down cognitive decline of AD patients at the Mild Cognitive Impairment (MCI) stage, but positive findings have been sporadic and no systematic study has investigated the biological basis of these effects. No study has tested the efficacy of a protocol of intensive cognitive stimulation (ICS) aimed at stimulating function of cognitive areas associated with temporal and limbic structures, the most vulnerable to the neuropathological effects of AD. The aim of this preliminary study was to verify whether a targeted programme of ICS can restore or consolidate function and connectivity in temporal and limbic structures. Treatment efficacy was evaluated using resting state functional Magnetic Resonance Imaging (fMRI), a technique that detects changes in function and connectivity in the Default Mode Network (DMN), a neurobiological system that includes functional circuits normally co-activated when the brain is at rest. **Methods:** Five participants with amnestic MCI and 5 healthy matched controls were enrolled. They underwent a detailed neuropsychological assessment and a Blood Oxygenation Level Dependent (BOLD) resting state fMRI brain scan at baseline and after completion of four weeks of ICS. The ICS consisted of twenty 90-minute sessions (one session per day, five sessions per week) of computerized exercises targeting semantic memory, logical reasoning, attention, and proper names retrieval. **Results:** Increases in BOLD activity in posterior DMN regions (temporal and precuneus), were observed when MCI and healthy controls were analysed together. Decreased frontal activity was also found. Independent analyses for each group showed similar significant findings in both groups, although improvements were more substantial in controls. There was a trend for behavioural improvement to parallel biological improvements. **Conclusions:** This targeted programme of ICS elicited a positive neural response both in controls and MCI individuals, with specific improvements in areas affected by AD neuropathology very early. The findings suggest that even at the MCI stage there is potential for neuroplasticity and targeted non-pharmacological interventions may prevent or mitigate the negative effects of AD progression.

P3-347 AN INTEGRATED COGNITIVE REHABILITATION MULTI FAMILY GROUP INTERVENTION FOR INDIVIDUALS WITH MILD COGNITIVE IMPAIRMENT AND THEIR CARE PARTNERS: PRELIMINARY DATA

Maureen Schmitter-Edgecombe[1], Chad Sanders[1], Christina Low[1], Lauren Warren[1], Diane Norell[2], Dennis Dyck[3], Lora Wu[1], *[1]Washington State University, Pullman, Washington, United States; [2]Eastern Washington University, Spokane, Washington, United States; [3]Washington State University, Spokane, Washington, United States.*

Background: Development of interventions that can improve the care, functional status, quality of life, and health services utilization of individuals with Alzheimer's disease and their caregivers is an important public health goal. We present preliminary data from an on-going randomized controlled study, which integrates cognitive rehabilitation with a multi-family group (MFG) psychoeducation format, to facilitate assimilation of new strategies into the everyday routines of care-dyads (i.e., individuals with mild cognitive impairment [MCI] and their care partners). **Methods:** Participants included 20 care-dyads. Ten care-dyads completed a 10-week intervention which provided information about MCI and dementia, guidelines for managing the disorder, training in practical memory strategies, practice in formulating and executing plans for incorporating new strategies into everyday living, and opportunity to exchange experiences and coping strategies with care-dyads in similar circumstances. Outcome measures were administered pre-treatment and 3 months later. The Medication Management Ability Assessment (MMAA) and Rivermead Behavioral Memory Test (RBMT) were modified to allow note taking. Measures of depressive symptoms, coping self-efficacy and quality of life were completed by both participants with MCI and their care-partners. Self-report and care-partner report of everyday compensatory strategy use was also assessed. **Results:** Group (intervention, standard care) by time (pre-treatment, 3 months) mixed-model ANOVAs revealed significant interactions for the MMAA, RBMT and self-report depressive symptoms. While participants with MCI in the intervention group performed significantly better post-treatment on these measures, there were no significant changes for the standard care group. Less conservative ANOVAs conducted across time separately for each group revealed that post-intervention participants with MCI self-reported significantly greater everyday strategy use, while their care-partners reported increased coping self-efficacy. No significant changes in outcome measures were found for standard care. **Conclusions:** Modified memory and everyday problem-solving tasks revealed improved post-intervention performances due to increased note taking behavior and more frequent referencing of notes. Reduced depression and increased everyday compensatory strategy

California Institute for Regenerative Medicine,

Grant Abstract: Developmental Candidates for Cell-Based Therapies for Parkinson's Disease

(last accessed Nov. 27, 2013)

CALIFORNIA INSTITUTE FOR REGENERATIVE MEDICINE
THE STATE STEM CELL AGENCY

Funding Type: Early Translational I
Grant Number: TR1-01267
Investigator:

PI
Evan Snyder
Sanford-Burnham Medical Research Institute

Partner-PI
Clare Parish
Howard Florey Institute

Funds Committed: $5,416,003
Disease Focus: Parkinson's Disease
Collaborative Funder: Victoria, Australia
Stem Cell Use: Adult Stem Cell
Status: Active
Public Abstract:

Parkinson's Disease (PD) is a devastating disorder, stealing vitality from vibrant, productive adults & draining our health care dollars. It is also an excellent model for studying other neurodegenerative conditions. We have discovered that human neural stem cells (hNSCs) may exert a significant beneficial impact in the most authentic, representative, & predictive animal model of actual human PD. Interestingly, we have learned that, while some of the hNSCs differentiate into replacement dopamine (DA) neurons, much of the therapeutic benefit derived from a stem cell action we discovered a called the “Chaperone Effect” – even hNSC-derived cells that do not become DA neurons contributed to the reversal of severe Parkinsonian symptoms by protecting endangered host DA neurons & their connections, restoring equipoise to the host nigrostriatal system, and reducing pathological hallmark of PD. While the ultimate goal may someday be to replace dead DA neurons, the Chaperone Effect represents a more tractable near-term method of using cells to address this serious condition. However, many questions remain in the process of developing these cellular therapeutic candidates. A major question is what is the best (safest, most efficacious) way to generate hNSCs? Directly from the fetal brain? From human embryonic stem cells? From skin cells reprogrammed to act like stem cells? Also, would benefits be even greater if, in addition to harnessing the Chaperone Effect, the number of stem cell-derived DA neurons was also increased? And could choosing the right stem cell type &/or providing the right supportive molecules help achieve this? This study seeks to answer these questions. Importantly, we will do so using the most representative model of human PD, a model that not only mimics all of the human symptomatology but also all the side-effects of treatment; inattention to this latter aspect plagued earlier clinical trials in PD. A successful therapy for PD would not only be of great benefit for the many patients who now suffer from the disease, or who are likely to develop it as they age, but the results will help with other potential disease applications due to greater understanding of stem cell biology (particularly the Chaperone Effect, which represents “low hanging fruit”) as well as their potential complications and side effects.

Statement of Benefit to California:

Not only is Parkinson's Disease (PD) a devastating disease in its own right-- impairing typically vibrant productive adults & draining our health care dollars -- but it is also an excellent model for studying other neurodegenerative diseases. We have discovered that stem cells may actually exert a beneficial impact independent of dopamine neuron replacement. As a result of a multiyear study performed by our team, implanting human neural stem cells (hNSCs) into the most authentic, representative, and predictive animal model of actual human PD, we learned that the cells could reverse severe Parkinsonian symptoms by protecting endangered host dopaminergic (DA) neurons, restoring equipoise to the cytoarchitecture, preserving the host nigrostriatal pathway, and reducing alpha-synuclein aggregations (a pathological hallmark of PD). This action, called the "Chaperone Effect" represents a more tractible near-term method of using cells to address an unmet medical need. However, many questions remain in the process of developing these cellular therapeutic candidates. A major question is what is the best (safest & most efficacious way) to generate hNSCs? Directly from the fetal brain? From human embryonic stem cells? From human induced pluripotent cells? Also, would benefits be even greater if, in addition to harnessing the Chaperone Effect, the number of donor-derived DA neurons was also increased? And could choosing the right stem cell type &/or providing the right supportive molecules help achieve this? This study seeks to answer these questions. Importantly, we will continue to use the most representative model of human PD to do so, a model that not only mimics all of the human symptomatology but also all the side-effects of treatment; inattention to this latter aspect plagued earlier clinical trials in PD. Because of the unique team enlisted, these studies can be done at a fraction of the normal cost, allowing for parsimony in the use of research dollars, clearly a benefit to California taxpayers. Not only might California patients benefit in terms of their well-being, and the economy benefit from productive adults re-entering the work force & aging adults remaining in the work force, but it is likely that new intellectual property will emerge that will provide additional financial benefit to California stakeholders, both citizens & companies.

Progress Report:

Year 1

Parkinson's Disease (PD) is a devastating disorder, stealing vitality from vibrant, productive adults & draining our health care dollars. It is also an excellent model for studying other neurodegenerative conditions. We have discovered that human neural stem cells (hNSCs) may exert a significant beneficial impact in the most authentic, representative, & predictive animal model of actual human PD (the adult African/St. Kitts Green Monkeys exposed systemically to the neurotoxin MPTP). Interestingly, we have learned that, while some of the hNSCs differentiate into replacement dopamine (DA) neurons, much of the therapeutic benefit derived from a stem cell action we discovered called the “Chaperone Effect” – even hNSC-derived cells that do not become DA neurons contributed to the reversal of severe Parkinsonian symptoms by protecting endangered host DA neurons & their connections, restoring equipoise to the host nigrostriatal system, and reducing pathological hallmark of PD. While the ultimate goal may someday be to replace dead DA neurons, the Chaperone Effect represents a more tractable near-term method of using cells to address this serious condition. However, many questions remain in the process of developing these cellular therapeutic candidates. A major question is what is the best (safest, most efficacious) way to generate hNSCs? Directly from the fetal brain? From human embryonic stem cells? From skin cells reprogrammed to act like stem cells? Also, would benefits be even greater if, in addition to harnessing the Chaperone Effect, the number of stem cell-derived DA neurons was also increased? And could choosing the right stem cell type &/or providing the right supportive molecules help achieve this? This international study – which involves scientists from California, Madrid, Melbourne -- has been seeking to answer these questions. Importantly, we have been doing so using the most representative model of human PD, a model that not only mimics all of the human symptomatology but also all the side-effects of treatment; inattention to this latter aspect plagued earlier clinical trials in PD. A successful therapy for PD would not only be of great benefit for the many patients who now suffer from the disease, or who are likely to develop it as they age, but the results will help with other potential disease applications due to greater understanding of stem cell biology (particularly the Chaperone Effect, which represents “low hanging fruit”) as well as their potential complications and side effects. To date, we have transplanted nearly 40 Parkinsonian non-human primates (NHPs) with a range of the different stem cell types described above. We have been able to generate neurons from some of these stem cells that appear to have the characteristics of the desired A9-type midbrain dopaminergic neuron lost in PD. Following transplantation, some of these stem cell derivatives appear to survive, integrate, & behave like dopaminergic neurons. Preliminary behavioral analysis of some engrafted NHPs offers encouraging results, suggesting an improvement in the Parkinsonism score in some of the animals. These NHPs will need to be followed for 1 year to insure that improvement continues & that no adverse events intervene. Over the next year, more stem cell candidates will be tested as we further optimize their preparation & differentiation.

Year 2

We have made substantial progress in what will amount to the largest and most comprehensive head-to-head behavioral analysis of stem cell transplanted MPTP-NHPs to date and have identified cell types that show dramatic improvement in this model. Compared to the improvement observed with undifferentiated fetal CNS-derived hNSCs (the stem cell type in used Redmond et al, PNAS, 2007), 3 human stem cell candidates have shown a larger improvement in PS. Summary of Achievements for this reporting period • Comprehensive Behavioral data collection of 84 monkeys comprising over 10,000 observation data points • Statistical analysis of Behavioral data collected to date identifies striking and statistically significant improvements in PS for several stem cell types. (Accordingly, NO-GO (or near NO-GO) cell types have been identified via comparison of levels of improvement or no improvement) [Figure 1] • DNA samples collected in order to pursue the first ever complete genome sequencing of the Vervet in collaboration with the Washington University Genome Center • Biochemistry sample processing and data collection of a 2nd large batch of samples completed.

Year 3

The identification and development of an ideal cell-based therapy for a complex neurodegenerative disease requires the rigorous evaluation of both efficacy and safety of different sources and subtypes of hNSCs. The objective of this project has been to fully evaluate and identify the optimal stem cell type for a cell based therapy for refractory Parkinson's Disease (PD) using the systemically MPTP-lesioned Old World non-human primate (NHP) (the St. Kitts Green Monkey) the most authentic animal model of the actual human disease. Among a list of plausible potentially therapeutic stem cell sources, 7 candidates have been evaluated head-to-head. The intent has been that the stem cell type (and its derivatives) safely producing the largest improvement in behavioral scores (based on a well-established NHP PD score – the Parkinson's Factor Score [PFS] or ParkScore (which closely parallels the Hoehn–Yahr scale used in human patients, and is an accurate functional read-out of nigrostriatal dopamine [DA] activity) -- as well as a Healthy Behaviors Score [HBS] (similar to the activities-of-daily-living [ADL] on the major Parkinson's rating scale and allows quantification of adverse events) -- will be advanced towards IND-enabling studies, to an actual IND filing, and ultimately a clinical trial. Candidate cells have been transplanted into specific sub-regions of the nigrostriatal pathway of MPTP-lesioned NHPs. Animals undergo behavioral scoring for analysis of severity of Parkinsonian behavior at multiple time points pre- and post-cell transplantation. At sacrifice, biochemical measurements of DA content are made. Tissue is also analyzed to determine the fate of donor cells; the status of the host nigrostriatal pathway; the number of alpha-synuclein aggregates; degree of inflammation; any evidence of adverse events (e.g., tumor formation, cell overgrowth, emergence of cells inappropriate to the CNS). We have made substantial progress in what will amount to the largest and most comprehensive head-to-head analysis of stem cell transplanted into any disease model to date, let alone behavioral analysis into a primate model of PD. Behavioral data have been collected on ~100 monkeys comprising >10,000 observation data points. We have identified a single Developmental Candidate (DC) that shows consistent and dramatic improvement in severely Parkinsonian NHPs (i.e., a significant decrease in Parkinsonian symptoms over the entire evaluation period), reflecting a restitution of DA function – human embryonic stem cell (hESC-derived) ventral mesencephalic (VM) precursors. We also suggest adding a mechanism to these cells for insuring unambiguous safety and invariant lineage commitment (a construct already generated and inserted into this DC, and recently engrafted into some initial monkeys). We believe are ready for IND-enabling studies, including additional long-term pre-clinical behavioral studies of hESC-derived hVM cells that bear the above-mentioned "safety construct" – combined with additional biochemical assays of DA metabolism, histological assessments, serial profiling to insure genomic stability. Scale-up conditions for this DC are defined and reproducible and a working cell bank has been established.

Streiffer,

At the Edge of Humanity: Human Stem Cells, Chimeras, and Moral Status,

KENNEDY INSTITUTE OF ETHICS JOURNAL, vol. 15, no. 4 (Dec. 2005)

Robert Streiffer

At the Edge of Humanity: Human Stem Cells, Chimeras, and Moral Status

ABSTRACT. Experiments involving the transplantation of human stem cells and their derivatives into early fetal or embryonic nonhuman animals raise novel ethical issues due to their possible implications for enhancing the moral status of the chimeric individual. Although status-enhancing research is not necessarily objectionable from the perspective of the chimeric individual, there are grounds for objecting to it in the conditions in which it is likely to occur. Translating this ethical conclusion into a policy recommendation, however, is complicated by the fact that substantial empirical and ethical uncertainties remain about which transplants, if any, would significantly enhance the chimeric individual's moral status. Considerations of moral status justify either an early-termination policy on chimeric embryos, or, in the absence of such a policy, restrictions on the introduction of pluripotent human stem cells into early-stage developing animals, pending the resolution of those uncertainties.

Some people object to human embryonic stem cell research (hES cell research) because of their beliefs about the moral status of the human embryos that are destroyed when the stem cells are derived. Some people object to using animals in biomedical research because of their beliefs about the moral status of animals. Most biomedical research advisory and regulatory bodies, however, believe that both types of objections can be overcome and are generally supportive of both hES cell research and biomedical research on animals. It is therefore somewhat surprising that many such bodies have expressed serious concern regarding a use of hES cells that combines both kinds of research: the creation of chimeras, organisms with parts from different species, through the xenotransplantation of hES cells into animals that are in the embryonic or early fetal stages of their development. Although the focus has been on the creation of chimeras using human embryonic stem cells, similar

Kennedy Institute of Ethics Journal Vol. 15, No. 4, 347–370 © 2005 by The Johns Hopkins University Press

concerns might arise with respect to transplants involving other types of human stem cells, as well as their more specialized progeny.

The emerging bioethics literature on the creation of such chimeras has analyzed several possible moral issues. Jason Robert and Françoise Baylis (2003) explore the possibilities that such research is unethical because of its unnatural results, because it violates species boundaries, or because it might harm society by leading down a slippery slope that undermines the categories presupposed by desirable legal and cultural practices. Phillip Karpowicz and colleagues (2004; 2005) also discuss the unnaturalness objection and look at whether chimeras might be problematic because they violate moral taboos, violate species integrity, or undermine human dignity.

With two notable exceptions (Karpowicz, Cohen, and van der Kooy 2004; 2005), the literature has neglected to address issues arising out of concern for the individual most directly affected by the research, namely the chimeric research subject itself. After outlining the relevant scientific and regulatory background, I argue that the effect that certain transplants could have on the moral status of chimeric research subjects raises novel and significant ethical issues. I distinguish between the two different views of moral status that are generating most of the controversy surrounding hES cell research and argue that on each of them certain kinds of human stem cell transplants could significantly enhance the chimeric individual's moral status. Given that the moral evaluation of research normally presupposes a fixed moral status for the subject, this raises novel ethical issues that are just now beginning to receive attention. I therefore construct a taxonomy of principles for evaluating moral status enhancements. I then argue that on the most plausible principle, the introduction of human stem cells into a nonhuman animal in a way that would substantially enhance its moral status is wrong, not because of the fact that the research subject's moral status is enhanced, which is a prima facie good, but rather because of the fact that the subsequent treatment of the subject likely will fall far below what its new moral status demands. Translating that ethical conclusion into a policy recommendation, however, is complicated by the fact that substantial empirical and ethical uncertainties remain about which transplants, if any, would significantly enhance the chimeric individual's moral status. I conclude by discussing various policy options, and I argue that the moral status framework justifies either an early-termination policy on chimeric embryos, or, in the absence of such a policy, restrictions on introducing human pluripotent stem cells into early-stage developing animals, pending the resolution of those uncertainties.

SCIENTIFIC AND REGULATORY BACKGROUND

A chimera is a single individual composed of cells that have different embryonic origins. Intraspecific chimeras, created when the cell donor and the cell recipient belong to the same species, have been an important research tool for decades (Nagy and Rossant 2001). Chimeras commonly are created by transplanting, or injecting, stem cells from one animal into another. Stem cells are cells that renew themselves and also give rise to more specialized kinds of cells. Although much of the research on chimeras involves injecting mouse stem cells into mouse blastocysts, I restrict my use of the term here to animal/human chimeras, by which I mean the individual that results from injecting human stem cells or their derivatives into a nonhuman animal.

Even though the term "chimera" evokes negative connotations for some, chimeras are often no more than animals with some human blood cells inside them. Such chimeras have been created for some time by transplanting stem cells derived from the bone marrow of adult humans, adult hematopoietic stem cells, into postnatal animals. By injecting cells that have been tagged with markers, researchers can observe where the cells and their progeny migrate, how they specialize, and how they interact with other tissues and systems in the animal's body. This provides researchers the opportunity to learn about how stem cells specialize in response to cues from their surrounding cellular environment, to explore their potential for repairing or replacing damaged tissue, and to explore their potential for creating animal models that more closely mimic humans (Okarma 2001; Thomson 2001). Although such research involves biomedical research on animals and, like all biomedical research on animals, raises important concerns within traditional animal ethics, it has not been regarded as especially or distinctively problematic by researchers or by bioethicists.

There is special interest in transplanting human stem cells into prenatal animals. Doing so helps to minimize the risk that the transplant will be rejected by the animal's immune system and provides the opportunity to learn about how stem cells act in a developing organism, which is of interest to developmental biologists. For future clinical therapies involving human stem cells, transplants might need to take place early in fetal or even embryonic development to help prevent complications before they start (Flake and Zanjani 1999). Early in utero transplants of human stem cells into animals would have to precede human clinical trials for such therapies.

Because adult hematopoetic stem cells are believed to be merely multipotent, restricted to differentiating only into types of blood cells, whereas hES cells are believed to be pluripotent, capable of differentiating into

any kind of cell (Wagers et al. 2002), there is now a growing interest in differentiating hES cells in vitro, and then transplanting these derivatives into prenatal animals. This would offer a wider range of opportunities to study early human development and potential therapies, not just for blood cells, but for any kind of tissue (Svendsen 2002). Researchers at the University of Wisconsin-Madison, for example, are interested in using chick embryos as a model for studying hES cell derived neural precursor cells (Basu 2005)—precursor cells differentiate into more specialized cells, but do not renew themselves as stem cells do. And there is growing interest in introducing undifferentiated hES cells into prenatal animals. One group has reported inserting hES cells into 1.5- to 2-day-old chick embryos to explore whether the chick embryo "may serve as an accessible and unique experimental system for the study of in vivo development of human ES cells" (Goldstein et al. 2002). Also, one test for pluripotency is to inject cells into a blastocyst and then see whether those cells contribute to the development of all the other tissues of the resulting organism (Kaiser Daily Reproductive Health Report 2002; Dewitt 2002; NAS 2004).

However, as I mentioned, transplanting hES cells into early-stage developing animals has been flagged for special concern by several regulatory and advisory bodies, bodies which view the typical concerns in both animal ethics and hES cell research as answerable. Geron, the company that funded James Thomson's original derivation of hES cells, has an ethics advisory board (EAB) that issued guidelines for hES cell research (Geron Ethics Advisory Board 1999). These guidelines are generally supportive of hES cell research but include a provisional prohibition on research involving "any creation of chimeras" until the EAB has undertaken more extensive analysis of the issues involved in doing so. The initial report on hES cell research of the University of Wisconsin's Bioethics Advisory Committee (1999) recommended that hES cells "not be used for introduction into a uterus without further University of Wisconsin Review and approval." WiCell Research Institute, the not-for-profit company that manages the University of Wisconsin's hES cell lines, went even further in their memorandum of understanding to which recipients of their cells must agree: "Recipient agrees that its research program will exclude (i) the mixing of Wisconsin Materials with an intact embryo, either human or nonhuman; (ii) implanting Wisconsin Materials or products of Materials in a uterus; and (iii) attempting to make whole embryos with Wisconsin Materials by any method" (WiCell 2001). The Clinton administration's proposed guidelines prohibited funding for "research in which human pluripotent

stem cells are combined with an animal embryo" (NIH 2000). (Interestingly, there appears to be no similar restriction in Bush's guidelines.)

Two consensus groups have taken up the task of establishing voluntary guidelines for hES cell research. The first was organized by the New York Academy of Sciences, but agreement was stymied because of disagreement over the creation of human/mouse embryonic chimeras (DeWitt 2002). The second was the National Academy of Sciences' Committee on Guidelines for Human Embryonic Stem Cell Research (NAS Committee), which published its guidelines in April 2005. Presentations to the NAS Committee on research involving the introduction of hES cells into embryonic animals ranged from supporting no special review whatsoever to supporting an outright ban, and although there seemed to be no inclination among the presenters to ban the introduction of hES cells into all fetal animals, there was disagreement as to where to draw the line between embryonic and fetal stages (NAS 2004; Weiss 2004). The final guidelines include a prohibition on research "in which hES cells are introduced into nonhuman primate blastocysts," and special review for all research "involving the introduction of hES cells into nonhuman animals at any stage of embryonic, fetal, or postnatal development" (NAS 2005, pp. 47–48).

HUMAN APPEARANCES, HUMAN EXPERIENCES, COGNITIVE CAPACITIES, AND MORAL STATUS

Some have suggested that the problematic aspect of chimeras is the aesthetics involved. William Hurlbut, a member of the President's Council on Bioethics, says that "visible chimeras," animals with visible parts that appear to be human, are unethical because "human appearance is something we should reserve for humans" (Shreeve 2005). The image of animals with human body parts, or even animals with the parts of other species of animals, is surely part of what is motivating the public's reaction to chimeras. It should go without saying that this view is a non-starter. It should go without saying, but evidently it does not, and so it is worth considering here.

Consider work by Yilin Cao and colleagues (1997), in which researchers evaluated whether a polymer template could be used to grow cartilage in the shape of a 3-year-old child's auricle. In order to provide a hospitable environment for the cartilage to form, the template was inserted under the skin on the back of a mouse. Pictures from this experiment showed a small mouse in a Petri dish with what appears to be a fully-formed human ear on its back. These pictures have been used by such anti-biotechnology organizations as the Turning Point Project to elicit negative aesthetic reac-

tions to biotechnology, which are then treated as if they were reactions that carried moral significance. But the ability to grow cartilage in the right shape is one step in the important process of being able to provide functional and aesthetically correct replacements for children who, due to deformity or accident, need total external ear reconstruction. Although such research on animals may be unethical because of traditional concerns in animal ethics, it is not remotely plausible to think that the mere visual appearance of the mouse makes such research wrong. The mere fact that one would be conferring a human appearance on a nonhuman animal is of no consequence.

Others who have discussed the introduction of human stem cells or their derivatives into an embryonic or fetal animal have focused on possible effects on the animal's neural tissue. As the NAS Committee stated, "Perhaps no organ that could be exposed to hES cells raises more sensitive questions than the animal brain, whose biochemistry or architecture might be affected by the presence of human cells" (NAS 2005, p. 41).

The possibility that transplantation of human stem cells or their derivatives could alter neural tissue is already well documented. Two groups have reported that they differentiated hES cells in vitro into neural precursor cells, which they then transplanted into the brains of neonatal mice (AAP 2001; Zhang et al. 2001). Su-Chun Zhang and colleagues (2001, p. 1129) report that the cells were then "incorporated into a variety of brain regions, where they differentiated into both neurons and astrocytes." Irv Weissman, a Stanford researcher, injected human neural stem cells into the brains of neonatal mice, with the result that "every part of the brain was populated with human cells" (Krieger 2002), although presumably only to a very small degree since Weissman also told the press that the human cells made up only 1 percent of the cells in the mice brains. Ronald Goldstein and colleagues (2002, p. 80) report that the hES cells they transplanted into chick embryos differentiated into neurons and penetrated into the developing central nervous system. Oliver Brüstle (1999, p. 537) reports that human neural precursor cells transplanted into embryonic rats differentiated into "all three major cell types of the nervous system" and generated "an extensive axonal network encompassing large areas of the host brain."

The focus on alterations of neural tissue might be justified in different ways. In its second report on hES cells, the University of Wisconsin Bioethics Advisory Committee (2001) explores the possibility that the introduction of human stem cells could result in a chimera capable of "human experiences":

> Mixing human stem cell lines with experimental animals early in the animal's fetal development may . . . result in the development of human neural tissue in the experimental animal, which raises at least the theoretical possibility that such tissue could become integrated in a way that human experiences become possible. After consulting with biologists, the Committee concluded, based on current knowledge of developmental biology, that this risk is extremely remote unless such mixing occurred very early in embryonic life. It is for this reason that introducing human stem cells into developing animals very early in embryonic life raises greater concerns about the creation of chimeras with human-like characteristics, and such experiments should receive careful ethical and scientific scrutiny.

This view appears to single out human experiences, and to require additional review for research that would provide animals with such experiences. The underlying moral principle appears to be something like the following:

> The Human Experience Principle: It is always morally problematic to enable a nonhuman individual to have human experiences.

The principle uses the phrase "human experience." How might this be defined? If a human experience is any experience that some humans are capable of having, then the experience of seeing red is a human experience. The Human Experience Principle then implies that it is always morally problematic to enable an animal to see color. This seems plainly false.

Perhaps a human experience is one that some humans are capable of having and no nonhumans are capable of having. That is, human experiences are experiences that are distinctively human. But what if it were true that only humans could see color? The Human Experience Principle would imply that, in those circumstances, it would be morally problematic to enable an animal to see color. Again, this is clearly false. It is not clear that the phrase "human experiences" picks out a morally relevant class of experiences.

A second possible justification for focusing on transplants that alter neural tissue is that neural tissue is the physical basis for those cognitive capacities that themselves form the basis for the robust moral agency and rational autonomy of which normal adult humans are, so far as we know, distinctively capable (DeGrazia 1996, pp. 199–210). Call such cognitive capacities "high-level cognitive capacities." Then, the underlying moral principle would be something like the following:

> The Cognitive Capacity Principle: It is always morally problematic to enable a nonhuman individual to have high-level cognitive capacities.

Because being able to experience red is not a high-level cognitive capacity, the Cognitive Capacity Principle does not imply that it would be morally problematic to enable a nonhuman to experience red, and thus it avoids the aforementioned problem with the Human Experience Principle.[1]

Nonetheless, the Cognitive Capacity Principle looks dubious. If one were to discover beings of another species, the normal adults of which had high-level cognitive capacities, it would not be morally problematic to cure one of them of severe brain damage—i.e., to enable *that* nonhuman individual to have high-level cognitive capacities.

Another possibility, which I think gets at something deeper than the previous principles, focuses on the possible impact of transplanting human stem cells into early-stage developing animals on the moral status of the resulting chimera:

> The Moral Status Principle: It is always morally problematic to cause an individual that would otherwise have a lower moral status to have the moral status of a normal, adult human.

Because being able to experience red is not what gives humans our moral status, the Moral Status Principle does not imply that it is morally problematic to enable an individual to experience red. Because the hypothetical beings of another species cognitively similar to our own presumably have the same moral status as we do, and retain their moral status even when they are brain damaged, the Moral Status Principle does not imply that it is morally problematic to return their cognitive capacities to normal.

In the remainder of the paper, I focus on the implications of the Moral Status Principle for the moral evaluation of chimeric research.

THE MORAL STATUS FRAMEWORK

Which effects would a transplant need to have to confer upon a chimeric research subject the moral status of a normal, adult human being, and which transplants, if any, would produce those effects? The answers depend on why normal human adults have the comparatively high moral status that they do.

On cognitive capacity views of moral status, an individual's cognitive capacities give it its moral status, and the high-level cognitive capacities that normal adult humans have is what gives them their relatively high moral status (VanDeVeer 1979). Although Karpowicz and colleagues (2005, p. 120) do not use the language of "moral status," they articulate one attractive view of these cognitive capacities in their discussion of human dignity:

> Human dignity is a widely shared notion that signifies that humans typically display certain sorts of functional and emergent capacities that render them uniquely valuable and worthy of respect. It is not only the capacities for reasoning, choosing freely, and acting for moral reasons, as Kant argues, or for entertaining and acting on the basis of self-chosen purposes, as Gewirth holds, that are at the core of what we mean by human dignity. The notion also encompasses such capacities as those for engaging in sophisticated forms of communication and language, participating in interweaving social relations, developing a secular or religious world view, and displaying sympathy and empathy in emotionally complex ways.

Given that high-level cognitive capacities are intimately related to the individual's neural tissue, this view obviously justifies focusing on transplants that could affect neural tissue in a way that enhances cognitive capacities.

As Karpowicz and his colleagues point out (2005, pp. 124–26), there are many constraints on the ability of differentiated human stem cells, particularly retinal and neural stem cells, to significantly enhance cognitive functions. In many cases, such enhancements likely would be prevented by the animal's smaller skull size and shorter gestation period, as well as by the surrounding nonhuman cellular environment that would provide developmental cues to transplanted cells. This is also the view that Dr. Fred Gage, a neuroscientist at the Salk Institute who specializes in neuroplasticity and neural stem cells, presented to the NAS Committee (NAS 2004).

It may be that many human stem cell xenotransplants would not confer any high-level cognitive capacities onto the resulting chimeric subjects, as seems to be the case with hematopoetic stem cell xenotransplants late in an animal's fetal development. But even with stem cells that are merely multipotent, there are still two uncertainties. First, the mechanism by which oocyte cytoplasm de-differentiates cells, a procedure involved in somatic cell nuclear transfer, is still unclear (NAS 2005, p. 35). Thus, there is the possibility that introducing multipotent stem cells into an embryonic environment might de-differentiate the cell, restoring it to a pluripotent state. Second, it is not yet clear what kinds of cognitive enhancements might be possible even within a constrained environment. For example, it is possible to use genetic engineering to enhance the learning and memory of mice without modifying skull size or gestational period (Tang et al. 1999).

Moreover, it would be premature to claim to know that the introduction of hES cells very early in development will not dramatically affect cognitive capacities. If a large enough quantity of hES cells were introduced, the cells themselves could induce changes that would eliminate some of the constraints mentioned above. As the NAS Committee concluded, "it

is not now possible to predict the extent of human contribution to such chimeras" (NAS 2005, p. 34). And Gage, when asked what the effects would be of introducing hES cells or neural stem cells into an animal early in development, said, "We don't know the answer to [that] question because the experiment hasn't been done, that I know of" (NAS 2004). Although some of the experiments cited above do involve the introduction of hES and neural progenitor cells early in development, experience in this area is limited. It therefore seems premature to place much confidence in our ability to draw a precise line between those introductions of human stem cells that will, and those that will not, confer high-level cognitive capacities.

It is also important to note that some researchers will be interested in the bases of the restrictions on cognitive development and in whether they can be overcome through the use of human stem cells. They will be interested in designing experiments that seek to overcome existing limitations on cognitive development in nonhuman animals. Other researchers will be interested in creating chimeras in which such limitations are overcome so that the chimeric individuals can be used as models that more closely mimic human beings. Such chimeras might be created to study diseases or injuries that impair high-level cognitive functions in humans, or to do basic research on the neurological development involved in language acquisition, mathematical concept acquisition, moral development, or any number of other cognitive capacities that are now limited to normally functioning human beings. As noted by the NAS Committee,

> [T]he idea that human neuronal cells might participate in "higher-order" brain functions in a nonhuman animal, however unlikely that may be, raises concerns that need to be considered. Indeed, if such cells are to be used in therapeutic interventions, one needs to know whether they could participate in that way in the context of a treatment. (NAS 2005, p. 33)

On anthropocentric views of moral status, normal human adults have the moral status they do simply because they are human beings, that is, because they are members of the species *homo sapiens* (Noonan 1970; Devine 1978; Schwarz 1990). As has often been noted, anthropocentric views seem to suffer from an explanatory gap: it is hard to see how being a member of a certain species could give an individual its moral status (Regan 1978; Feinberg 1980; DeGrazia 1996, pp. 56–61).[2] Nonetheless proponents of these views argue that they provide the only way to explain adequately the equal moral status of all human beings, even human beings who lack high-level cognitive capacities, and thereby to avoid the so-called "marginal humans" problem that afflicts cognitive capacity views.

Karpowicz and colleagues (2005, p. 120), for example, maintain that human's special moral status is "attributable equally to all human beings," but, quite clearly, the capacities they cite as the basis of human's special value are not held equally by all human beings, and some human beings lack them altogether. The authors recognize that their view may exclude infants and seriously disabled individuals and respond:

> [W]e tend to ascribe [human dignity] to all humans, no matter how seriously impaired or ill they may be, because there is no clear agreement about just how many dignity-associated capacities a person must possess to be said to have human dignity. To avoid the possibility of mistakenly failing to treat those with severe disabilities as ends in themselves, human dignity proponents ascribe dignity to all humans. (Karpowicz, Cohen, and van der Kooy 2005, pp. 121–22)

But an appeal to uncertainty and disagreement seems implausible given that there is no real uncertainty or disagreement that a newborn fails to have the capacities they cite and so would, on their view, *clearly* lack the special moral status that accompanies individuals with human dignity.

Anthropocentric views raise difficult questions about how much human material an individual needs in order to be a human being. Since normal human embryos are both human and organisms, they are human beings, albeit ones at the earliest stages of development (Feinberg 1980, pp. 288–91). But when faced with an organism that has some human cells and some nonhuman cells, how is one to decide whether the organism is human, and hence, whether it is a human being? It is not plausible to suppose that the individual in question has to have a human brain: an anencephalic infant is a human being and would possess human moral status on an anthropocentric view. Thus, on anthropocentric views, the focus on alterations of neural tissue is overly narrow, and the focus on alterations of neural tissue that affect cognitive capacities even more so. Presumably, replacing the entire inner cell mass of an animal blastocyst with hES cells would suffice to make the resulting individual a human being since, in normal human development, the inner cell mass is what goes on to form the fetus. In such cases, one could, at least in principle, end up with a human being surrounded by a nonhuman trophectoderm. On the other hand, having only a few nonhuman cells in the final individual would not suffice since a human with a porcine heart valve is still a human being. But where to draw the line is unclear, as it is with other objects, such as the ship of Theseus, that have vague identity conditions (Parfit 1984, pp. 231–43; Thomson 1987; Thomson 1997).

It seems, then, that on both anthropocentric and cognitive capacity views of moral status—views that, in some form or other, generate most of the controversy about hES cell research—the transplantation of human stem cells or their derivatives into developing animals could, at least in principle, significantly enhance the chimeric research subject's moral status. The question remains, though: why think that significantly enhancing an individual's moral status is always morally problematic?

EVALUATING ENHANCEMENTS OF MORAL STATUS

What are the moral principles regarding enhancements in moral status? To focus the discussion, I shall concentrate primarily on issues that arise from concern for the altered individual, concerns from the perspective of that individual itself.

There would seem to be the following possibilities. First, an enhancement in moral status might always be an unequivocal good from the individual's perspective. Any deleterious effects an enhancement might have on other factors that are relevant from the individual's perspective are always outweighed by the enhancement itself. I will call this view the Millian View since it echoes Mill's remark that it is better to be Socrates unsatisfied than a pig satisfied.

Second, whether an enhancement in moral status is good or bad from the individual's perspective might be entirely derivative upon its effects on other, independently relevant factors. For example, if the chimeric research subject suffers more than it would have, and if the research has no other morally relevant effects, then the enhancement in status would be bad from its perspective just to the degree that its suffering was bad. Because the relevant baseline in this case is how the individual would have fared had it not received the enhancement, I will call this view the Instrumentalist View with the Non-Moral Baseline.

The third view is the Instrumentalist View with the Moral Baseline: how good an enhancement is from the individual's perspective depends entirely upon how the individual's life compares, in terms of other, independently relevant factors, to the life it would have were its new moral status fully respected. To the extent that the individual's life meets this moral baseline, the enhancement is good from the individual's perspective; but to the extent that its life falls short of the moral baseline, the enhancement is bad from the individual's perspective. The two Instrumentalist views disagree in cases where the chimeric research subject's life is better than it would have been had its status not been enhanced, but given its new moral status, it deserves to have its life be even better.

The fourth and fifth possibilities are mixed views that attach some positive moral weight to the fact that the individual's status has been enhanced, but allow that this improvement might be outweighed by deleterious effects on other factors that are independently relevant from the individual's perspective. According to the Mixed View with the Non-Moral Baseline, the relevant question is how the individual's life, taking into account both the *prima facie* good of the enhancement and its other effects, compares to the life it would have had had it not received the enhancement. And according to the Mixed View with the Moral Baseline, the relevant comparison is to the life it would have if its new moral status were fully respected.

Finally, a sixth possibility holds that conferring an enhanced moral status on an individual is always bad from the individual's perspective. I will call this the No-Enhancing View. There are other logical possibilities, but these are the most interesting ones.

If any of these views is to underwrite a general moral objection to status-enhancing research, such as the one expressed in the Moral Status Principle, it would have to be the No-Enhancing View. The others allow that, in some circumstances, an enhancement could be good from the individual's perspective. But of the views, the No-Enhancing View is the least plausible. Imagine conferring an enhanced moral status on an entity, and then ensuring that it lives a life in which it receives much better treatment than it would have gotten otherwise and in which it is given everything it is owed in virtue of that enhanced moral status. It is hard to see how this outcome could be bad from the individual's perspective. I conclude, then, that if the Moral Status Principle is to be sustained at all, it must be by appeal to some factors other than the ones relevant from the individual's perspective.

It is worth considering briefly whether the Moral Status Principle might be justified on such grounds. Perhaps bringing new individuals into existence that have the distinctive moral status of normal human adults somehow lessens the value of that status for extant humans, as expanding membership in an exclusive club might lessen its value to its already existing members. That thought, however, is belied by the fact that every time people reproduce, they engage in just such an activity, and they produce far more individuals with human moral status than would ever be produced by chimeric research.

Another thought might be that the extension of human moral status to "lesser animals" somehow diminishes the value of that status for humans, as extending a university diploma to those who do not deserve it lessens the value of that diploma for those who do. If, however, a transplant truly has

enhanced the moral status of the chimeric research subject, then in whatever sense we humans "deserve" our special status, it now does as well, and so the value placed on human moral status will not be lessened.

Undoubtedly, there are other possible arguments that support the No-Enhancing View—e.g., enhancing is unnatural, enhancing is playing God, and so on. Although I have seen no conclusive refutation of all such arguments, I am dubious that any of them are sound and maintain that many would be unacceptable grounds for public policy even if they were (Streiffer 2003; Streiffer and Hedemann 2005).

The opposite extreme of the No-Enhancing View is the Millian View, according to which moral status enhancements are always an unequivocal good from the perspective of the enhanced individual. This view is also implausible: what kind of life an individual with an enhanced status will lead surely matters. My life is better than the life of even a *very* satisfied pig, but if my life were filled with enough pain and misery, and with extremely limited prospects, it arguably would be worse.[3]

The Instrumentalist Views might be motivated by a hedonistic view of how good an animal's life is. Since an enhanced moral status it not itself pleasurable or painful, an Instrumentalist View must be true. But there are two problems.

First, it is doubtful that hedonism is the correct view regarding animals. Hedonism about animal welfare seems to presuppose that all nonhuman animals have an exceedingly limited mental life, incapable of being interested in anything other than experiencing pleasure and avoiding pain. There is substantial empirical research against this idea (see DeGrazia 1996, pp. 97–257, for extensive discussion of the mental life of animals and its relationship to welfare).

There is also a lively debate in the agricultural biotechnology literature about reducing an animal's suffering by genetically altering it so as to eliminate its natural desires and capacities (Thompson 1997, pp. 96–99; Cooper 1998; Rollin 1998). Consider the following example. In the crowded conditions of industrial agriculture, chickens frequently hurt and kill one other (Cheng and Ali 1985). Since this is economically inefficient, producers often cut off the chickens' beaks, combs, and toes to minimize the damage the chickens can do (Duncan 2001). Although this measure mitigates the problem, it does not eliminate it. It is also economically costly to producers and painful to the chickens. Scientists have known for some time about genetically blind chickens, the result of a chemically-induced genetic mutation (Smyth, Boissey, and Gawron 1977). These chickens are

of interest to producers because their blindness makes them less mobile, which means that they use less feed, and makes them unable to cannibalize their eggs, which means that they have increased egg productivity (Cheng et al. 1980). Their blindness also limits their ability to injure or kill their cagemates, and flocks of blind chickens suffer less feather damage and fewer injuries (Cheng and Ali 1985). Even assuming that blind chickens experience substantially less suffering than they would have experienced without the alteration, it is arguable that such alterations are still morally problematic from the individual's perspective. (For a discussion of similar cases, see Gavrell Ortiz 2004; Comstock 2000, pp. 95–138.) If so, then hedonism with respect to animals cannot be correct.

Second, whatever plausibility hedonism might have when it is applied to animals, it surely has even less plausibility when applied to humans. Since status-enhanced chimeric research subjects may be similar to humans in the morally relevant respects, it is dubious to suppose that hedonism about animals extends to hedonism about chimeric research subjects in the kind of research at issue.

The Mixed Views have the advantage of accommodating the Millian intuition that status enhancement could be good from the individual's perspective even if it had some harmful consequences. Also, the plausible idea that status diminishments are *prima facie* bad from the individual's perspective lends plausibility to the idea that status enhancements are *prima facie* good from the individual's perspective. I thus conclude, albeit tentatively, that one of the Mixed Views is correct.

All that remains, then, is to determine which baseline, the moral or the non-moral, is the relevant one. Consider an example from the literature on exploitation in which a transaction provides someone with a benefit, but with far less benefit than they deserve: an employer who pays an employee a wage that is beneficial compared to the alternatives, but is still substantially less than what justice requires. In such cases, the relevant baseline for the evaluation of the transaction is the moral baseline: given that the employee deserves more, it is no defense of the employer's behavior to say that the employee is better off than he would have been without the job (see Wertheimer 1996 for extended discussion). Similarly, the relevant question for evaluating status-enhancing transplants is surely whether the subject's new entitlements are respected. I therefore conclude that the Mixed View with the Moral Baseline is correct.

ETHICAL AND POLICY IMPLICATIONS

If a Mixed View is correct, then transplants that confer the moral status of a normal human being onto a chimeric research subject are *prima facie* good. But because the relevant baseline is the moral baseline, transplants that enhance an animal's moral status to that of a normal human adult raise the following problem. The view institutionalized by animal research oversight committees is that almost any valid research objective justifies sacrificing even the most fundamental interests of animals (Francione 1995). In contrast, the view institutionalized by human subjects research oversight committees is that humans have a moral status which provides them with substantial moral protections, including a very stringent prohibition on harmful research without informed consent. So long as experiments that involve the xenotransplantation of human stem cells into animals are overseen by animal research oversight committees, or by human subjects committees only attentive to concerns of those who provided the gametes or embryos from which the stem cells were derived, the wrong, or an incomplete, set of moral protections is likely to be afforded to status-enhanced chimeric research subjects. If the relevant baseline were the non-moral baseline, then transplants that enhanced moral status probably would be no more problematic than other kinds of biomedical research on animals. But because the relevant baseline is the moral baseline, sacrificing the fundamental interests of the chimeric research subject as they would have been sacrificed in any other animal research is the moral equivalent of sacrificing the fundamental interests of a fully functional adult human being. On all but the most extreme animal rights views, this makes status-enhancing chimeric research much worse than other biomedical research on animals, and on any plausible view, makes it absolutely unacceptable.

Alternatively, if researchers guaranteed adequate protections for any chimeric research subject whose status had been enhanced to that of a normal adult human, then at least from that individual's perspective, there would be no objection to the research. It is difficult to see, however, how researchers could do that without undermining their research objectives, since most biomedical research on animals involves procedures that plainly would be unacceptable if performed on individuals with the moral status of a normal human adult. And if it were acceptable to do the research on something with the moral status of a normal human adult, then actual humans presumably would provide a better model in which to learn about human development and in which to test possible therapies intended for

human beings. Why then go to the trouble of creating a chimera and introducing the need to ascertain whether the results obtained from the chimera are generalizable to humans?

If adequate research protections cannot be guaranteed, then the Mixed View with the Moral Baseline implies that status-enhancing transplants are unethical. But which transplants run an unacceptably high risk of enhancing the status of the chimeric research subject? The epistemological difficulties here are daunting, especially in light of the moral stakes. How does one know whether the harms being imposed are no more morally problematic than those usually imposed in biomedical research on animals, or, whether despite outward appearances, the harms being imposed amount to the moral equivalent of Nazi-style research? This question is especially problematic on cognitive capacity views of moral status because high-level cognitive capacities do not manifest themselves without substantial care and education, treatment unlikely to be provided to most research animals.

The empirical uncertainties regarding the effects that various kinds of xenotransplants would have and the moral uncertainties regarding which effects would be status-enhancing seem to me to be the crux of the practical problems about how to set an acceptable policy. I conclude by evaluating some of the existing policy proposals from the perspective of the moral status framework.

Karpowicz and his colleagues (2004; 2005) focus their attention on transplants of disassociated retinal and neural stem cells and citing the constraints mentioned above—smaller skull size, shorter gestation period, and nonhuman environment—conclude that such cells would "not be able to achieve human brain size and the human brain organization needed to give rise to human neural functions and behaviors [that form that basis of human dignity] when transplanted to nonhuman hosts" (2005, p. 26). Presumably, such transplants also would not result in a human being and so would not enhance moral status on an anthropocentric view either. The introduction of multipotent, but not pluripotent, stem cells, then, looks promising as a class of research that could be permissible, but there are two issues that need to be resolved.

The first is the empirical issue already mentioned, namely whether retinal and neural stem cells could revert to a more pluripotent state by being introduced into an embryonic environment. The second has both an empirical and an ethical component. As previously discussed, the list of robust cognitive capacities that Karpowicz and his colleagues say are necessary for human dignity and its associated moral status looks exces-

sively demanding. So even if a transplant would not confer all of those cognitive capacities, the question remains whether it nonetheless might confer cognitive capacities that, although less robust, are still sufficient for significantly enhancing the moral status of the chimeric research subject. If the answer to both of those question is no, then such transplants would seem to be acceptable within the moral status framework. Transplants of other non-neural, multipotent stem cells, such as hematopoetic stem cells, presumably would be even easier to justify.

More difficult questions arise for transplants of hES cells, which are pluripotent and not merely multipotent, and still more difficult questions arise for transplants of hES cells during the embryonic or early fetal stages of development. As already discussed, such transplants might induce changes in the animal that would alter features of the animal that otherwise would have constrained the transplant's effects.

Regarding the introduction of hES cells into developing animals, the NAS Committee proposes special review by a newly instituted committee, the Embryonic Stem Cell Research Oversight (ESCRO) Committee. The ESCRO Committee would be an important institutional mechanism for assuring that the kinds of considerations raised in this article, which fall outside the types of concerns normally addressed by animal care and use committees or human subjects committees, would have an opportunity to be addressed. The ESCRO Committee's special review would address the following:

> the number of hES cells transferred, what area of the animal body will be involved, and whether the cells might migrate through the animal's body. The hES cells may affect some animal organs rather than others, raising questions about the number of organs affected, how the animal's functioning would be affected, and whether some valued human characteristic might be exhibited in the animal, including physical appearance. (NAS 2005, p. 41)

These are quite general considerations, and, with the exception of physical appearance, which is irrelevant to moral status, the moral status framework offers a constructive way to sharpen them. What effects would a transplant have to have in order to significantly enhance the moral status of the chimeric research subject? What is the likelihood that a given transplant would have those effects? And would the researcher be able to provide adequate research protections for the resulting chimeric individual, were the research to proceed?

With respect to the introduction of hES cells into embryonic animals, the NAS Committee's guidelines include a ban on the introduction of any hES cell into a nonhuman primate blastocyst (NAS 2005, pp. 47–48). The

restricted focus on hES cells appears not to represent a substantive claim that transplants of more specialized human stem cells are unproblematic. The restriction instead appears to be an artifact of the Committee's restricted mandate (NAS 2005, p. 4). The Committee explicitly says that other kinds of human stem cells can raise issues similar to those raised by hES cell transplants. And if pluripotent human stem cells were to become available from another source besides human embryos, these too would surely raise the issues highlighted by the moral status framework.

According to the moral status framework, this ban is both overly permissive and unnecessarily restrictive. It is overly permissive because the moral status framework would not sharply distinguish between primate and nonprimate blastocysts or between blastocyst stages and slightly later developmental stages. If one introduces enough pluripotent human stem cells into an animal embryo, primate or otherwise, one could, in principle at least, end up with a human inner cell mass surrounded by a nonhuman trophectoderm, affecting both its species and its potential to develop robust cognitive capacities. Furthermore, because brain development occurs after the blastocyst stage, it seems likely that even a transplant that occurred after the blastocyst stage still could affect the characteristics relevant to the individual's cognitive capacities.

From the perspective of the moral status framework, the ban on introducing pluripotent human stem cells into a nonhuman primate blastocyst is also unnecessarily restrictive. From an anthropocentric view of moral status, a transplant would not significantly enhance the individual's moral status so long as two conditions are met. First, the number of cells introduced into the animal blastocyst is sufficiently low so that the original transplant itself does not constitute the creation of a human being. Second, the chimeric individual is terminated before the human cells increase in sufficient proportion to result in the entity's being deemed a human being. From a cognitive capacity view of moral status, early termination prior to the onset of consciousness would ensure that a transplant would not significantly enhance the individual's moral status. So on either view, a general ban on the introduction of pluripotent human stem cells into a nonhuman primate blastocysts is overly broad. The Committee's position cannot reflect any general concern about early termination policies and potential entanglement in the abortion debate since it requires early termination of *human* embryos used in research at 14 days of development, or the appearance of the primitive streak, whichever comes earlier (NAS 2005, p. 46). If an early termination policy is acceptable with respect to

human embryos, it surely is acceptable with respect to chimeric embryos, even those with some human pluripotent stem cells in them.

Any early termination policy that permits the creation of human beings but requires termination prior to the onset of any cognitive capacities, as the Committee's policy does with respect to human embryos, will be acceptable according to cognitive capacity views of moral status, but not according to anthropocentric views. Such policies therefore will directly entangle the chimera policy debate with the abortion policy debate. This is surely an unwelcome result for proponents of this research, but perhaps is unavoidable if certain lines of research are to be pursued.

At any rate, in the absence of an early termination policy, a general ban on the introduction of pluripotent human stem cells into nonhuman primate blastocysts is a reasonable response to the present empirical and ethical uncertainties. One might object to such a ban that, in the face of uncertainty, potentially beneficial research should be allowed to proceed rather than be restricted, but in other areas of basic and therapeutic biomedical research, experiments that pose risk of serious harm to individuals with the moral status of normal human adults can only be carried out once they have been shown to be reasonably safe. That is, there is a clear moral requirement to perform such experiments in animals first, even if doing so slows down research and the provision of medical benefits. Given the uncertainties as to which human stem cell transplants into embryonic or early fetal animals would result in research on something that has the moral status of a normal adult human, it seems reasonable to require, in a similar fashion, that further research be done on the transplantation of *animal* pluripotent stem cells into embryonic or early fetal animals before similar work is done with human pluripotent stem cells. If such research confirms the view that some transplants of pluripotent human stem cells into early-stage developing animals will not substantially enhance the individual's moral status, then the moral problems discussed here will be laid to rest regarding those transplants. And if such research disconfirms the view, then it is surely best to know that before the research goes any further.

For their many helpful comments on this topic, I thank the University of Wisconsin Bioethics Advisory Committee, Antonio Rauti, Alan Rubel, Brad Majors, Mark Brown, Justine Wells, Sara Gavrell Ortiz, Christopher Ciocchetti, Norm Fost, Alta Charo, Su-Chun Zhang, Clive Svendsen, Ian Duncan, Dan Hausman, Elliott Sober, the chimera discussion group at Bioethics Retreat 2004, and anonymous reviewers for the *Kennedy Institute of Ethics Journal*. This material is based upon work supported by the University of Wisconsin Graduate School.

NOTES

1. Karpowicz and colleagues (2005, p. 121) seem to adopt something close to the Cognitive Capacity Principle. They claim that it is always wrong to confer the physical basis for high-level cognitive capacities onto a individual unable to exercise those capacities to a significant degree because, in so doing, the researcher "would diminish or eliminate the very capacities associated with human dignity." But if conferring the physical basis also confers the capacities, then the individual's capacities are enhanced in such cases, not eliminated or diminished, compared to what they would have been. And if the physical basis is present without the high-level cognitive capacities being present, then it is still not true that the researcher eliminated or diminished the capacities, since they were never there to begin with.
2. One way to try to avoid being blatantly anthropocentric and yet still accord equal moral status to all human beings is to hold a view that attributes equal moral status to all individuals that are members of species, the normal adult members of which have high-level cognitive capacities (Fox 1978, p. 110). Such a view, however, suffers an explanatory gap of its own: why should mere membership in the same species as individuals who have high-level cognitive capacities confer equal moral status on those members who do not have high-level cognitive capacities?
3. Indeed, the Millian view is even too extreme for Mill (1979 [1861], p. 9), who agrees that some people's lives are filled with "unhappiness so extreme" that they would be better off exchanging "their lot for almost any other, however undesirable in their own eyes."

REFERENCES

AAP. Australian Associated Press. 2001. Scientists to Use Stem Cells to Fix Brain Damage. AAP Newsfeed, 1 December.

Basu, Paroma. 2005. Scientists Grow Critical Nerve Cells. Available at *http://www.news.wisc.edu/packages/stemcells/10648.html*. Accessed 25 October 2005.

Brüstle, Oliver. 1999. Building Brains: Neural Chimeras in the Study of Nervous System Development and Repair. *Brain Pathology* 9: 527–45.

Cao, Yilin; Vacanti, Joseph P.; Paige, Keith T.; et al. 1997. Transplantation of Chondrocytes Utilizing Polymer-Cell Construct to Produce Tissue-Engineered Cartilage in the Shape of a Human Ear. *Plastic and Reconstructive Surgery* 100: 297–302.

Cheng, Kimberly M., and Ali, Ahmed. 1985. Early Egg Production in Genetically Blind (rc/rc) Chickens in Comparison with Sighted (RC+/rc) Controls. *Poultry Science* 65: 789–94.

Cheng, K. M.; Shoffner, R. N.; Gelatt, K. N.; et al. 1980. An Autosomal Recessive Blind Mutant in the Chicken. *Poultry Science* 59: 2179–82.

Comstock, Gary. 2000. *Vexing Nature? The Ethical Case against Agricultural Biotechnology*. Boston: Kluwer Academic Publishers.

Cooper, David. 1998. Intervention, Humility and Animal Integrity. In *Animal Biotechnology and Ethics*, ed. Alan Holland and Andrew Johnson, pp. 145–55. London: Chapman and Hall.

DeGrazia, David. 1996. *Taking Animals Seriously: Mental Life and Moral Status*. Cambridge: Cambridge University Press.

Devine, Philip. 1978. *The Ethics of Homicide*. Ithaca, NY: Cornell University Press.

DeWitt, Natalie. 2002. Biologists Divided Over Proposal to Create Human-Mouse Embryos. *Nature* 420: 255.

Duncan, Ian. 2001. Animal Welfare Issues in the Poultry Industry: Is There a Lesson to Be Learned? *Journal of Applied Animal Welfare Science* 4: 207–21.

Feinberg, Joel. 1980. Abortion. In *Matters of Life and Death*, 2d ed., ed. Tom Regan, pp. 256–93. New York: Random House.

Flake, Alan, and Zanjani, Esmail. 1999. In Utero Hematopoietic Stem Cell Transplantation: Ontogenic Opportunities and Biologic Barriers. *Blood* 94: 2179–91.

Fox, Michael. 1978. "Animal Liberation": A Critique. *Ethics* 88: 106–18.

Francione, Gary L. 1995. *Animals, Property, and the Law*. Philadelphia: Temple University Press.

Gavrell Ortiz, Sara. 2004. Beyond Welfare: Animal Integrity, Animal Dignity, and Genetic Engineering. *Ethics and the Environment* 9 (1): 94–120.

Geron Ethics Advisory Board. 1999. Research with Human Embryonic Stem Cells: Ethical Considerations. *Hastings Center Report* 29 (2): 30–36.

Goldstein, Ronald S.; Drukker, Micha; Reubinoff, Benjamin E.; and Nissam Benvenisty. 2002. Integration and Differentiation of Human Embryonic Stem Cells Transplanted to the Chick Embryo. *Development Dynamics* 225: 80–86.

Kaiser Daily Reproductive Health Report. 2002. Stem Cell Researchers at New York Forum Disagree About Creation of Mixed-Species Embryos. Available at *http://www.kaisernetwork.org/daily_reports/print_report.cfm?DR_ID=14743&dr_cat=2*. Accessed 25 October 2005.

Karpowicz, Phillip; Cohen, Cynthia; and van der Kooy, Derek. 2004. Is It Ethical to Transplant Human Stem Cells into Nonhuman Embryos. *Nature Medicine* 10: 331–35.

———. 2005. Developing Human-Nonhuman Chimeras in Human Stem Cell Research: Ethical Issues and Boundaries. *Kennedy Institute of Ethics Journal* 15: 107–34.

Krieger, Lisa. 2002. Scientists Put a Bit of Man into a Mouse. *Mercury News*. Available at *http://www.mercurynews.com/mld/mercurynews/4698610.htm*. Accessed 24 August 2004.

Mill, John Stuart. 1979 [1861]. *Utilitarianism*, ed. George Sher. Indianapolis: Hackett Publishing Company.

Nagy, Andras, and Rossant, Janet. 2001. Chimeras and Mosaics for Dissecting Complex Mutant Phenotypes. *International Journal of Developmental Biology* 45: 577–82.

NAS. National Academy of Sciences. Committee on Guidelines for Human Embryonic Stem Cell Research. 2004. Workshop on Guidelines for Human Embryonic Stem Cell Research, 12–13 October. Washington, DC.

———. 2005. Guidelines for Human Embryonic Stem Cell Research. Washington, DC: National Academies Press.

NIH. National Institutes of Health. 2000. National Institutes of Health Guidelines for Research Using Human Pluripotent Stem Cells. Available at *http://www.bioethics.gov/reports/stemcell/appendix_d.html*. Accessed 25 October 2005.

Noonan, John T., Jr. 1970. *The Morality of Abortion*. Cambridge, MA: Harvard University Press.

Okarma, Thomas. 2001. Human Embryonic Stem Cells: A Primer on the Technology and Its Medical Applications. In *The Human Embryonic Stem Cell Debate*, ed. Suzanne Holland, Karen Lebacqz, and Laurie Zoloth, pp. 3–13. Cambridge, MA: MIT Press.

Parfit, Derek. 1984. *Reasons and Persons*. Oxford: Clarendon Press.

Regan, Tom. 1978. Fox's Critique of Animal Liberation. *Ethics* 88: 126–33.

Robert, Jason Scott, and Baylis, Françoise. 2003. Crossing Species Boundaries. *American Journal of Bioethics* 3 (3): 1–13.

Rollin, Bernard E. 1998. On *Telos* and Genetic Engineering. In *Animal Biotechnology and Ethics*, ed. Alan Holland and Andrew Johnson, pp. 156–71. London: Chapman and Hall.

Schwarz, Stephen. 1990. *The Moral Question of Abortion*. Chicago: Loyola University Press.

Shreeve, Jamie. 2005. The Other Stem-Cell Debate. *New York Times Magazine* (10 April): 42–47.

Smyth, J. R., Jr.; Boissey, R. E.; Gawron, M. F. 1977. An Inherited Delayed Amelanosis with Associated Blindness in the Domestic Fowl. *Poultry Science* 56: 1758.

Streiffer, Robert. 2003. In Defense of the Moral Relevance of Species Boundaries. *American Journal of Bioethics* 3 (3): 37–38.

———, and Thomas Hedemann. 2005. The Political Import of Intrinsic Objections to Genetically Engineered Food. *Journal of Agricultural and Environmental Ethics* 18: 191–210.

Svendsen, Clive. 2002. Stem Cells. In *A Companion to Genethics*, ed. Justine Burley and John Harris, pp. 7–17. Malden, MA: Blackwell Publishing Ltd.

Tang, Ya-Ping; Shimizu, Eiji; Dube, Gilles Ru.; et al. 1999. Genetic Enhancement of Learning and Memory in Mice. *Nature* 401: 63–69.

Thompson, Paul. 1997. *Food Biotechnology in Ethical Perspective*. London: Blackie Academic and Professional.

Thomson, James. 2001. Human Embryonic Stem Cells. In *The Human Embryonic Stem Cell Debate*, ed. Suzanne Holland, Karen Lebacqz, and Laurie Zoloth, pp. 14–26. Cambridge, MA: MIT Press.

Thomson, Judith Jarvis. 1987. Ruminations on an Account of Personal Identity. In *On Being and Saying: Essays for Richard Cartwright*, ed. Judith Jarvis Thomson, pp. 215–40. Cambridge, MA: MIT Press.

———. 1997. People and their Bodies. In *Reading Parfit*, ed. Jonathan Dancy, pp. 202–29. Oxford: Blackwell Publishers.

University of Wisconsin, Bioethics Advisory Committee. 1999. Final Report of the Bioethics Advisory Committee on Human Stem Cell Research at the University of Wisconsin-Madison. Available at *http://www.news.wisc.edu/packages/stemcells/bac_report.html*. Accessed 25 October 2005.

———. 2001. Second Report of the Bioethics Advisory Committee on Human Stem Cell Research at the University of Wisconsin-Madison. Available at *http://www.news.wisc.edu/packages/stemcells/bac_report2.html*. Accessed 25 October 2005.

VanDeVeer, Donald. 1979. Interspecific Justice. *Inquiry* 22: 55–70.

Wagers, Amy; Sherwood, Richard; Christensen, Julie, and Weissman, Irving. 2002. Little Evidence of Developmental Plasticity of Adult Hematopoietic Stem Cells. *Science* 297: 2256–59.

Weiss, Rick. 2004. Of Mice, Men and In-Between: Scientists Debate Blending of Human, Animal Forms. *Washington Post* (20 November): A01.

Wertheimer, Alan. 1996. *Exploitation*. Princeton, NJ: Princeton University Press.

WiCell. 2001. Memorandum of Understanding. Available at *http://www.news.wisc.edu/story.php?get=6457*. Accessed 25 October 2005.

Zhang, Su-Chun; Wernig, Marius; Duncan, Ian; et al. 2001. *In Vitro* Differentiation of Transplantable Neural Precursors from Human Embryonic Stem Cells. *Nature Biotechnology* 19: 1129–33.

Hagglund,

Patentability of Human-Animal Chimeras,

25 SANTA CLARA COMP. & HIGH TECH. L. J. 51 (2008)

25 SANTA CLARA COMP. & HIGH TECH. L. J. 51

ARTICLE: PATENTABILITY OF HUMAN-ANIMAL CHIMERAS, 25 Santa Clara Computer & High Tech. L.J. 51 (Copy citation)

November, 2008

Reporter: **25 Santa Clara Computer & High Tech. L.J. 51**

Length: 26974 words

Author: Ryan Hagglund*

LexisNexis Summary

… Still others argue that the creation of chimeras violates the rights of human and animal species to exist in an uncorrupted manner and denigrates humanity by commingling human and animal organisms. … These rejections were based on one of two grounds: (1) considering living things unpatentable products of nature under the product of nature doctrine, which excludes products of nature from the realm of patentable subject matter; and (2) rejecting the idea that living things are patentable subject matter under § 101 because Congress provided for plant patents separately in the 1930 Plant Patent Act and the 1970 Plant Variety Protection Act, apparently indicating that plants and other living things were not covered by § 101 and that Congress intended for plants covered by these statutes to be the only living things afforded intellectual property protection. … While the issuance of these patents indicates that even the PTO believes that the mere presence of human genes or individual cells derived from humans in an invention does not render it unpatentable, an argument for the patentability of human-animal chimeras, especially those that are considered human, is less than convincing because chimeras (and full human beings) are readily distinguishable from transgenic animals and cells. … Nonetheless, the above analysis suggests that a court would likely find that Congress's intent to restrict patentability of human organisms in enacting the Weldon Amendment, an appropriations act, did not constitute the clear and manifest intent to repeal or modify § 101 as required by the Supreme Court and, accordingly, that the Weldon Amendment does not wholly foreclose the patentability of human organisms, including human-animal chimeras considered human. … Thus, as patents do not confer possessory rights in the patented article, the intangible property rights that inhere in a patent as well as markets in patent rights do not impinge on the autonomy of a patented human creature itself. … Given the vestigial nature of the moral utility doctrine, courts will not likely apply it to defeat the patentability of human inventions or human animal-chimeras. … Some also object to chimera research because they feel that the commingling of human embryos with animal embryos, potentially conferring human characteristics on animals, offends the dignity of humanity. … As the Supreme Court concluded in Chakrabarty, policy arguments raised against the patentability of living things, such as human-animal chimeras, are best addressed by Congress and that courts should not foreclose the patentability of inventions that are included within the scope of patentable subject matter under § 101 on such grounds.

Highlight

The chimera was a mythological creature with a lion's head, goat's body, and serpent's tail. Because of recent advances in biotechnology, such permutations on species are no longer the stuff of myth and legend. The term "chimera" has come to describe a class of genetically engineered creatures composed of some cells from one species, which thus contain genetic material derived entirely from that species, and some cells from another species, containing only genetic material from that species. Scientists have created a goat-sheep chimera or "geep" which exhibits physical characteristics of both animals. Likewise, scientists have also used the tools of modern molecular biology to create human-animal chimeras containing both human and animal cells. While none of the human-animal chimeras hitherto created have exhibited significant human characteristics, the synthesis of human-animal chimeras

raises significant ethical concerns. The advent of human-animal chimera technology naturally raises the issue of whether development of human-animal chimeras should be encouraged by the issuance of patents to inventors of human-animal chimeras.

This article explores the patentability of human-animal chimeras. First, it surveys the law governing the patentability of living things. Prior to the 1970s, the courts evinced great hostility to the patentability of living things. However, courts became more amenable to the patentability of living things and have held that manmade living things, such as microorganisms, plants, and animals, that do not appear in nature are patentable subject matter under 35 U.S.C. § 101. Although the federal courts have never passed on the patentability of human-animal chimeras or other forms of human inventions, the United States Patent and Trademark Office (PTO) has **[52]** indicated that human inventions are unpatentable and rejected an application claiming certain human-animal chimeras because the broadest reasonable interpretation of the claimed chimeras encompassed a human being. Furthermore, although Congress has failed to expressly exclude human beings from the scope of patentable subject matter under § 101, Congress has restricted the patentability of human organisms through its appropriations power by enacting the Weldon Amendment that proscribed the use of federal funds provided for the operation of the PTO for the issuance of patents on human organisms. However, no statutory or constitutional source provides a definition for humanity despite the obvious importance of one in the human-animal chimera context. This article also evaluates various proposed standards for a chimera to qualify as human and concludes that the preferred standard that best reflects moral, intuitional, and biological conceptions of humanity classifies an organism as human if it is characterized by the higher mental faculties and physical characteristics associated with human beings to a significant degree.

Under the Supreme Court's broad interpretation of § 101 in holding living things patentable, indicating that anything made by man is patentable subject matter, human-animal chimeras, including those considered human, as well as other human inventions are patentable subject matter. Despite Congress's apparent attempt to foreclose the patentability of human inventions using its appropriations power, analysis of the patent law and the Weldon Amendment and its legislative history indicates that Congress did not intend to create a conflict with § 101's broad scope of patentable subject matter when it enacted the Weldon Amendment. Thus, a court would likely hold that the Amendment did not completely foreclose patentability of human inventions. Likewise, a patent for a human invention does not run afoul of the Thirteenth or Fourteenth Amendments. Furthermore, human-animal chimeras satisfy the patent law's utility requirement inasmuch as they have practical utility and would not be found unpatentable under the moral utility doctrine. Similarly, the patent law doctrines of novelty and nonobviousness do not foreclose the patentability of human-animal chimeras. Therefore, at least some human-animal chimeras may be patentable under some circumstances, and this universe of potentially patentable human-animal chimeras may include chimeras that are considered human.

Text

[53]

Introduction

In Greek mythology, the chimera was a monster that breathed fire and had a lion's head, goat's body, and serpent's tail. [1] While this creature remains a thing of myth and legend, recent advances in biotechnology have allowed scientists to create permutations of species that are eerily similar to the mythological chimera. [2] Today, the term "chimera" is used to describe one of these permutations. The precise nature of a chimera is most readily explained by comparing it to the hybrid, a more commonly known biotechnological invention. [3] A hybrid is the result of a genetic cross between a male of one species and a female of another. [4] Accordingly "every cell in hybrid contains one set of chromosomes from one species and one set from another." [5] Thus, 50% of the genetic material in each cell of a hybrid, and accordingly 50% of the genetic material of the entire hybrid animal, is derived from one species while the other 50% is derived from another species. In a chimera, the genetic material of the two species used to engender the creature does not mix in the same cell. [6] Rather, a chimera is composed of some cells from one species, which contain genetic material derived entirely from that species, and some cells from another species containing only genetic material from that species. [7] Thus, the cells in a chimeric animal always remain segregated by species, and no cell contains genetic material from both species. [8] For instance, the brain of a human-chimpanzee chimera might contain

some human neurons and some chimpanzee neurons, but none of the neurons would contain both human and chimpanzee genetic material. [9]

One method of creating chimeras entails collecting embryos from recently impregnated females of each species to be represented in the chimera at the eight-cell-stage of development. [10] The embryos are combined by a grafting procedure where the two embryos, one of **[54]** each species to be represented in the chimera, are pushed together and fuse when they are incubated at 37 [degrees] C. [11] The fused embryo begins to grow and divide in vitro as one embryo. [12] When the embryo reaches the blastocyst stage of development, it is implanted into the womb of a pseudopregnant female, a naturally impregnated pregnant female from which the naturally created embryo has been surgically removed, of one of the species present in the chimera. [13] This female serves as a surrogate mother for the chimeric animal as it develops and is born naturally. [14] The chimeric animal contains some cells that are derived from each species used to engender it and thus displays characteristics of both animals. This embryo fusion technique was first successfully used in 1984 when scientists created a goat-sheep chimera or "geep." [15] Parts of the "geep" derived from the goat portion of the chimeric embryo were hairy, and those that grew from the sheep portion were wooly. [16]

The embryo fusion technique is not the only method that researchers employ to create chimeras. For instance, chimeras can also be generated by injecting stem cells of one species into an embryo, fetus, or even a newborn of another species. [17] However, chimerism in chimeras created by this method is of a lesser extent than in those created by the fusion method, and cells derived from the injected cells are usually restricted to certain organs and/or are present in small numbers. [18] Furthermore, the simplest and oldest technique **[55]** for generating a chimera is xenotransplantation, the transplantation of organs from one species to another. [19]

Prior to the advent of modern molecular biology, humans created human-animal chimeras inasmuch as clinical xenotransplantation has long been used for medical treatment. [20] For instance, pig and cow heart valves are used to replace faulty human heart valves. [21] Formally speaking, such a surgery makes the recipient a chimera, although nobody would seriously dispute that the recipient is anything other than human. [22]

Scientists have also used the tools of modern molecular biology to create human-animal chimeras of the types discussed above. In 2003, Chinese scientists created human-rabbit chimeric embryos. [23] The embryos were allowed to develop for several days in a laboratory dish before the scientists destroyed them to harvest their stem cells for research purposes. [24] Also, a human-sheep chimera was created by injecting a sheep fetus with human stem cells half-way though gestation, too early for the animal's immune system to have developed to reject the human cells, but after the animal's body plan had formed. [25] Thus, the resulting animals look like normal sheep rather than strange chimeras having the physical characteristics of both species such as the "geep," which are created by embryo fusion at an early stage before either embryo develops a body plan. [26] However, these sheep have human cells in some organ systems and have livers that are 7 to 15% human. [27] Scientists have created other human-animal chimeras, including pigs with human blood formed by injecting human blood-forming cells into pig fetuses and mice with brains containing 1% human cells by injection of human neural stem cells into the brains of fetal or newborn mice. [28] Research is currently under way at Stanford University to create a mouse with an entirely **[56]** human brain by injecting human neural stem cells into mouse embryos. [29]

The synthesis of human-animal chimeras creates significant ethical dilemmas. On one hand, human-animal chimeras have the potential to create great benefits for the human race. They may become valuable sources of organs for transplantation into humans as a human is less likely to reject a chimeric organ than an animal organ. [30] Furthermore, chimeras will be useful in research as models for studies on human embryonic development and on the effects of pharmaceutical agents on humans. [31] Despite the potential benefits of this technology to human health, human-animal chimera research raises legitimate ethical concerns. Some consider any research involving manipulation of human embryos morally wrong, and chimera research may lead to needless human and animal suffering stemming from both research and potential deformities resulting from chimerism. [32] This point is especially important with regard to chimeras that have enough human genetic material or characteristics to qualify as human. These chimeras would receive heightened legal rights and protections, and it would be fantastic to believe that scientists could compel a chimera that was predominately human, such as a xenotransplant recipient, to involuntarily act as a research subject. Some people also raise a religious objection to synthesis of chimeras, arguing that the creation of new types of animals should only be

the province of God. [33] Still others argue that the creation of chimeras violates the rights of human and animal species to exist in an uncorrupted manner and denigrates humanity by commingling human and animal organisms. [34] Finally, there is much debate over the standard for determining whether a chimera is sufficiently human to constitute a human being entitled to the legal rights extended to humans both in terms of the factors considered in assessing the humanity of an organism and the degree of humanity required for an organism to be classified as human. [35]

[57] As chimera research continues, the courts and Congress will have to determine whether they should encourage development of chimeras by permitting the creators of human-animal chimeras to obtain patents for them, thus allowing the creators to assert exclusive rights to make and use the chimeras. In Diamond v. Chakrabarty, [36] the Supreme Court held that living things created by man that do not occur in nature are patentable subject matter in the recombinant microorganism context. [37] This holding has subsequently been extended to multicellular organisms such as plants and animals. [38] Human-animal chimeras are living things that do not occur in nature and are created by man, and nothing in the language of any opinions addressing the patentability of living things suggests that human-animal chimeras or even non-naturally occurring fully-human organisms, such as transgenic humans, are not patentable. Nonetheless, the United States Patent and Trademark Office (PTO) rejected the first and only patent application for a human-animal chimera reasoning that it encompassed a human and that human organisms are not patentable subject matter. [39] Furthermore, Congress has used its appropriations power to prohibit the PTO from using federal funds for the issuance of patents on human organisms, seemingly foreclosing their patentability. [40] However, Congress failed to define human organisms and thus did not articulate a standard for determining whether a particular chimera is sufficiently human to constitute a human.

This Article explores the patentability of human-animal chimeras. [41] Part I traces the evolution of the treatment of the patentability of living things by the courts and the PTO. It also describes the PTO's handling and rejection of an application for a patent for a human-animal chimera and Congressional action related to the patentability of human-animal chimeras. Part II analyzes the **[58]** patentability of human-animal chimeras and related human inventions. Part II initially examines the confusion surrounding the definition of humanity to be applied in the chimera context and concludes that the definition which best reflects modern conceptions of humanity is one that considers a chimera that possesses significant human cognitive and physical characteristics human. Then, it explores the patentability of human-animal chimeras in light of the Patent Act and case law interpreting it. First, it concludes that under conventional patent law, human-animal chimeras, even those chimeras considered human, constitute patentable subject matter. Second, given this conclusion, Part II suggests that Congress's withholding of federal funds from the PTO for the issuance of human patents does not necessarily foreclose the patentability of chimeras considered human. Third, Part II examines whether the rights conferred by the Thirteenth and Fourteenth Amendments proscribe the patentability of human-animal chimeras considered human and determines they do not. Finally, Part II concludes that the statutory patentability requirements of utility, novelty, and nonobviousness may be met in the human-animal chimera context. Thus, it appears that at least some human-animal chimeras are patentable.

I. Patentability of Living Things

The scope of patentable subject matter is defined in 35 U.S.C. § 101. [42] In order to be eligible for patent protection, an invention must fall within one of the statutory categories of process, machine, manufacture, or composition of matter. [43] Congress deliberately crafted these categories to be broad, and they seldom pose an obstacle to an inventor's endeavors to patent his invention. [44] However, the Supreme Court has determined that certain categories of invention or discovery, including laws of nature, products of nature, physical or natural phenomenon, abstract ideas, and unapplied mathematical **[59]** algorithms, exceed the statutory boundaries of patentable subject matter. [45]

This Part surveys the treatment of the patentability of living things by courts and the PTO. First, it describes the situation prior to the Supreme Court's 1980 decision in Chakrabarty. Second, this Part explores the Chakrabarty Court's reasoning in holding that manmade microorganisms that do not occur in nature are patentable. Third, this Part traces the extension of this holding to multicellular organisms, such as plants and animals, by courts and the PTO. Fourth, the PTO's rejection of a patent for human-animal chimeras is examined. Finally, this Part describes the role Congress has hitherto taken in regulating the patentability of chimeras.

A. Historical Treatment of the Patentability of Living Things

In 1873, the PTO issued the first American patent for a living thing to Louis Pasteur for purified yeast as an article of manufacture under § 101. [46] However, prior to Chakrabarty, patents for living organisms independent of their use, such as the one issued to Pasteur, were very much an anomaly. [47] The PTO and the courts almost **[60]** invariably rejected patents that pertained to living organisms regardless of whether the organism is found in nature in the form claimed or not. [48] These rejections were based on one of two grounds: (1) considering living things unpatentable products of nature under the product of nature doctrine, which excludes products of nature from the realm of patentable subject matter; [49] and (2) rejecting the idea that living things are patentable subject matter under § 101 because Congress provided for plant patents separately in the 1930 Plant Patent Act [50] and the 1970 Plant Variety Protection Act, [51] apparently indicating that plants and other living things were not covered by § 101 and that Congress intended for plants covered by these statutes to be the only living things afforded intellectual property protection. [52]

B. Diamond v. Chakrabarty-Patentability of Microorganisms as Living Subject Matter

In Chakrabarty, the Supreme Court directly addressed the question of whether living things that did not occur in nature themselves were patentable subject matter. The patentee created a genetically engineered bacterium capable of degrading crude oil by introducing certain plasmids harboring genes that confer the ability to break down multiple components of crude oil into a naturally occurring strain of bacteria which had no capacity to degrade crude oil. [53] The Court held that a living non-natural microorganism is patentable subject matter under § 101. [54] Also, the Court noted that, in choosing terms such as "manufacture" and "composition of matter," **[61]** Congress contemplated that patent laws be given wide scope based on the common usages of these terms. [55] This was consistent with the legislative history of the Patent Act indicating that "Congress intended statutory subject matter to "include anything under the sun that is made by man.'" [56] For instance, the Court adopted a broad definition of "manufacture" as an article produced "for use from raw or prepared materials by giving these materials new forms, qualities, properties, or combinations, whether by hand-labor or by machinery." [57] Similarly, the Court defined "composition of matter" to include "all compositions of two or more substances and … all composite articles, whether they be the results of chemical union, or of mechanical mixture, or whether they be gases, fluids, powders or solids." [58] The genetically engineered bacterium in the case plainly met both of these definitions as it had different properties than the naturally occurring bacterium, which served as a raw material in its production, and it was a composition of the original bacterium and the plasmids. Thus, the Court concluded that the bacterium was patentable subject matter because it was a non-naturally occurring manufacture or composition of matter - "a product of human ingenuity "having a distinctive name, character, [and] use'" from the natural bacterium from which it was synthesized. [59]

In addition, the Court distinguished Funk Brothers Seed Co. v. Kalo Inoculant Co., [60] where it rejected the patentability of a mixture of naturally occurring bacteria used by farmers for their natural ability to help plants fix nitrogen. [61] The Funk Bros. Court reasoned that the patentee did not create any new bacteria and, when mixed together, the bacteria performed the same function they performed in nature. [62] In contrast, the genetically engineered bacterium at issue in Chakrabarty was a new bacterium with markedly different characteristics from any bacterium appearing in nature. [63] Therefore, **[62]** the Court concluded that the non-naturally occurring genetically engineered bacterium did not constitute an unpatentable product of nature because its creation was the patentee's handiwork rather than nature's. [64]

Furthermore, the Court also rejected the argument that Congress needed to expressly authorize protection for this new subject matter because it was not contemplated when the patent laws were enacted, reasoning that it was encompassed by the broad scope of the statutes that Congress already had authorized precisely because inventions are often unforeseeable. [65] A rule that unanticipated inventions cannot be patented would conflict with the core concept of patent law that anticipation undermines patentability. [66]

The Court also rejected the argument that Congress had impliedly excluded living organisms from patentability by enacting the 1930 Plant Patent Act [67] and the 1970 Plant Variety Protection Act, [68] which provided for intellectual property protection for plants but not other living things, because these acts would have been unnecessary if living things, such as plants, were patentable by evaluating the legislative history of these acts. [69] Nothing in the language of the legislative histories of either act

suggested that Congress enacted them because it believed § 101 did not include living things. [70] The Court noted that Congress believed that the work of the plant breeder in aid of nature constituted a patentable invention and that these acts were passed to ensure intellectual property protection for plants in the face of two factors that were impairing the patenting of plants: (1) the belief evinced by the PTO that plants, even artificially bred ones, were products of nature and (2) "the fact that plants were thought not amenable to the "written description' requirement of the patent law." [71] Furthermore, the legislative history indicated Congressional acknowledgment that a plant discovery resulting from artificial breeding, not repeated in nature or reproduced by nature and unaided by man, is different than the discovery of a product of nature, such as a mineral, which is created wholly without **[63]** the assistance of man. [72] Therefore, the Court concluded that the statutes specifically protecting plants draw a distinction between products of nature, whether living or not, and manmade inventions, rather than between living and inanimate things. [73] Since genetically engineered bacteria are the result of human ingenuity and research, the mere existence of statutes specifically protecting plants does not support the conclusion that genetically engineered bacteria are not patentable. [74]

Finally, the Court rejected arguments against patentability of genetically engineered microorganisms based on assertions "that genetic research … may spread pollution and disease, that it may result in a loss of genetic diversity, and that its practice may tend to depreciate the value of human life." [75] The Court reasoned Congress was better suited to make such policy determinations. [76]

The Court's decision in Chakrabarty was a seminal event in the evolution of patent law. The Court repudiated the rationales previously employed to reject patents claiming living things. Furthermore, the Court's broad language permitting patents for any living thing created by man seemed to permit patents for all living things, including larger organisms such as plants, animals, and even humans.

C. Extension of Chakrabarty to Multicellular Organisms

1. Plants

In 1985, the Board of Patent Appeals and Interferences (Board) applied the Supreme Court's reasoning in Chakrabarty in holding that an artificially bred corn plant that contained abnormally high levels of the amino acid tryptophan was patentable subject matter under § 101. [77] The Board reasoned that in light of Chakrabarty, the scope of § 101 encompassed manmade life forms, including plant life. [78] Furthermore, the Board noted that the Chakrabarty Court's analysis of the acts giving specific intellectual property protection to plants clarified that the legislative intent was to extend intellectual property protection to plant breeders who were hindered in procuring patents **[64]** and that it did not evince an intent to limit the scope of patentable subject matter under § 101. [79] Thus, the availability of plant-specific protection did not foreclose the availability of patent protection for manmade plants. [80]

In 2001, the Supreme Court adopted the Board's position and explicitly held that artificially developed plant breeds were patentable subject matter under § 101 in J.E.M. Ag Supply, Inc. v. Pioneer Hi-Bred International, Inc. [81] The Court reaffirmed its conclusions in Chakrabarty that the patent laws were to be given wide scope considering the broad language Congress employed in § 101 and that the relevant distinction in determining the patentability of a living thing is not between living and inanimate things, but between products of nature, living or not, and manmade inventions. [82] Thus, the Court concluded that artificially bred plants were patentable subject matter because they fall within the broad terms of § 101 that include manufactures and compositions of matter. [83] Furthermore, the Court adopted the Board's conclusion that the statutes providing for plant-specific intellectual property protection do not limit the scope of patentable subject matter under § 101 because Congress did not give any indication that it intended to do so, and accordingly, such statutes do not foreclose the patentability of artificially bred plants. [84] Given these decisions, the PTO now routinely grants patents for manmade plants. [85]

2. Animals

In contrast to plants, neither the Supreme Court nor the Federal Circuit have squarely faced the issue of whether non-naturally occurring animals developed by man are patentable, although they appear to be encompassed by the Chakrabarty Court's broad language in a published decision. Even in the wake of Chakrabarty, the PTO refused to grant patents for multicellular animals on the **[65]** ground that it required explicit judicial or congressional authorization to do so. [86]

In 1987, the Board faced this issue when it decided Ex parte Allen. [87] In Allen, the patentee sought to patent polyploid oysters on the basis that their polyploidy was induced by the application of pressure on oyster zygotes by the patentee. [88] The Board noted that under Chakrabarty's holding that § 101 included manmade life forms, "the issue … in determining whether the claimed subject matter is patentable … is simply whether that subject matter was made by man." [89] Thus, the Board concluded that a non-naturally occurring animal made by man was patentable subject matter. [90] As the claimed oysters did not occur naturally without the intervention of man, the Board held that they were non-naturally occurring manufactures or compositions of matter and thus were patentable subject matter. [91]

Only four days after the Board delivered its decision in Allen, the PTO issued a notice stating that the PTO considered non-naturally occurring nonhuman multicellular living organisms patentable subject matter as compositions of matter or manufactures. [92] The PTO also indicated that a manufacture or composition of matter occurring in nature, such as an animal, would not be patentable unless "given a new form, quality, properties or combination not present in the original article existing in nature in accordance with existing law." [93] This statement was also the first by either a court or the PTO concerning the patentability of human beings. The PTO stated that "a human being will not be considered … patentable subject matter under [§] 101 [because t]he grant of a limited, but exclusive property right in a human being is prohibited by the Constitution." [94] Accordingly, the PTO required that any claim "directed to a non-plant multicellular organism which would include a human being within its **[66]** scope include the limitation "non-human' to avoid this ground of rejection." [95] However, the PTO did not specify a precise provision of the Constitution that it relied on in reaching the conclusion that humans were not patentable. Commentators speculate that the PTO was referring to the Thirteenth Amendment's ban on slavery. [96]

After the announcement, various animal rights groups, animal husbanders, and farmers challenged the PTO's notice by filing a lawsuit claiming it was not properly promulgated under the Administrative Procedure Act. [97] The Federal Circuit held that the suit should be dismissed because the plaintiffs lacked standing. [98] However, it suggested in dicta that it considered non-naturally occurring animals patentable subject matter as it noted that the Chakrabarty Court held that all manmade life forms were patentable and pointed out that it affirmed the Board's decision in Allen, which expressly included animals in the realm of patentable subject matter, albeit in a summary unpublished opinion. [99]

In April 1988, the PTO issued the first patent for a multicelluar animal. [100] The patent was issued for a transgenic mouse known as the Harvard oncomouse, a mouse in which at least one additional gene has been introduced into the germ cells of the animal. [101] Harvard researchers introduced a human oncogene into the mouse that made it particularly disposed to breast cancer. [102] Since 1988, the PTO has granted numerous patents for animals not occurring in nature, including other transgenic animals containing additional human and nonhuman genes and a rabbit infected with the HIV virus. [103]

D. Rejection of an Application for a Patent for a Human-Animal Chimera by the PTO

On December 18, 1997, Stuart Newman, a biology professor at New York Medical College, and Jeremy Rifkin, a biotechnology **[67]** activist, filed a patent application for human-animal chimeras that could be up to 50% human and for several processes to make them. [104] Newman and Rifkin did not actually create a human-animal chimera nor did they express any intention to do so. [105] They filed their application for the purposes of preventing other scientists from creating human-animal chimeras and engaging in human-animal chimera research for the twenty-year patent term. [106] The application sparked a debate about the morality of patenting such life forms, pressured policymakers to develop a set of formal rules regarding the patentability of these life forms, and convinced the American public to support an outright ban on the synthesis of human-animal chimeras. [107]

Newman and Rifkin publicized their application in April 1998. [108] The PTO immediately responded by putting out a press release stating that human-animal chimeras might not be patentable because they would fail to meet the public policy and morality components of § 101's requirement that an invention must be useful to be patentable. [109] Early judicial decisions held that this requirement encompasses moral or beneficial utility, rendering inventions injurious to the well-being, good policy, or sound morals of society unpatentable. [110] As authority for its position, the PTO cited one of these decisions, Lowell v. Lewis, [111] a case decided by Justice Story in 1817. Furthermore, in the wake of the Newman-Rifkin application, PTO Commissioner Bruce Lehman indicated that human-animal chimeras were unpatentable

[68] when he stated in an interview that "there will be no patents on monsters." [112]

However, when the PTO officially rejected the Newman-Rifkin application in June 1999, it did not do so on moral utility grounds. [113] Rather, the PTO concluded that the human-animal chimeras claimed in the application were not patentable subject matter under § 101. [114] The PTO reasoned that the broadest reasonable interpretation of the claimed invention, the human-animal chimeras, encompassed a human being. [115] The PTO went on to summarily conclude that an invention that was not limited to nonhuman creatures and included a human being in it scope was not patentable subject matter because Congress did not intend for § 101 to include the patenting of humans. [116] However, the PTO cited no authorities for this proposition. The PTO's logic gave rise to several questions. First, the PTO did not explain its holding that the claimed chimeras, which could be at most 50% human, embraced a human being. This suggests that the PTO believes that a creature need not be completely human to constitute a human being and that degrees of humanity less than 100% and even less than 50% are sufficient to render a creature human. Thus, one commentator surmised that although the PTO did not mention moral utility in its rejection of the application, the rejection was "in part, a rejection of a patent based on (moral) utility grounds." [117] Second, the PTO did not distinguish transgenic animals containing human genetic material, such as the Harvard oncomouse, that it had previously held patentable and did not explain why such transgenic animals do not embrace a human being.

The PTO issued its final rejection of the Newman-Rifkin patent application in its final office action in the matter in August 2004. [118] In the final rejection, the PTO reaffirmed its conclusory finding that human beings are not patentable subject matter. [119] However, the PTO provided additional explanation for its conclusion that the chimeras **[69]** claimed in the application could encompass humans. The PTO noted that chimeric embryos could produce animals with only one chimeric organ or that exhibit no chimerism at all (either entirely human or animal). [120] Thus, the patent examiner concluded that the claims presented in the Newman-Rifkin patent were written in such a way as to encompass a creature that was completely human since chimeric embryos covered by the patents could produce "an animal of one cell type or predominately one cell type." [121] The language "predominately one cell type" suggests that, despite its clarification, the PTO considers animals human even though they are less than completely human genetically or in terms of cell composition. However, the PTO does not provide any standards for determining whether a creature is an animal or human based on the percentages of animal and human cells it contains.

Newman and Rifkin did not respond to the PTO's final rejection of their patent application for human-animal chimeras. In 2005, the PTO found the application abandoned. [122]

E. Congressional Regulation of Human-Animal Chimera Research and Patentability

Recently, Senator Sam Brownback of Kansas introduced a bill dubbed the Human Chimera Prohibition Act of 2005. [123] The bill would have prohibited any person from creating or attempting to create a human chimera. [124] For the purposes of the Act, a human chimera was broadly defined to include various methods of introducing non-human cells into human embryos. [125] However, the bill never made it out of committee. Also, despite the fact that the United States Food and Drug Administration (FDA) has asserted jurisdiction over human cloning, it has not attempted to extend its regulatory reach over research involving human-animal chimeras. [126]

[70] No legislation or proposed legislation has hitherto directly addressed the patentability of chimeras. However, several bills have been introduced in Congress to ban the patenting of human tissues and human beings, but none of them have become law. [127] Most notable of these is the proposed Transgenic Animal Patent Reform Act of 1988, otherwise known as the 1988 Animal Patent Act, that included an amendment to § 101 that expressly excluded "human beings" from the scope of patentable subject matter. [128] The Transgenic Animal Patent Reform Act of 1988 passed in the House. [129] However, the Senate never approved the bill, and it failed to become law. [130]

Even though Congress has failed to expressly exclude human beings from the scope of patentable subject matter under § 101, it has restricted the patentability of human organisms using its appropriations power. In an amendment to the federal budget for 2004 introduced by Rep. David Weldon of Florida, known as the Weldon Amendment, Congress stated that federal funds provided for the operation of the PTO "may [not] be used to issue patents on claims directed to or encompassing a human organism." [131] Thus, even if human organisms are patentable subject matter under § 101, [132] the PTO is prohibited from issuing patents for them. This provision serves to codify the PTO's position,

expressed in its 1987 statement and its denial of the Newman-Rifkin patent, that human organisms are not patentable. [133] Indeed, Rep. Weldon, the sponsor of the amendment that bears his name, stated that the amendment was intended to codify the PTO's previous position against the patentability of human **[71]** organisms. [134] Likewise, the PTO interprets the Weldon Amendment in this manner. [135]

The Weldon Amendment does not expressly address chimeras or bar their patentability in all cases. However, the legislative history suggests that the Amendment tracks the PTO's policy concerning chimeras articulated in its rejection of the Newman-Rifkin patent and proscribes the patentability of at least some chimeras. [136] While debating the amendment, Rep. Weldon noted without disapproval that the PTO has granted patents for transgenic organisms, such as the Harvard oncomouse, that are modified to include a few human genes allowing the production of a human protein or antibody, suggesting that such organisms are patentable under the Weldon Amendment. [137] However, Rep. Weldon also stated that the PTO has "rejected patents on … half-human embryos because [they] can broadly but reasonably be construed as human organisms." [138] Thus, the legislative history of the Weldon Amendment suggests that the amendment's prohibitions are not confined to organisms that are entirely human and foreclose the patentability of chimeras that contain at least 50% human cells as they may be considered human organisms. However, neither the Weldon Amendment nor its legislative history articulate a precise definition of a human organism - that is, the minimum amount of human cells, genes, or characteristics an organism must have to be considered human for the purposes of the statute. Rather, Rep. Weldon stated during floor debate that the amendment "leaves the USPTO free to address new or borderline issues on the same case-by-case basis as it already does." [139] The PTO has not determined the percentage of human cells or genetic material or the degree of human characteristics required to **[72]** justify patent prohibition. [140] Thus, the Weldon Amendment does little to resolve the question of how many human characteristics, genes, or cells are necessary to render an organism within its prohibitions.

However, because the Weldon Amendment was part of an appropriations bill for 2004, it was limited in its impact as it only affected patents that were to issue in 2004. [141] A similar bill would have to be passed and signed into law by the President each year for its prohibitions to remain in effect. [142] After its initial passage, Congress reenacted the Weldon Amendment in the federal budgets for 2005 and 2006. [143] The 2006 enactment of the Weldon Amendment expired on September 30, 2006. [144] Congress did not expressly reenact the Weldon Amendment in the 2007 federal budget. [145] However, although the barrier to patentability of human organisms erected by the Weldon Amendment was not expressly attached to the funds expressly appropriated for the PTO in 2007, the continuing act appropriating these funds provided that "except as otherwise expressly provided in [the act], the requirements, authorities, conditions, limitations, and other provisions of the [appropriations acts in force for 2006] shall continue in effect." [146] Nothing in the **[73]** 2007 continuing appropriations act appropriating funds for the PTO expressly repudiates the Weldon Amendment's restriction on the patentability of human organisms. Thus, despite the fact that Cognress did not formally reenact it, the Weldon Amendment's restrictions applied to the funds appropriated for the operation of the PTO in 2007. However, Congress explicitly reenacted the Weldon Amendment as part of the 2008 federal budget. [147] This latest ban on the patentability of human organisms formally expired on September, 30 2008, [148] and Congress has failed to formally reenact the Weldon Amendment as part of the 2009 federal budget. [149] However, Congress enacted a continuing appropriations act providing for funding for the PTO until March 6, 2009, or the passage of an appropriations act providing for, or otherwise applicable to, PTO funding for the 2009 budget that cannot be used for activities that appropriations, funds, or other authority were not available for under the 2008 federal budget. [150] Thus, since Congress expressly made funds allocated to the PTO unavailable for the patenting of human organisms in the 2008 federal budget, the restriction on patentability of human organisms imposed by the Weldon Amendment remains in force as long as the continuing appropriations act is in effect.

The Weldon Amendment was in force when the PTO issued its final rejection of the Newman-Rifkin patent application for human-animal chimeras on the ground that the claims embraced human organisms. However, the PTO based its rejection on its previous conclusion that § 101 did not embrace human organisms rather than on the Weldon Amendment. [151] The PTO's rationale would, at least at first blush, provide an unambiguous statutory basis for its decision. [152] Although it is unclear why the PTO did not rely on the Weldon **[74]** Amendment in the final rejection, it is possible that the PTO did not want to rely on statutory language that expires on an annual basis and thus might not always be present, especially when the patent application process usually takes longer than one year.

II. Patenting Human-Animal Chimeras

Neither the courts nor the Board have broached the issue of the patentability of human-animal chimeras. The PTO office actions rejecting the Newman-Rifkin patent do not create a legal precedent. [153] The abandonment of the Newman-Rifkin patent ensures that it will not be litigated.

This Part explores the legal issues concerning the patentability of human-animal chimeras. Congress, the courts, and the PTO have all failed to provide a standard for determining whether a given chimera has enough human cells, genes, or characteristics to qualify as human. This question is of immense importance as it appears to determine the class of human-animal chimeras that are patentable, at least as long as Congress continues to reenact the Weldon Amendment in its appropriations bills. Indeed, courts and the PTO are unable to effectively and consistently apply the Weldon Amendment in the chimera context until the courts adopt a workable definition of "human being." [154]

Thus, this Part begins by evaluating various standards proposed for a chimera to qualify as a human being and concludes that the best standard is one that defines humanity based on the higher faculties and physical characteristics associated with human beings. The definition of humanity bears on the constitutional issues related to the patentability of human-animal chimeras, such as whether the Thirteenth Amendment, which applies to humans and not animals, forecloses the patentability of chimeras. Then, this Part explores the statutory and constitutional issues related to the patentability of human-animal chimeras. The first such issue is whether human-animal chimeras constitute patentable subject matter under § 101. This Part concludes that human-animal chimeras, even those which qualify as humans (as well as fully-human manmade inventions) are patentable subject matter. Second, this Part examines the implications of this conclusion for the effectiveness of the Weldon Amendment as a vehicle to foreclose patentability of human organisms, which Congress believed was in concordance with § 101 when it was **[75]** passed. Third, this Part examines the constitutionality of patents on human-animal chimeras, including those which may reasonably be classified as humans, under the Thirteenth and Fourteenth Amendments and concludes that these constitutional provisions do not foreclose the patentability of these chimeras. Fourth, this Part examines the argument set forth by the PTO that human-animal chimeras are not patentable because they lack the requisite moral utility and concludes that a court is unlikely to accept this argument. Finally, this Part concludes that the patent law doctrines of novelty and nonobviousness do not foreclose patentability of human-animal chimeras.

A. Standards for Humanity

As Congress has failed to articulate standards for the amount of human cells, genes, or characteristics a creature must possess to be considered human, this task is left to the courts. However, the courts have never discussed the requirements for an organism to be considered human in any context. The PTO and commentators are generally in agreement about several easy cases. Transgenic animals containing one or a handful of human genes are not rendered human by virtue of the fact that they contain these genes. [155] Indeed, the PTO has granted patents on transgenic animals containing human genes, such as the Harvard oncomouse, and the legislative history of the Weldon Amendment, foreclosing patentability of human organisms, indicates that the amendment does not affect the patentability of transgenic animals in any way. [156] These transgenic animals share two characteristics. First, they contain a relatively small percentage of human genes. Second, unlike chimeras, transgenic animals contain no fully human cells. Rather, each cell in the animal contains a small number of human genes. At the other end of the spectrum, no one would seriously question a conclusion that a transplant patient who received an animal organ was still human after the transplant. [157] Outside of these extreme situations, there is little consensus as to the humanity of chimeras.

Commentators have proposed both quantitative and qualitative models for determining humanity. [158] In quantitative models, the issue **[76]** of what type of biological material to use as a criterion for determination of the human character of an organism is a complex one. [159] Possibilities include quantities of genetic material (DNA), proteins, and metabolites and the number of genes, cells, tissues, and organs. [160] Some commentators have suggested that a creature is human if 50% or more of its genetic material is of human origin. [161] From a common sense point of view, this approach seems appealing and reasonable. [162] However, it is somewhat simplistic and artificial. [163] A determination that an animal possessing 49% human genetic material is not human although the animal displays substantial human characteristics for the purposes of patentability seems arbitrary. Furthermore, this rule produces some absurd results. Chimpanzees share 95% or greater genetic homology with humans, yet no one would ever consider a chimpanzee human. [164] Although one might base this approach on the percentage of the creature's genetic material actually derived from human sources, it appears

illegitimate for the source of genetic material to affect the analysis of whether it renders an organism human.

Further complications in using the percentage of genetic material criterion arise in the context of human-animal chimeras that are not present in the transgenic animal context. Unlike transgenic animals, which contain a given percentage of human genetic material in all of their cells, chimeras contain a given percentage of cells that contain only human genes with the rest of the cells containing entirely nonhuman genetic material. [165] Thus, chimeras contain portions that **[77]** are fully human while transgenic animals do not. Therefore, they might be considered more human than a transgenic animal with the identical percentage of human genetic material. Indeed, many people would consider human certain chimeras consisting of much less than 50% human genetic material. [166] As chimeras possess some cells that are completely human, it could be possible to create a chimera that has the body and outward appearance of an animal, but the brain and central nervous system of a human. [167] Such a creature would contain far less than 50% human genetic material or human cells, but many people would consider such a creature to be human, depending on its cognitive abilities. [168] Thus, the use of quantitative standards for humanity is problematic, especially in the human-animal chimera context.

These problems and inconsistencies associated with a quantitative standard are ameliorated under a qualitative standard focusing on the higher faculties associated with humanity. One commentator has suggested that humanity be determined by a case-by-case evaluation of whether a creature "possesses significant human characteristics" in terms of possessing higher faculties such as:

The ability to reason (including, but not limited to, the ability to use facts and argue them, to arrive at conclusions from premises in a logical manner, to explain observed phenomena and to form beliefs based on facts); the ability to evaluate principles and observations to arrive at reasoned decisions; the ability to formulate speech and communicate; the ability to write; the ability to develop meaningful personal relationships with other human beings on the basis of equality; the demonstration of awareness of self as a unique and separate being; the ability to feel concern for others; or any other higher faculty. [169]

[78] A related standard that has been proposed considers human-animal chimeras human if the chimera itself would consider itself human, demonstrating the ability to reason in a manner known as self-awareness. [170] By focusing on a creature's qualitative characteristics, these approaches track people's ideas of what creatures are human and what characteristics make a creature human better than the quantitative approaches. For instance, a creature with a human brain and central nervous system that outwardly resembles an animal would qualify as human under this standard while it would not under a quantitative standard. More specific to the patent context, a creature with these higher faculties characteristic of humans is capable of suffering psychic harm by being property or being enslaved. The Supreme Court considers the Thirteenth Amendment to strive to prevent the psychic harm caused by slavery as it stated that the amendment was directed at the institution of slavery as well as its "badges and incidents." [171] Thus, if a chimera possessed the mental faculties to suffer such psychic harm, courts would likely consider it human for the purposes of entitlement to the protections afforded by the Thirteenth Amendment. [172]

However, these approaches also have serious flaws inasmuch as they fail to precisely track people's conceptions of the definition of humanity. Under such conceptions, reasoning ability and other types of higher mental faculties alone do not determine humanity. No one could reasonably contend that a seriously mentally handicapped individual who does not possess the higher faculties discussed above and is unable to understand that she is human is less than human. Thus, considering such an individual property would be unethical and in violation of the rights accorded a human being by the law. Likewise, many people might consider a chimera with many of the physical characteristics of a human being, but without the mental capacities associated with a normal human a human being and entitled to the legal rights accorded to humans.

Perhaps in order to abate these concerns with qualitative approaches to assessing humanity based on reasoning ability, at least one commentator has proposed a standard where an organism is considered human if it either possesses the high mental faculties **[79]** associated with humanity or was begotten of human gametes (i.e., human egg and sperm), regardless of whether the genetic material of the gametes or the resulting embryo was genetically altered. [173] While a severely mentally handicapped

individual would be considered human under this rubric, in keeping with people's general conceptions of humanity, this framework proves unsatisfactory in the human-animal chimera context. Consider a chimera with a human body and appearance but an animal brain and central nervous system. Such a creature would neither have the higher mental faculties nor be born from human parents or gametes. However, people would likely be chary to treat such a chimera, which is identical to a human with respect to outward appearance, as less than human. From a moral and intuitional standpoint, it is highly unlikely that society would brook the creation of a genetically engineered underclass consisting of chimeras that outwardly appear human but are not considered human and, accordingly, are not afforded the legal protections and rights granted to humans with severe defects in reasoning ability. Indeed, it seems unseemly to distinguish human organisms of like physical appearance and reasoning ability based on how they were engendered, whether by natural birth or the tinkering of man with human organisms.

A preferred standard would accurately reflect people's conception of humanity. Such a standard takes both higher mental faculties and physical characteristics of human beings into account in terms of a sliding scale. As the above discussion suggests, both of these aspects shape people's conception of humanity. Generally speaking, people perceive reasoning ability to be the touchstone for humanity yet do not consider humans that have severe mental disabilities and that are wholly incapable of reasoning divested of their humanity. Thus, a chimera that possesses human higher faculties but that physically resembles an animal will be considered human. The more a given chimera physically resembles a human, the fewer mental faculties are required for it to be considered to "possess significant human characteristics" [174] and thus constitute a human organism. Likewise, the more mental faculties a chimera possesses, the less physical resemblance to a human is required for it to be considered human. Under this scheme, chimeras that have significant human characteristics, and thus would be considered human by community standards, will be recognized as human, and those that are **[80]** more like animals, even those animals that are close genetic relatives to humans such as chimpanzees, will not be.

B. Human-Animal Chimeras and Human Organisms Are Patentable Subject Matter Under the Supreme Court's Broad Interpretation of § 101

Although the Weldon Amendment appears to effectively render human-animal chimeras considered human unpatentable at first blush, it does not cover chimeras that are not considered human. Furthermore, a discussion of whether human-animal chimeras considered human and human organisms themselves constitute patentable subject matter under § 101 is still germane after the Weldon Amendment for several reasons. First, the Weldon Amendment and its successors are parts of appropriation bills that expire annually. Thus, the Weldon Amendment must be reenacted every year, and there is no guarantee that Congress will renew it annually, especially since Congress has failed to formally reenact it on more than one occasion. Second, as demonstrated by the analysis below, the PTO's conclusion that § 101 does not encompass human inventions is inconsistent with the language of § 101 and its interpretation by the Supreme Court. Thus, in regard to the patentability of human organisms, the Weldon Amendment, an appropriations statute, appears to conflict with the substantive authorization in § 101. This conflict serves as the basis for a weighty argument against the ability of the Weldon Amendment to foreclose the patentability of human organisms.

1. Human-Animal Chimeras and Human Inventions Are Patentable Subject Matter Under § 101

In holding that living microorganisms that were made by man and did not occur in nature were patentable subject matter in Chakrabarty, the Supreme Court employed broad language indicating that any living thing made by man that was not naturally occurring, which would include human-animal chimeras and human beings themselves, constitutes patentable subject matter. The Court defined the realm of patentable subject matter as "anything under the sun that is made by man." [175] In extending Chakrabarty to multicellular animals in Allen, the Board stated that the issue in determining **[81]** whether a living thing was patentable "is simply whether that subject matter was made by man." [176]

Human-animal chimeras do not occur in nature. Thus, as they do not exist in nature without the intervention of man, human-animal chimeras are patentable subject matter under Chakrabarty and Allen. This is the case regardless of whether the chimera is considered human by any applicable legal standard. It also applies to transgenic humans, fully human creatures genetically engineered to contain a gene-one encoding a bacterial enzyme, for instance-that does not naturally occur in humans. Therefore, straightforward application of the language defining patentable subject matter in

Chakrabarty and Allen indicates that human-animal chimeras, whether considered human or not, or other manmade human inventions, are patentable subject matter under § 101. Nothing in Chakrabarty or its progeny suggests that chimeras that are considered humans or even human beings themselves are not patentable subject matter. [177] Under the reasoning set forth in these cases, transgenic humans and human-animal chimeras cannot be distinguished from nonhuman transgenic and chimeric animals that are patentable under Allen.

The PTO cited no precedent or authorities to support its conclusion that humans and chimeras considered human were not patentable subject matter under § 101. [178] Furthermore, the PTO's reasoning that human inventions are unpatentable because Congress did not intend for § 101 to encompass their patentability was rejected by the Supreme Court in Chakrabarty. The Chakrabarty Court explicitly rejected the argument that Congress needed to expressly authorize patentability of new areas of subject matter, such as living things, because they were not contemplated when the patent laws were enacted. [179] The Court reasoned that new subject matter areas were patentable because Congress authorized a broad scope of patentable subject matter to include all inventions made by man precisely for the reason that new inventions are often unforeseeable and that a rule that unanticipated inventions cannot be patentable conflicts with the long-standing patent law concept that anticipation undermines patentability. [180] In addition, the Court found that legislative history and intent, as well as the statutory purpose **[82]** underlying § 101, are not relevant considerations in evaluating the scope of patentable subject matter because Congress unambiguously cast § 101 in broad terms to fulfill the constitutional and statutory goal of promoting the progress of science and the useful arts. [181] Congress did not likely anticipate the creation of human-animal chimeras or manmade human beings when it enacted § 101. [182] Under Chakrabarty, this fact does not foreclose their patentability because, as discussed above, they fall under the broad scope of § 101, and any invention within this scope is patentable subject matter. Even if the PTO correctly determined that Congress did not intend for § 101 to encompass human inventions, despite offering no evidence from the legislative history or otherwise to support this conclusion, Congress's actual intention with regard to their patentability is immaterial because Congress unambiguously provided for a broad scope of patentable subject matter that encompassed human inventions in § 101. [183] Therefore, the PTO's assertions that inventions involving humans are not patentable subject matter under § 101 run contrary to the Supreme Court's interpretation of the statute and constitute "a unilateral reinterpretation of the law." [184]

Several commentators have suggested that the fact that the PTO has issued patents on living things derived from human beings, such as transgenic animals containing human genes and human cell lines, cuts in favor of the patentability of human-animal chimeras, including those classified as human, as well as other human inventions. [185] While the issuance of these patents indicates that even the PTO believes that the mere presence of human genes or individual cells derived from humans in an invention does not render it unpatentable, an argument for the patentability of human-animal chimeras, especially those that are considered human, is less than convincing because chimeras (and full human beings) are readily distinguishable from transgenic animals and cells. Unlike chimeras, transgenic animals and cells do not display a significant amount of human characteristics and cannot fairly be considered to constitute a human being. Relatively speaking, transgenic animals such as the Harvard oncomouse contain a very **[83]** small percentage of human genes. [186] These animals physically resemble animals and do not have significant human characteristics. In contrast, a chimera containing a sufficient percentage of human cells might resemble a human. Furthermore, unlike human-animal chimeras, which may contain a significant percentage of entirely human cells, transgenic animals contain no human cells. Human cell lines do not constitute an organism, but rather are free living cells maintained under laboratory conditions. [187] They are unable to be used in generating a human being and share few, if any, characteristics with human beings, save for the fact they are derived from human tissue. In addition, human cell lines contain mutations in their DNA, including additional chromosomes in some cases, so they do not truly contain the same genetic material as humans. [188] However, extended discussion of the extension of the PTO's determination that certain genetically engineered inventions are patentable to the human-animal chimera context is unwarranted given the analysis above based on § 101 and cases interpreting it, demonstrating that human-animal chimeras are patentable subject matter.

2. Implications for the Effectiveness of the Weldon Amendment in Foreclosing Patentability of Human Organisms

The conclusion that the scope of patentable subject matter as defined by § 101 encompasses human-animal chimeras considered human as well as other human inventions serves as the foundation for a

persuasive argument that the Weldon Amendment does not effectively foreclose patentability of such creatures. When Congress enacted the Weldon Amendment, it assumed that the amendment explicitly codified the PTO's practice of forbidding patents for human organisms as outside the scope of patentable subject matter under § 101. [189] Indeed, statements by Rep. Weldon, the sponsor of the amendment, in the legislative history indicated that the Weldon **[84]** Amendment "was not meant to change existing policies." [190] Thus, the legislative history indicates that Congress did not intend to alter the scope of patentable subject matter under § 101 and that it did not contemplate that the amendment would conflict with § 101. [191]

The Supreme Court has held that Congress may modify existing authorization statutes (or substantive statutes), such as § 101, by an amendment to an appropriations bill. [192] However, the Court has indicated that the doctrine disfavoring repeals by implication applies with greater force when the claimed repeal rests on an appropriations act. [193] Indeed, the House Rules state that appropriations bills should not change existing law. [194] For a statute to be repealed by implication by a subsequent Act of Congress, "the intention … to repeal must be clear and manifest." [195] In evaluating the intention, courts look to both legislative history and the traditional separation between **[85]** appropriations and authorization. [196] Given the House Rule against changes in existing law by appropriations measures and the fact that Congress enacted the Weldon Amendment under an erroneous interpretation of § 101 with no apparent intention to repeal or modify it, even expressly indicating that the Weldon Amendment was not intended to modify patent law, there is a convincing argument that Congress did not express a clear and manifest intent to foreclose the patentability of human organisms that were patentable subject matter under § 101. In the absence of express intention to repeal, "the only permissible justification for a repeal by implication is when the earlier and later statutes are irreconcilable." [197] The Weldon Amendment may be interpreted not as foreclosing patentability of human organisms, but as withdrawing federal subsidy for PTO analysis of applications involving human organisms. Thus, the PTO might comply with the amendment by charging higher fees for patent applications directed to human subject matter, reflecting a lack of federal subsidy. Therefore, the Weldon Amendment is not irreconcilable with § 101, indicating that the amendment did not repeal § 101.

On the other hand, the legislative history of the Weldon Amendment unmistakably evinces a clear congressional intent to foreclose patentability of human organisms. [198] Furthermore, the Weldon Amendment, addressing the patentability of human organisms, is more specific than the extremely broad § 101 and thus might overcome it given the principle of statutory construction "that a more specific statute will be given precedence over a more general one." [199] However, while Congress intended to codify the PTO's practice that human inventions were unpatentable, it neither contemplated that the PTO's conclusion was inconsistent with § 101 as interpreted by the Supreme Court nor intended to repeal § 101 with respect to human inventions. Furthermore, Congress has expressly foreclosed patentability of inventions useful solely in connection with certain nuclear materials and atomic weapons. [200] Rather than preclude the use of federal funds for PTO processing of applications related to **[86]** these inventions, Congress explicitly stated that "no patent shall … be granted for [them]." [201] Thus, if Congress had wanted to expressly proscribe patentability of human organisms, it certainly knew how to do so.

Neither the Weldon Amendment nor the PTO's conclusion that human inventions, such as chimeras considered human, fall outside the scope of patentable subject matter under § 101 have been challenged in the courts. Indeed, the ability of the Weldon Amendment to foreclose patentability of human inventions is a close issue. Nonetheless, the above analysis suggests that a court would likely find that Congress's intent to restrict patentability of human organisms in enacting the Weldon Amendment, an appropriations act, did not constitute the clear and manifest intent to repeal or modify § 101 as required by the Supreme Court and, accordingly, that the Weldon Amendment does not wholly foreclose the patentability of human organisms, including human-animal chimeras considered human.

C. The Thirteenth Amendment Does Not Foreclose the Patentability of Human-Animal Chimeras or Human Inventions

The fact that human-animal chimeras, including those qualifying as human, and other forms of human inventions fall within the scope of patentable subject matter articulated in § 101 does not necessarily render them patentable. For instance, if patentability of human inventions ran afoul of a constitutional provision, such as the Thirteenth Amendment, as suggested by the PTO, it would not be patentable although it fits within the statutory scope of patentable subject matter. [202] The PTO provided no

explanation for this conclusion. The Thirteenth Amendment prohibits slavery and involuntary servitude. [203] As the amendment was enacted to eradicate any remaining vestiges of slavery, the Supreme Court has interpreted it to permit Congress to eliminate the "badges and incidents" and "relics of slavery." [204] The Court defined "badges of slavery" as the ""burdens and disabilities' [associated with slavery] including restraints upon "those fundamental rights which are the essence of civil freedom, namely, the … rights … to inherit, purchase, lease, **[87]** sell and convey property.'" [205] Impairments of autonomy give rise to social inferiority and, at some point, may lead to subjugation. [206] Despite the PTO's conclusion, the Thirteenth Amendment does not proscribe patentability of chimeras considered human and other human inventions, as the patentability of such inventions does not appear to parallel slavery.

"There is no reason to suppose that the Thirteenth Amendment addresses the type of right [or monopoly] conferred by a patent." [207] A patent merely confers the right to stop others from making, using, or selling a patented invention. [208] A patent does not give the patentee an affirmative right to practice or use the invention or even to possess a physical embodiment of it. [209] For instance, in the pharmaceutical context, a patent holder must not make, use, or sell a patented drug without approval from the FDA, and may not even possess her patented pharmaceutical invention if it is contraband or it is distributed only by prescription and she does not have a prescription. [210] Further, while some states prohibit the use of radar detectors, and these prohibitions apply equally to the inventors of such radar detectors, courts have nevertheless found them patentable. [211] Thus, while the holder of a patent for a chimera considered human or other human invention could prohibit others from making, using, or selling such a creature, these rights do not permit the patentee to impress the patented creature into bondage or servitude or otherwise "own" the living creature itself. [212] Indeed, the rights conferred to a patent holder do not encompass such control over individual embodiments of the invention. Patent rights are distinct from any given embodiment of an invention. [213] A person can buy, sell, or trade away patent rights without buying, selling, or trading a physical embodiment of the patented invention. [214] Thus, as patents do not confer possessory rights in the patented article, the **[88]** intangible property rights that inhere in a patent as well as markets in patent rights do not impinge on the autonomy of a patented human creature itself. [215] The patentee's exercise of his rights "to exclude others from manufacturing, using or selling the human invention would not by itself give rise to socially imposed inferiority" or "result in subjugation of the [patented individual]." [216] A patentee's rights to a human invention with a particular cellular or genetic constitution do not engender the burdens and disabilities of slavery as they do not interfere with such an individual's fundamental rights, such as the right to own or dispose of property. Impairment of property rights is the prototypic example of a badge of slavery. [217] Therefore, the patentee's rights conferred by a patent for a chimera considered human or another type of human invention do not of themselves constitute badges of slavery. [218] Patentability of human inventions is not a relic of slavery inasmuch as non-possessory rights conferred by a patent do not press a patented individual directly into slavery or bondage. Thus, patentability of chimeras considered human does not run afoul of the Thirteenth Amendment.

The Thirteenth Amendment is only potentially applicable to chimeras that are considered human. [219] It has no effect on the patentability of chimeras that fall short of humanity even if a court were to find that it foreclosed patentability of chimeras considered **[89]** human and other human inventions. Just like the applicability of the Weldon Amendment, the Thirteenth Amendment's applicability depends on the definition of humanity adopted by courts and cannot be effectively and consistently applied until courts adopt a workable definition, like the one suggested above. [220] Several commentators have suggested that courts apply a narrower standard for humanity in the Thirteenth Amendment context than that suggested by the PTO and restrict its potential applicability to organisms that are entirely human, excluding all chimeras from its scope. [221] However, such an approach is unsatisfying because it forecloses Thirteenth Amendment protections for chimeras with considerable human characteristics, including those that would suffer psychic harm from the badges and incidents of slavery, such as subjugation. [222]

D. The Fourteenth Amendment Substantive Due Process Right to Privacy and Reproductive Autonomy Does Not Foreclose Patentability of Human Animal Chimeras, Including Those Considered Human

The Supreme Court has recognized that one aspect of the "liberty" protected by the Due Process Clause of the Fourteenth Amendment is "a right of personal privacy" encompassing reproductive freedom and autonomy. [223] Indeed, the Court considers the decision whether or not to reproduce at the heart of this substantive due process right. [224] Patenting human-animal chimeras considered human and other human inventions might conflict with the right of reproductive autonomy and freedom of the individual patented human creature itself. [225] A patent restricts the right of others besides the

patentee to reproduce the invention. [226] Thus, some commentators have suggested that a patented chimera or human might violate the patentee's right to restrict others from manufacturing it whenever it reproduced naturally. [227] Accordingly, this right might conflict with the patented human organism's right to **[90]** reproductive autonomy. [228] While this position makes some intuitive sense, it is untenable, especially in the chimera context. Foremost, natural reproduction by chimeras will not produce chimeric offspring. A human-animal chimera contains cells that are either entirely human or entirely animal. There are no hybrid cells. Thus, a chimera will produce gametes that are entirely human or entirely animal. [229] As the chimera cannot produce chimeric offspring, any offspring generated by a patented chimera would not fall within the scope of the patent for a chimera. [230] However, other forms of human inventions, such as transgenic humans, may produce offspring that have the same transgenic properties as the parent(s). Even in these cases, the right to reproductive autonomy does not foreclose patentability. The fact that the Constitution negates a patentee's exclusionary rights when a patented human is made by the procreation of a patented individual as to that individual does not render the patentee's remaining rights, such as the right to prevent others from synthesizing the patented human organism by genetic engineering, a nullity - "the entire fabric of the patent grant need not be unraveled by clipping one thread." [231] The reasoning presented here also defeats the related argument that a patent's possible abrogation of a patented human organism's reproductive rights constitutes a violation of Thirteenth Amendment because its infringement on a fundamental right of the patented organism represents a badge and incident of slavery.

[91]

E. The Moral Utility Doctrine Does Not Foreclose the Patentability of Human-Animal Chimeras

In addition to prescribing the scope of patentable subject matter, § 101 requires that an invention be useful to be patentable. [232] Courts require patentable inventions to possess practical or specific utility, meaning some real world use. [233] Early U.S. judicial decisions required moral or beneficial utility, rendering inventions "injurious to the well-being, good policy, or sound morals of society" unpatentable, [234] but the Federal Circuit has rejected these earlier decisions and considers the modern standard for utility as excluding morality as a relevant consideration in determining the patentability of an invention. [235] Human-animal chimeras have practical or specific utility and are not rendered unpatentable by notions of moral or beneficial utility.

1. Practical or Specific Utility

The substantive threshold for satisfying the utility requirement is relatively low. [236] "An invention is "useful' … if it is capable of providing some identifiable benefit." [237] Although an invention need not have more than one use, that use must be credible to a person of ordinary skill in the art. [238] The Supreme Court requires that an invention have substantial utility or a real world use. [239] Use as a research tool, or scientific curiosity, does not qualify as a substantial use and thus does not satisfy the utility requirement. [240] Under this rule, an asserted use for "basic research such as studying the properties of the claimed product itself" fails to satisfy the utility requirement. [241] Likewise, "inventions whose asserted utility requires further research to identify or reasonably confirm" do not meet the **[92]** utility requirement. [242] However, an invention inciting amusement in the public possesses the requisite utility to be patentable. [243] The PTO Utility Examination Guidelines state the standard in a similar manner and require a "specific and substantial" utility and exclude ""throw-away,' "insubstantial,' or "nonspecific' utilities, such as the use of a complex invention as landfill, as a way of satisfying the utility requirement." [244]

Human-animal chimeras satisfy the utility requirement. Human-animal chimeras could create a valuable new source for organ transplantation, as human donors are in short supply. [245] Xenotransplantation of animal organs into humans has only achieved limited success because humans often reject animal organs as foreign. [246] Chimeric organs more closely resemble human organs and thus might not be rejected. [247] Moreover, some chimeras might have some wholly human organs, which would not pose the rejection risk associated with animal or even chimeric organs. If an organ or organ system developed entirely from one of the human cells used to make the chimeric embryo, that organ or organ system will be entirely human. Furthermore, despite the general rule that use as a research tool is insufficient to meet the utility requirement, the PTO has indicated that a use for "analyzing compounds" in a research or laboratory environment constitutes a specifically identified substantial utility and thus satisfies the utility requirement. [248] Thus, the inventor of a human animal-chimera might assert a use for studying the effects of drugs on humans as a given drug is likely to affect chimeras, and in

particular their human cells, in a manner more similar to the way it **[93]** affects humans than the way it affects animals. [249] In addition, some chimeras, such as Minotaurs, might be used for amusement purposes in zoos and sideshows provided that the chimeras used did not qualify as human under the Thirteenth Amendment. [250]

2. Moral Utility

Early U.S. judicial decisions recognized a morality component within the utility requirement restricting utility to inventions with "some beneficial use in society" and rendering unpatentable those that were injurious to the well-being, good policy, sound morals, health, or good order of society. [251] Applying this standard, courts invalidated patents on gambling devices and patents on inventions designed to be deceptive in the early Twentieth Century. [252] However, the Federal Circuit has noted that this principle has not been applied broadly in recent years. [253] In 1977, the Board upheld the patentability of a slot machine, reasoning that it could not find any basis in § 101 to hold that gambling machines were unpatentable for want of utility, although some consider gambling immoral and injurious to public order. [254] In 1999, the Federal Circuit held patentable an arguably deceptive product designed to appear to be something it is not, **[94]** reasoning that cases invalidating patents on deceptive products on moral utility grounds "do not … represent[] the correct view of the doctrine of utility." [255] Thus, although the Federal Circuit did not expressly disclaim moral utility entirely, it suggested that the modern standard of utility does not attempt to judge the morality of an invention. [256] The Court reasoned that the PTO is not the proper arbiter of whether an invention is moral, deceptive, or illegal as this is the realm of Congress and other agencies such as the FDA. [257] Congress is free to declare classes of inventions unpatentable and has done so in the case of inventions useful solely in connection with certain nuclear materials and atomic weapons. [258] Lower courts have held that radar detectors, which are used only to circumvent the law, are patentable. [259] However, the moral utility doctrine is not completely dead, as lower courts have recited it as the standard for utility without applying it. [260]

Given the vestigial nature of the moral utility doctrine, courts will not likely apply it to defeat the patentability of human inventions or human animal-chimeras. Even if a court contemplated applying the moral utility doctrine, as discussed above, human-animal chimeras have beneficial uses in society and thus have moral utility. However, the moral objections to the patentability of human-animal chimeras are far from trivial. Thus, if the moral utility doctrine has survived at all, human-animal chimeras, especially those considered human, are a context where a court might apply it. However, moral arguments against the patentability of human-animal chimeras are neither airtight nor decisive as there are weighty arguments on the other side of the ledger.

The primary ethical objections to the development and creation of human-animal chimeras are deontological. [261] Some oppose human-animal chimera research on the basis that human-animal chimeras are made from human embryos as they contend that human **[95]** embryos are to be afforded a special dignity that is offended by their use in any kind of experimentation, much more commingling cells taken from them with cells from animal embryos. [262] This reasoning provides the basis for precluding patentability of human-animal chimeras under European Union rules. [263] However, giving embryos legal status in the patent context is inconsistent with the established right to an abortion, as patenting is a far lesser affront to the integrity of the embryo than an abortion is. Thus, given the weak legal position of embryos in the abortion context and the lack of consensus on this issue in society, a court would be unlikely to give significant weight to this objection. [264]

A related objection is that the human species, and animal species as well, have a right to their uniqueness and not to be corrupted by the formation of chimeras. [265] However, a court might consider this objection specious as it is difficult to say that species have rights because they are not static, but rather are constantly evolving, and breeding is routinely used to alter species. [266] Some also object to chimera research because they feel that the commingling of human embryos with animal embryos, potentially conferring human characteristics on animals, offends the dignity of humanity. [267] Thus, creating creatures that are partially human is morally wrong. Furthermore, some have advanced a religious objection to chimera research on the ground that the creation of new types of animals by man that do not occur in nature is encroaching on a domain that is reserved for God. [268] Finally, although it might seem like farfetched science fiction, human-animal chimeras, which combine human brains and central nervous systems that confer human reasoning abilities, with the bodies of animals possessing certain physical capabilities greater than humans, such as apes or lions, could create grave danger to humanity as superhuman warriors in combat fighting alongside humans or if they turned on the humans that created them and sought to establish chimeric hegemony on the Earth. [269] While **[96]** these

objections are not without merit, the beneficial uses of human-animal chimeras likely outweigh them. [270] In addition to their use in organ transplantation, as discussed above, human-animal chimeras have valuable uses in studying the effects of drugs on humans. [271] They are also useful as models for human embryonic development. [272]

A final moral objection to human-animal chimeras is that construction of chimeras and research involving them may result in needless animal suffering. [273] This argument is disputable because a human-animal chimera would suffer no more than other animals used in research, patented or otherwise. [274] However, the suffering of a human-animal chimera, especially one considered human, would be weighed more heavily than that of an animal. [275] A retort is that any suffering incurred by chimeras and the increased opprobrium associated with chimera, as opposed to animal, research "would be offset by the increased value of using chimeras, rather than animals, in research … designed to benefit humanity." [276] Although it is strictly regulated, human research is a staple of medical science. Thus, the possibility of the suffering of chimeras, including those considered human, in research endeavors is not a reason to remove incentives for scientists to engage in chimera research by withholding patent protection. [277]

[97]

F. Human-Animal Chimeras Meet the Patent Law's Novelty Requirement

The novelty provisions of the patent law are conveyed in 35 U.S.C. § 102. [278] Under § 102, an invention is not patentable if "the invention was known or used by others in this country, or patented or described in a printed publication in this or a foreign country" or if "the invention was made in this country by another inventor who had not abandoned, suppressed, or concealed it" before the patentee invented it. [279] Human-animal chimeras do not appear in nature and are entirely created by man. Thus, if a human-animal chimera has not been previously described by other researchers and was first created by a patent applicant, the patent law's novelty provisions do not foreclose its patentability.

G. Human-Animal Chimeras Meet the Patent Law's Nonobviousness Requirement

Under 35 U.S.C. § 103(a), an invention is not patentable,

if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which [the] subject matter pertains. [280]

In Graham v. John Deere Co., [281] the Supreme Court provided analytical guidance for determining the issue of nonobviousness under § 103. [282] The Court articulated four criteria to be weighed in analyzing nonobviousness: (1) the scope and content of the prior art; (2) differences between the prior art and the claimed invention; (3) the level of ordinary skill in the art; and (4) secondary considerations that are objective indicia of nonobviousness including commercial success, long felt but unsolved needs, and failure of others. [283] The Supreme Court recently reaffirmed this framework in KSR International Co. v. Teleflex Inc. [284] In assessing the obviousness of an **[98]** invention, the prior art references are combined, and the entire prior art is compared to the invention as a whole. [285] Thus, while a single reference is required to anticipate an invention, the combination of multiple prior art references can render an invention obvious, although no single one of them does. In combining prior art references, a court must be careful to "avoid aggregating pieces of prior art through hindsight which would not have been combined absent the inventor['s] insight." [286] The obviousness inquiry is highly fact specific, making formulation of specific rules difficult. [287] However, in this nonobviousness analysis, the Federal Circuit considers two factors:

(1) whether the prior art would have suggested to those of ordinary skill in the art that they should make the claimed composition or device … and (2) whether the prior art would also have revealed that in so making or carrying out, those of ordinary skill would have a reasonable expectation of success. [288]

When the prior art suggests that an invention should be made and/or suggests the use of a technique to make an invention, but does not convey to those of ordinary skill a reasonable expectation that the invention could be made by suggesting how to create the invention using the technique and providing evidence that this could be accomplished successfully, the invention is not obvious. [289] Thus, both factors must be fulfilled to render an invention unpatentable as **[99]** obvious; prior art only satisfying the first one is insufficient "as it serves merely as an invitation to try to create the invention." [290]

[100] In the human-animal chimera context, a court would likely consider the relevant prior art to include animal chimeras and human-animal chimeras that have already been synthesized and the techniques used to synthesize them. In the xenotransplantation context, the prior art would include the current state of xenotransplantation and transplantation technologies.

Under this standard, the synthesis of some types of chimeras, those created by xenotransplantation, for instance, are likely obvious. A court would likely consider the prior art to have suggested to those of ordinary skill in the art that they use xenotransplantation. Xenotransplantation has been used in many situations where a human transplant is not available or practicable. [291] Also, the prior art would likely reveal that an attempted xenotransplantation would have a likelihood of success. Scientists and physicians have a relatively good understanding of when xenotransplantation is appropriate and when a human patient may reject an animal transplant. [292] Furthermore, although research involving surgical techniques is not regulated to the same degree as other forms of medical research such as testing of experimental drugs, an ethical surgeon would not attempt a xenotransplantation procedure on a human patient unless the surgeon believed that there was some likelihood of success. [293] However, nonobviousness calls for a very case-specific analysis. One might envision a xenotransplant, such as transplanting an animal heart or liver into a human, that would not be obvious if perfected because the prior art would not provide evidence of success. [294]

[101] In contrast, a court would likely find that a human-animal chimera created using molecular biological methods not hitherto described, is not obvious. One might argue that previous chimeras would render future chimeras obvious. Indeed, a court would probably consider the prior art to have suggested to a person with ordinary skill in the art to create a human-animal chimera. Numerous chimeric animals and human-animal chimeras have been created and numerous prior art references suggest avenues of research that chimeras may be used in. However, this is not sufficient to render a **[102]** given human-animal chimera obvious. [295] Specific difficulties may attend the creation of specific chimeras such that knowledge of previous chimeras will not suggest that the desired chimera will be created successfully. Successful creation of one human-animal chimera does not create a reasonable expectation that a different chimera can be successfully created using the same technique. Scientists find the results unpredictable when adopting molecular biological techniques involving embryos, such as cloning and embryo fusion to create chimeras, from one species to another. [296] For instance, the fact that a chimera can be created between humans and one animal does not mean that a chimera can be created containing cells of humans and a different animal. This suggests that a human-animal chimera with a new species is not obvious. Certain chimeras may be difficult to create. An example is a mouse with an entirely human brain. In this situation, difficulties may arise because a mouse has a smaller cranial cavity than a human, and a human brain may not develop properly in it. Furthermore, the embryo fusion method used to create the "geep" has never been used to create a true human-animal chimera that was allowed to develop to birth. Researchers have encountered problems in human cloning experiments that they have not observed with other species, including the termination of development of cloned human embryos in vitro at an early stage. [297] If allowed to develop further, the same problem might be encountered with chimeric embryos containing human cells. These problems make it unlikely that a court would find that the prior art involving the previous synthesis of human-animal chimeras engendered a reasonable expectation of success in a researcher attempting to create a human-animal chimera that has not been hitherto created.

Conclusion

Analysis of patentability involves many fact-specific factors that could not reasonably be considered in this article. However, the above analysis indicates that at least some human-animal chimeras may be patentable under some circumstances. The purpose of the patent system is to induce the discovery of new inventions by granting inventors the right to exclude others from making, selling, or using **[103]** the invention for a limited period of time. [298] Those who raise ethical objections to human-animal chimeras argue that the patent system should not encourage the creation of such potentially unethical inventions. However, the above analysis reveals that, under the current state of the patent law, at

least some human-animal chimeras may be patented. As the Supreme Court concluded in Chakrabarty, policy arguments raised against the patentability of living things, such as human-animal chimeras, are best addressed by Congress and that courts should not foreclose the patentability of inventions that are included within the scope of patentable subject matter under § 101 on such grounds. [299]

Courts consider living things, including plants, animals, and microorganisms, patentable subject matter so long as they are created by man and do not occur in nature. [300] Human-animal chimeras that do not rise to the level of humanity constitute patentable subject matter as they are wholly manmade living things that do not occur in nature and could not exist but for the intervention of man. However, as discussed above, the language of the Supreme Court in holding living things patentable, indicating that anything made by man is patentable subject matter, brings human-animal chimeras considered human and entirely human inventions into the scope of patentable subject matter under § 101. [301] However, the question of patentability of a chimera considered human, just like a wholly human invention, is complicated in several respects. First, Congress has enacted the Weldon Amendment and its progeny in appropriations bills proscribing the use of federal funds by the PTO to issue patents on human organisms. Second, humanity triggers Thirteenth and Fourteenth Amendment rights which give a creature autonomy rights and curtail the ability of others to assert property rights over it. However, neither the Weldon Amendment nor any constitutional source provides a definition of humanity despite the obvious importance of one in the chimera context. The preferable definition best reflecting moral and intuitional, as well as biological, conceptions of humanity classifies an organism as human if it is characterized by higher faculties and physical characteristics associated with human beings to a significant degree.

[104] However, careful analysis of the patent law and the Thirteenth and Fourteenth Amendment indicates that despite the uneasiness associated with the patentability of chimeras that are considered human and wholly human inventions, a court would likely hold that Congress did not intend to create a conflict with § 101's broad scope of patentable subject matter when it enacted the Weldon Amendment and, accordingly, that the Amendment did not completely foreclose patentability of human inventions and that a patent for a human invention does not run afoul of the Thirteenth and Fourteenth Amendments. Although the state of chimera technology has not developed to the point where the creation of mythological part-human monsters that physically resemble human beings and would be considered human, such as the Minotaur, is a reality; it is possible, if not likely, that the technology will eventually reach such a point. If Congress wishes to effectively prevent tinkerers in the realm of the unnatural from obtaining patent protection for such creatures, it must do what it has failed to do several times and enact an explicit restriction on the patentability of human organisms (or human-animal chimeras) as it has done for certain nuclear materials and nuclear weapons.

Santa Clara Computer and High Technology Law Journal

Santa Clara Computer & High Technology Law Journal

Footnote *

Associate, Intellectual Property and Technology Group, Skadden, Arps, Slate, Meagher & Flom LLP; B.A., 1999, Northwestern University; M.S., 2001, Ph.D., 2003, J.D., 2006, University of Chicago.

Footnote 1

Thomas A. Magnani, The Patentability of Human-Animal Chimeras, 14 Berkeley Tech. L.J. 443, 443 (1999).

Footnote 2

Id.

Footnote 3

Id. at 445.

Footnote 4

Id.

Footnote 5

Id.

Footnote 6

Id.

Footnote 7

Id.

Footnote 8

Id. at 445 n.17.

Footnote 9

Id. at 445.

Footnote 10

See Bruce Alberts et al., Molecular Biology of the Cell 1225-26 (4th ed. 2002); Magnani, supra note 1, at 446.

Footnote 11

See, e.g., Alberts et al., supra note 10, at 1225. As the chimera is the result of the fusion of two embryos of different species, each with parents of the same species, a chimera can be said to have four parents, two of each species present in the chimera. In contrast, a hybrid has two parents, one of each species present in the chimera.

Footnote 12

See id.; Magnani, supra note 1, at 446.

Footnote 13

See Alberts et al., supra note 10, at 1225; Magnani, supra note 1, at 446.

Footnote 14

See Alberts et al., supra note 10, at 1225.

Footnote 15

See Sabine Meinecke-Tillmann, Experimental Chimaeras - Removal of Reproductive Barrier Between Sheep and Goat, 307 Nature 637 (1984); Carole B. Fehilly et al., Interspecific Chimaerism Between Sheep and Goat, 307 Nature 634 (1984).

Footnote 16

See Meinecke-Tillmann, supra note 15; Fehilly et al., supra note 15.

Footnote 17

See, e.g., John Rennie, Human-Animal Chimeras, Sci. Am., June 27, 2005, http://www.sciam.com/article.cfm?id=human-animal-chimeras; Maryann Mott, Animal-Human Hybrids Spark Controversy, Nat'l Geographic, Jan. 25, 2005, http://news.nationalgeographic.com/news/2005/01/0125 050125 chimeras.html; Rick Weiss, Of Mice, Men and In-Between, Wash. Post, Nov. 20, 2004, at A1; Sylvia Pagan Westphal, Growing Human Organs on the Farm, New Scientist, Dec. 20, 2003, at 4; Magnani, supra note 1, at 446.

Footnote 18

See, e.g., Westphal, supra note 17 at 4.

Footnote 19

See, e.g., Bratislav Stankovic, Patenting the Minotaur, 12 Rich. J.L. & Tech. 5, 6 (2005), available at http://law.richmond.edu/jolt/v12i2/article5.pdf.

Footnote 20

See id.

Footnote 21

Mott, supra note 17.

Footnote 22

See id.

Footnote 23

Id.

Footnote 24

Id.

Footnote 25

Westphal, supra note 17, at 4.

Footnote 26

See id.

Footnote 27

Id.

Footnote 28

See Mott, supra note 17.

Footnote 29

Id.

Footnote 30

See Magnani, supra note 1, at 456. Depending on the division of cells between each species in the chimera, it is possible that some organs in a human-animal chimera could consist entirely of human cells.

Footnote 31

Id.

Footnote 32

See id. at 457.

Footnote 33

See Stankovic, supra note 19, at 31.

Footnote 34

See Magnani, supra note 1, at 457.

Footnote 35

See infra Part II.A.

Footnote 36

Diamond v. Chakrabarty, 447 U.S. 303 (1980).

Footnote 37

Id. at 309.

Footnote 38

See J.E.M. Ag Supply, Inc. v. Pioneer Hi-Bred Int'l, Inc., 534 U.S. 124 (2001); Ex parte Allen, 2 U.S.P.Q.2d 1425 (B.P.A.I. 1987); Ex parte Hibberd, et al., 227 U.S.P.Q. 443 (B.P.A.I. 1985).

Footnote 39

Robert Patrick Merges & John Fitzgerald Duffy, Patent Law and Policy: Cases and Materials 129-30 (4th ed. 2007).

Footnote 40

See infra Part I.E.

Footnote 41

This article is restricted in scope to product claims for the human-animal chimera itself. Process claims for making human-animal chimeras do not raise the complex issues relating to the patenting of humanity. See Magnani, supra note 1, at 450. These claims are patentable if they meet the statutory requirements for patentability including novelty, nonobviousness, and utility.

Footnote 42

35 U.S.C. § 101 (2000) ("Whoever invents or discovers any new and useful process, machine, manufacture, or composition of matter, or any new and useful improvement thereof, may obtain a patent therefor … .").

Footnote 43

Id.

Footnote 44

See Alan L. Durham, Patent Law Essentials 23 (1999); see also S. Rep. No. 82-1979, at 5 (1952) (stating that patents are available for "anything under the sun that is made by man"); H.R. Rep. No. 82-1923, at 6 (1952) (also stating that patents are available for "anything under the sun that is made by man").

Footnote 45

See, e.g., Diamond v. Chakrabarty, 447 U.S. 303, 309 (1980) (relating cases where certain categories of invention were deemed unpatentable subject matter); Janice M. Mueller, An Introduction to Patent Law 234 (2d ed. 2006).

Footnote 46

U.S. Patent No. 141,072 (filed May 9, 1873).

Footnote 47

See David G. Scalise & Daniel Nugent, International Intellectual Property Protections for Living Matter: Biotechnology, Multinational Conventions and the Exception for Agriculture, 27 Case W. Res. J. Int'l L. 83, 95 (1995). In 1937, a leading commentator opined that Pasteur's patent would be refused because it did not cover patentable subject matter. See P.J. Frederico, Louis Pasteur's Patents, 86 Sci. 327 (1937). The only other examples of patents for living things, independent of their use, issued by the PTO prior to 1980, identified in the case law and literature, are two patents claiming living microorganisms issued in 1967 and 1969. See, e.g., Chakrabarty, 447 U.S. at 314 n.9. However, while the PTO and lower courts refused to permit patents for living organisms themselves, they repeatedly permitted patents for compositions containing living things and processes utilizing them. See, e.g., Milwaukee v. Activated Sludge, Inc., 69 F.2d 577, 578 n.1 (7th Cir. 1934) (recognizing a patent for a septic tank utilizing bacteria); Union Solvents Corp. v. Guar. Trust Co., 61 F.2d 1041 (3d Cir. 1932) (upholding a patent for a bacterial process used in the synthesis of alcohol); Cameron Septic Tank Co. v. Saratoga Springs, 159 F. 453, 462-63 (2d Cir. 1908) (holding a patent claiming a septic tank using anaerobic bacteria valid). However, courts have long held that biological substances purified from living things were patentable because they did not exist in a purified form in nature. See, e.g., Parke-Davis & Co. v. H.K. Mulford Co., 189 F. 95, 103, 115 (C.C.S.D.N.Y. 1911). This reasoning was extended to purified microorganism cultures much later as such purified cultures of microorganisms do not exist in nature and are manmade as they can only be produced under carefully controlled laboratory conditions. See In re Bergy, 563 F.2d 1031, 1035-37 (C.C.P.A. 1977), vacated sub nom. Parker v. Bergy, 438 U.S. 902 (1978) (mem.), aff'd on remand sub nom. In re Bergy, 596 F.2d 952 (C.C.P.A. 1979), aff'd sub nom. Diamond v. Chakrabarty, 447 U.S. 303 (1980). Naturally occurring higher plants and animals, such as human beings and human-animal chimeras, are "large enough that their identification and isolation does not require a scientist's ingenuity in developing experimental culture conditions." Mark L. Rohrbaugh, The Patenting of Extinct Organisms: Revival of Lost Arts, 25 AIPLA Q.J. 371, 385 (1997). Thus, a higher organism may only be patentable if the organism itself results from the application of human ingenuity and effort rather than from the routine cultivation of a free-living organism. See id.; see also infra Part I.C.

Footnote 48

See, e.g., Scalise & Nugent, supra note 47, at 95.

Footnote 49

See Valerie J. Phillips, Half-Human Creatures, Plants & Indigenous Peoples: Musings on Ramifications of Western Notions of Intellectual Property and the Newman-Rifkin Attempt to Patent a Theoretical Half-Human Creature, 21 Santa Clara Computer & High Tech. L.J. 383 (2005).

Footnote 50

35 U.S.C. § 161 (2000).

Footnote 51

§ 2402 (2000).

Footnote 52

See Scalise & Nugent, supra note 47, at 95; see also Chakrabarty, 447 U.S. at 309 (indicating that products of nature are unpatentable).

Footnote 53

See Chakrabarty, 447 U.S. at 305. Plasmids are circular pieces of DNA which encode bacterial genes and function as hereditary units that are physically separate from the bacterial chromosome. Id. at 305 n.1.

Footnote 54

See id. at 318.

Footnote 55

See id. at 308.

Footnote 56

Id. at 309. (quoting S. Rep. No. 82-1979, at 5 (1952); H.R. Rep. No. 82-1923, at 6 (1952)).

Footnote 57

Id. at 308. (quoting Am. Fruit Growers, Inc. v. Brogdex Co., 283 U.S. 1, 11 (1931)).

Footnote 58

Id. (quoting Shell Dev. Co. v. Watson, 149 F.Supp. 279, 280 (D.D.C. 1957)).

Footnote 59

Id. at 309-10 (quoting Hartranft v. Wiegmann, 121 U.S. 609, 615 (1887) (alteration in original)).

Footnote 60

Funk Bros. Seed Co. v. Kalo Inoculant Co., 333 U.S. 127 (1948); see also Philip McGarrigle & Vern Norviel, Laws of Nature and the Business of Biotechnology, 24 Santa Clara Computer & High Tech. L.J. 275 (2008).

Footnote 61

Funk Bros., 333 U.S. at 131.

Footnote 62

See id.

Footnote 63

Chakrabarty, 447 U.S. at 310.

Footnote 64

Id.

Footnote 65

Id. at 314-15.

Footnote 66

Id. at 316.

Footnote 67

35 U.S.C. § 161 (2000).

Footnote 68

7 U.S.C. § 2402 (2000).

Footnote 69

See Chakrabarty, 447 U.S. at 313.

Footnote 70

Id.

Footnote 71

Id. at 311-12 (quoting 35 U.S.C. § 112 (1976)).

Footnote 72

Id. at 313.

Footnote 73

Id.

Footnote 74

Id.

Footnote 75

Id. at 316-17.

Footnote 76

Id. at 317.

Footnote 77

Ex parte Hibberd, 227 U.S.P.Q. 443, 443-44 (B.P.A.I. 1985).

Footnote 78

Id. at 444.

Footnote 79

Id. at 445.

Footnote 80

See id. at 446.

Footnote 81

J.E.M. Ag Supply, Inc. v. Pioneer Hi-Bred Int'l, Inc., 534 U.S. 124, 145-46 (2001).

Footnote 82

Id. at 130.

Footnote 83

See id. at 131-32.

Footnote 84

See id. at 145-46.

Footnote 85

Edmund J. Sease, From Microbes, to Corn Seeds, to Oysters, to Mice: Patentability of New Life Forms, 38 Drake L. Rev. 551, 563 (1989).

Footnote 86

Patents and the Constitution: Transgenic Animals: Hearing Before the Subcomm. of Courts, Civil Liberties and the Admin. of Justice on the H. Comm. on the Judiciary, 100th Cong. 160 (1988); Paul Blunt, Selective Breeding and the Patenting of Living Organisms, 48 Syracuse L. Rev. 1365, 1369 (1998).

Footnote 87

Ex parte Allen, 2 U.S.P.Q.2d 1425 (B.P.A.I. 1987), aff'd, 846 F.2d 77 (Fed. Cir. 1988) (unpublished

table decision).

Footnote 88

Id. at 1426.

Footnote 89

Id.

Footnote 90

Id. at 1427.

Footnote 91

Id.

Footnote 92

Donald J. Quigg, Animals-Patentability, 69 J. Pat. & Trademark Off. Soc'y 328, 328 (1987).

Footnote 93

Id.

Footnote 94

Id.

Footnote 95

Id.

Footnote 96

Magnani, supra note 1, at 448. See, e.g., Elizabeth Joy Hecht, Beyond Animal Legal Defense Fund v. Quigg: The Controversy Over Transgenic Animal Patents Continues, 41 Am. U. L. Rev. 1023, 1024 (1992).

Footnote 97

Animal Legal Def. Fund v. Quigg, 932 F.2d 920, 922 (Fed. Cir. 1991).

Footnote 98

Id. at 925.

Footnote 99

Id. at 927-28 (citing In re Allen, 846 F.2d 77 (Fed. Cir. 1988) (unpublished table decision)).

Footnote 100

U.S. Patent No. 4,736,866 (filed June 22, 1984) (issued Apr. 12, 1988).

Footnote 101

See, e.g., Warren D. Woessner, The Evolution of Patents on Life - Transgenic Animals, Clones and Stem Cells, 83 J. Pat. & Trademark Off. Soc'y 830-31 (2001).

Footnote 102

Id. at 832.

Footnote 103

See, e.g., Woessner, supra note 101, at 833-34.

Footnote 104

See, e.g., Merges & Duffy, supra note 39, at 129; David Dickson, Legal Fight Looms over Patent Bid on Human/Animal Chimaeras, 392 Nature 423 (1998); Rick Weiss, Patent Sought on Making of Part-Human Creatures, Wash. Post, Apr. 2, 1998, at A12.

Footnote 105

Merges & Duffy, supra note 39, at 129; Weiss, supra note 104. An invention need not be built to be patentable. E.g., Weiss, supra note 104. An invention is patentable as constructively reduced to practice if the inventor's application discloses information that would enable a person skilled in the art to make and use the invention without undue expectation. See In re Strahilevitz, 668 F.2d 1229, 1232 (C.C.P.A. 1982).

Footnote 106

Merges & Duffy, supra note 39 at 129; Dickson, supra note 104.

Footnote 107

See, e.g., Merges & Duffy, supra note 39, at 129; Magnani, supra note 1, at 443; Weiss, supra note 104.

Footnote 108

E.g. Merges & Duffy, supra note 39, at 129.

Footnote 109

Merges & Duffy, supra note 39, at 225 (citing Press Release, U.S. Patent & Trademark Office, Media Advisory (Apr. 1, 1998), available at www.uspto.gov/web/offices/com/speeches/98.06.htm.). Section 101 permits patents only for inventions that are "new and useful." 35 U.S.C. § 101 (2000).

Footnote 110

Lowell v. Lewis, 15 F. Cas. 1018, 1019 (C.C.D. Mass. 1817) (No. 8568); see also Bedford v. Hunt, 3 F. Cas. 37 (C.C.D. Mass. 1817) (No. 1217).

Footnote 111

Lowell v. Lewis 15 F. Cas. 1018 (C.C.D. Mass. 1817) (No. 8568).

Footnote 112

Merges & Duffy, supra note 39, at 225 (quoting "Morality" Aspect of Utility Requirement Can Bar Patent for Part-Human Inventions, 55 Pat. Trademark & Copyright J. (BNA) 555 (Apr. 9, 1998)).

Footnote 113

See id. at 130-31.

Footnote 114

Id. at 131 (quoting Patent Application Is Disallowed as "Embracing' Human Being, 58 Pat. Trademark & Copyright J. (BNA) 203 (June 17, 1999)).

Footnote 115

Id.

Footnote 116

Id.

Footnote 117

Stankovic, supra note 19, P 23.

Footnote 118

U.S. Patent Application No. 08/993,564 (filed Dec. 19, 1997), Office Action from Deborah Crouch, Primary Examiner (Feb. 9, 2004).

Footnote 119

Id. at 21.

Footnote 120

Id.

Footnote 121

Id.

Footnote 122

See U.S. Patent Application No. 08/993,564, Notice of Abandonment from Deborah Crouch, Primary Examiner (Feb. 25, 2005).

Footnote 123

Human Chimera Prohibition Act of 2005, S. 1373, 109th Cong. (2005).

Footnote 124

Id. at § 302. Even if Congress were to prohibit the development of human-animal chimeras, such a law does not necessarily proscribe patentability without an express provision doing so. Indeed, courts have found radar detectors patentable even though their use, including that by the inventor, is illegal in some states. See Whistler Corp. v. Autotronics, Inc., 14 U.S.P.Q.2d 1885 (N.D. Tex. 1988).

Footnote 125

S. 1373, 109th Cong. § 301(1) (2005).

Footnote 126

Nicole E. Kopinski, Human-Nonhuman Chimeras: A Regulatory Proposal on the Blurring of Species Lines, 45 B.C. L. Rev. 619, 620 (2004).

Footnote 127

See, e.g., S. 387, 103d Cong. (1993) (proposing a moratorium on patenting animal and human tissues); Transgenic Animal Patent Act, H.R. 4970, 100th Cong. (1988) (proposing an amendment to § 101 excluding human beings from the scope of patentable subject matter).

Footnote 128

Transgenic Animal Patent Reform Act, H.R. 4970, 100th Cong. (1988). Under the Animal Patent Act, § 101 would have read: "Whoever invents or discovers any new and useful process, machine, manufacture, or composition of matter, or any new and useful improvement thereof, may obtain a patent therefore, subject to the conditions and requirements of this title, except that human beings are not patentable subject matter." Id.

Footnote 129

House Passage of Animal Patent Bill, 36 Pat. Trademark & Copyright J. (BNA) 499, 502 (1988).

Footnote 130

Daniel J. Kevles, Diamond v. Chakrabarty and Beyond: The Political Economy of Patenting Life, in Private Science 65, 76 (Arnold Thackray ed., 1998).

Footnote 131

Consolidated Appropriations Act of 2004, Pub. L. No. 108-199, § 634, 118 Stat. 3, 101 (2004).

Footnote 132

See infra Part II.B.1.

Footnote 133

Kopinski, supra note 126, at 635.

Footnote 134

E.g., Kopinksi, supra note 126, at 635 n.133 (citing Rick Weiss, Hill Negotiators Agree to Bar Patents for Human Organisms, Wash. Post, Nov. 25, 2003, at A19).

Footnote 135

See Letter from James E. Rogan, Under Sec'y and Dir., U. S. Patent and Trademark Office, to Hon. Ted Stevens, Chairman, Comm'n on Appropriations, U.S. Senate, at 2 (Nov. 20, 2003), available at http://www.nrlc.org/Killing Embryos/Human Patenting/WeldonamendUSPTO.pdf (indicating that the Weldon Amendment "does not alter the [PTO] policy on the non-patentability of human life-forms at any stage of development and is fully consistent with [PTO] policy"); accord Kopinski, supra note 126, at 635 n.133; Weiss, supra note 134.

Footnote 136

149 Cong. Rec. E2234 (daily ed. Nov. 5, 2003) (statement of Rep. Weldon).

Footnote 137

See Kopinski, supra note 126, at 636 n.136 (quoting 149 Cong. Rec. E2234 (daily ed. Nov. 5, 2003) (statement of Rep. Weldon)).

Footnote 138

Id.

Footnote 139

Id.

Footnote 140

See Kopinski, supra note 126, at 636-37 n.140.

Footnote 141

Judith L. Toffenetti & Thomas A. Haag, Biotech and Ethics Collide in Patent-Funding Debate, Wash. Bus. J., Aug 20-26, 2004.

Footnote 142

Id.

Footnote 143

Science, State, Justice, Commerce and Related Agencies Appropriations Act of 2006, Pub. L. No. 109-108, § 623, 119 Stat. 2290, 2342 (2005); Consolidated Appropriations Act of 2005, Pub. L. No. 108-447, § 626, 118 Stat. 2809, 2920 (2004).

Footnote 144

See Science, State, Justice, Commerce and Related Agencies Appropriations Act of 2006, Pub. L. No. 109-108, § 626, 119 Stat. 2290, 2290 (2005).

Footnote 145

A bill providing for the reenactment of the Weldon Amendment as part of the 2007 federal budget passed in the House but was not passed in the Senate. H.R. 5672, 109th Cong. § 618 (2006).

Footnote 146

See Continuing Appropriations Resolution of 2007, Pub. L. No. 110-5, §§104, 20934, 121 Stat. 8, 9, 45 (2007) (appropriating funds for the operation of the patent office). The amended Continuing Appropriations Resolution of 2007, which expressly provided for funding for the PTO, was enacted on Feb. 15, 2007. The Weldon Amendment was in force for the period between the expiration of the 2006 enactment on Sept. 30, 2006 and the passage of the amended Continuing Appropriations Resolution of 2007 on the basis of the passage of continuing appropriations acts providing for PTO funding until the passage of an appropriations act providing for, or otherwise applicable to, PTO funding for the 2007 budget that could not be used for activities that appropriations, funds, or other authority were not available for under the 2006 federal budget. See Continuing Appropriations Resolution, 2007, 109 Pub. L. No. 289, div. B,§§101, 104, 106, 120 Stat. 1257 (2006); Pub. L. No. 109-369, 120 Stat. 2678 (2006); Pub. L. No. 383, 120 Stat. 2678 (2006). Since the Weldon Amendment was enacted as part of the 2006 budget and expressly rendered funds appropriated for the PTO unavailable for patenting of human organisms, the restriction on patentability of human organisms imposed by the Weldon Amendment remained in force during this period.

Footnote 147

Consolidated Appropriations Act of 2008, Pub. L. No. 110-161, § 520, 121 Stat. 1844, 1928 (2007).

Footnote 148

Id. §§6, 520.

Footnote 149

A bill providing for the reenactment of the Weldon Amendment as part of the 2009 federal budget has been introduced in the Senate, but has not been voted on as of the time of printing. S. 3182, 110th Cong. § 518 (2008).

Footnote 150

See Consolidated Security, Disaster Assistance, and Continuing Appropriations Act, 2009, Pub. L. No. 110-329,§§101, 104, 106, 122 Stat. 3574 (2008). See also id. § 101 (providing that funds available for 2009 under the continuing appropriations act were available under the "authority and conditions of" the 2008 appropriations acts). The continuing appropriations act also provides that funds available under it were available under the "authority and conditions of" the 2008 appropriations acts. See id. § 101. The Weldon Amendment prescribing that none of the funds appropriated or made available by Congress be used for the patenting of human organisms can be viewed as a condition attached to the funds.

Footnote 151

See PTO Final Rejection, supra note 118.

Footnote 152

Id.

Footnote 153

Stankovic, supra note 19, P 25.

Footnote 154

See Magnani, supra note 1, at 450.

Footnote 155

See, e.g., Kopinski, supra note 126, at 636 n.136 (quoting 149 Cong. Rec. E2234 (daily ed. Nov. 5, 2003) (statement of Rep. Weldon)); Magnani, supra note 1, at 449.

Footnote 156

See supra note 137 and accompanying text.

Footnote 157

See, e.g., Magnani, supra note 1, at 449.

Footnote 158

See, e.g., Stankovic, supra note 19, P 34.

Footnote 159

See id.

Footnote 160

See id.

Footnote 161

See Magnani, supra note 1, at 449.

Footnote 162

See id. at 449-50.

Footnote 163

Id. at 450.

Footnote 164

Stankovic, supra note 19, at 18 n.93 (citing Roy J. Britten, Divergence Between Samples of Chimpanzee and Human DNA Sequences Is 5%, Counting Indels, 99 Proc. Nat'l Acad. Sci. U.S. 13633, 13633 (2002)).

Footnote 165

A human-animal chimera does not necessarily contain the same percentage of human cells and human genetic material because human and animal cells do not necessarily contain the same amount of genetic material. See, e.g., Alberts et al., supra note 10, at 20. For instance, the human genome is larger than the mouse genome. Thus, a human-mouse chimera with a given percentage of human cells contains a greater percentage of human genetic material (DNA) because each human cell in the chimera contains a greater amount of human DNA than the amount of mouse DNA present in each mouse cell. Thus, a tenable argument can be made that percentage of cells rather than percentage of genetic material should be examined in gauging the humanity of a chimera. The percentage of cells, rather than the sheer amount of genetic material derived from a given species, which depends in part on the size of the species' genomes (in addition to the number of cells), determines the character of the chimera. See, e.g., Press Release, National Human Genome Research Institute, International Team of Researchers Assembles Draft Sequence of Mouse Genome (May 6, 2002), http://www.genome.gov/10002983 ("The mouse genome is contained in 20 chromosome pairs and the current results suggest that it is about 2.7 billion base pairs in size, or about 15 percent smaller than the human genome. The human genome is 3.1 billion base pairs spread out over 23 pairs of chromosomes.").

Footnote 166

Magnani, supra note 1, at 450.

Footnote 167

See id. Indeed, efforts are underway to create a mouse with a brain comprised of entirely human tissue. See supra note 30 and accompanying text.

Footnote 168

See Magnani, supra note 1, at 450. Thus, one of the flaws with quantitative approaches is that the number of cells required for people to consider a chimera human might fluctuate depending on which organs and/or organ systems are of human origin.

Footnote 169

Rachel E. Fishman, Patenting Human Beings: Do Sub-Human Creatures Deserve Constitutional Protection?, 15 Am. J.L. & Med. 461, 480-81 (1989).

Footnote 170

See Magnani, supra note 1, at 450.

Footnote 171

Civil Rights Cases, 109 U.S. 3, 20 (1883).

Footnote 172

If the kind of property rights in a human organism conferred by a patent were foreclosed by the Thirteenth Amendment, such chimeras would not be patentable. See infra Part II.C.

Footnote 173

Fishman, supra note 169, at 480-81.

Footnote 174

Id. at 481.

Footnote 175

Diamond v. Chakrabarty, 447 U.S. 303, 309 (1980).

Footnote 176

Ex parte Allen, 2 U.S.P.Q.2d 1425, 1426 (B.P.A.I. 1987).

Footnote 177

See Stankovic, supra note 19, at 14.

Footnote 178

See id.

Footnote 179

See Chakrabarty, 447 U.S. at 314-15.

Footnote 180

See id. at 314-16.

Footnote 181

See id. at 315.

Footnote 182

See Stankovic, supra note 19, at 17.

Footnote 183

See, e.g., Tenn. Valley Auth. v. Hill, 437 U.S. 153, 184 n.29 (1978) ("When confronted with a statute which is plain and unambiguous on its face, [courts] ordinarily do not look to legislative history as a guide to its meaning. [In such cases,] it is not necessary to look beyond the words of the statute.").

Footnote 184

Stankovic, supra note 19, at 14.

Footnote 185

See, e.g., id. at 9-10; Magnani, supra note 1, at 448.

Footnote 186

See, e.g., Rebecca M. Bratspies, Glowing in the Dark: How America's First Transgenic Animal Escaped Regulation, 6 Minn. J.L. Sci. & Tech 457, 457 n.3 (2005).

Footnote 187

See, e.g., Moore v. Regents of Univ. of Cal., 793 P.2d 479, 481 n.2 (Cal. 1990); Alberts et al., supra note 10, at 472.

Footnote 188

See Cell line - definition from Biology-Online.org, Sep. 29, 2006, http://www.biology-online.org/dictionary/Cell line; Definition: cell line from Online Medical Dictionary, Mar. 26, 1998, http://cancerweb.ncl.ac.uk/cgi-bin/omd?cell+line. See, e.g., Alberts et al., supra note 10, at 472-75, 1323-24; Merryn Macville et al., Comprehensive and Definitive Molecular Cytogenetic Characterization of HeLa Cells by Spectral Karyotyping, 59 Cancer Res. 141 (1999).

Footnote 189

See, e.g., Kopinski, supra note 126, at 635 & n.133, 636 & nn. 136-37.

Footnote 190

Id. at 636 n.137 (citing 149 Cong. Rec. E2235 (statement of Rep. Weldon)).

Footnote 191

One might argue that Congress's assumption that the foreclosure of patentability of human organisms by the Weldon Amendment was consistent with § 101 indicates that § 101 did not provide for the patentability of human organisms, despite the Supreme Court's interpretation of it in Chakrabarty, abating the conflict discussed here. While this argument may have some appeal, it is inconsistent with the precepts of statutory interpretation articulated by the Supreme Court. The Supreme Court has noted that "the views of a subsequent Congress form a hazardous basis for inferring the intent of an earlier one." United States v. Phila. Nat'l Bank, 374 U.S. 321, 348-49 (1963)(quoting United States v. Price, 361 U.S. 304, 313 (1960)). This holds true despite the fact that misunderstanding of the original statute may have played some part in the passage of the subsequent statute, as is the case with § 101 and the Weldon Amendment. Id. at 349. The Court considers "subsequent history … less illuminating than contemporaneous evidence." Hagen v. Utah, 510 U.S. 399, 420 (1994). "Thus, even when it would otherwise be useful, subsequent legislative history will rarely override a reasonable interpretation of a statute that can be gleaned from its language and legislative history prior to its enactment." Consumer Prod. Safety Comm'n v. GTE Sylvania, Inc., 447 U.S. 102, 118 n.13 (1980); accord Doe v. Chao, 540 U.S. 614, 626-27 (2004). The language of § 101 authorizes a wide scope of patentable subject matter seemingly including anything made by man. See Diamond v. Chakrabarty, 447 U.S. 303, 308-09, 314-16 (1980). Likewise, the contemporaneous legislative history provides that "anything under the sun that is made by man" is patentable under § 101. S. Rep. No. 82-1979, at 5 (1952); accord H.R. Rep. No. 82-1923, at 6 (1952); see also Chakrabarty, 447 U.S. at 308-09. Given the broad, inclusive language of § 101 and the unambiguous legislative history that § 101 rendered anything made by man patentable, a court would not likely consider Congress's incorrect assumption that § 101 allowed for the exclusion of human organisms from the realm of patentable subject matter at the time it passed the Weldon Amendment to restrict the scope of patentable subject matter under § 101 contrary to its plain language and contemporaneous legislative history. Another argument undermining the contention that § 101 excluded human organisms from the realm of patentable subject matter is that if it did so, the Weldon Amendment would have been unnecessary and redundant.

Footnote 192

See United States v. Dickerson, 310 U.S. 554, 555 (1940).

Footnote 193

See Tenn. Valley Auth. v. Hill, 437 U.S. 153, 190 (1978).

Footnote 194

See id. at 191.

Footnote 195

See id. at 189. (quoting Posadas v. Nat'l City Bank, 296 U.S. 497, 503 (1936)).

Footnote 196

See Neal E. Devins, Regulation of Government Agencies Through Limitation Riders, 1987 Duke L.J. 456, 482.

Footnote 197

Tenn. Valley Auth., 437 U.S. at 190 (quoting Morton v. Mancari, 417 U.S. 535, 550 (1974)).

Footnote 198

See, e.g., Kopinski, supra note 126, at 635-36 & n.137 (indicating that Congress intended to bar patentability of human organisms in the Weldon Amendment).

Footnote 199

Busic v. United States, 446 U.S. 398, 406 (1980) (citing Preiser v. Rodriguez, 411 U.S. 475, 489-90 (1973)); accord Sullivan v. Augusta, 511 F.3d 16, 27 (1st Cir. 2007).

Footnote 200

See 42 U.S.C. § 2181 (2000).

Footnote 201

Id.

Footnote 202

See supra notes 94-96 and accompanying text.

Footnote 203

U.S. Const. amend. XIII, § 1.

Footnote 204

Jones v. Alfred H. Mayer Co., 392 U.S. 409, 440-43 (1968).

Footnote 205

Id. at 441 (quoting Civil Rights Cases, 109 U.S. 3, 22 (1883)).

Footnote 206

See Kevin D. DeBre, Patents on People and the U.S. Constitution: Creating Slaves or Enslaving Science?, 16 Hastings Const. L.Q. 221, 230 (1989).

Footnote 207

Dan L. Burk, Patenting Transgenic Human Embryos: A Nonuse Cost Perspective, 30 Hous. L. Rev. 1597, 1647-48 (1993).

Footnote 208

See 35 U.S.C. § 154 (2000).

Footnote 209

See Mueller, supra note 45, at 14; Burk, supra note 207, at 1648.

Footnote 210

See Burk, supra note 207, at 1641, 1648.

Footnote 211

See Whistler Corp. v. Autotronics, Inc., 14 U.S.P.Q.2d 1885, 1885-86 (N.D. Tex. 1988).

Footnote 212

Burk, supra note 207, at 1648.

Footnote 213

Id.

Footnote 214

Id.

Footnote 215

See id.; DeBre, supra note 206, at 232.

Footnote 216

DeBre, supra note 206, at 232.

Footnote 217

See Jones v. Mayer, 392 U.S. 409, 441 (1968) (quoting Civil Rights Cases, 109 U.S. 3, 22 (1883)).

Footnote 218

See Burk, supra note 207, at 1648; DeBre, supra note 206, at 232. Some commentators have argued that the badges and incidents of slavery may arise from "genetic bondage" occurring when genetic manipulation, of a human organism, such as generating a human-animal chimera, gives rise to a sort of character determination. See DeBre, supra note 206, at 230. Genetic bondage may involve genetically engineering a human creature that possesses a disabling condition(s) infringing on its autonomy, such as low intelligence, or a more subtle intrusion on autonomy, such as mass production of like human organisms leading to an erosion of their sense of individuality. Id. While this argument has some merit, it stretches the meaning of the Thirteenth Amendment and, in particular, the terms "slavery" and "involuntary servitude." Burk, supra note 207, at 1648. This argument is also inconsistent with the Supreme Court's interpretation of the Thirteenth Amendment. Id. The Court has

interpreted the Thirteenth Amendment in light of its historical setting as directed toward eradicating the condition and badges of slavery inflicted upon African-Americans. See, e.g., id. (collecting cases). The Court does not recognize genetic determinism as a component of that history of bondage. Id. at 1648-49. A broad reading of the Thirteenth Amendment that would encompass genetic determinism could also lead to absurd and socially undesirable results, such as a conclusion that individuals with a formative influence on children, such as parents and teachers, are subjecting them to the badges of slavery. Id. at 1649.

Footnote 219

See, e.g., Magnani, supra note 1, at 450.

Footnote 220

See supra Part II.A; Magnani, supra note 1, at 450.

Footnote 221

See Stankovic, supra note 19, at 19-20; See Magnani, supra note 1, at 450.

Footnote 222

See supra Part II.A.

Footnote 223

Carey v. Population Servs. Int'l, 431 U.S. 678, 684-86 (1977).

Footnote 224

Id. at 685.

Footnote 225

Burk, supra note 207, at 1649.

Footnote 226

See Mueller, supra note 45, at 14. See also 35 U.S.C. § 271(a) (2000).

Footnote 227

See Burk, supra note 207, at 1649.

Footnote 228

The patentee's ability to control the reproduction of a self-replicating living invention is called into question by the doctrine of patent exhaustion which provides that a patentee who sells or transfers a patented article cannot restrain subsequent resale, transfer, or use of the article. See id. at 1638-39 n.320, 1650 n.396. However, the doctrine of patent exhaustion does not apply when the article

embodying the invention is not sold by the patentee. See, e.g., Monsanto Co. v. McFarling, 302 F.3d 1291, 1299 (Fed. Cir. 2002). No sale by the patentee to the creature itself occurs when the patentee creates a human organism. However, the Thirteenth Amendment prohibition on slavery seemingly prohibits sale of a human organism. Thus, a court might consider a patentee's act in creating a patented human organism a constructive sale to the organism itself for the purposes of patent exhaustion.

Footnote 229

See Alberts et al., supra note 10, at 1225-26.

Footnote 230

In sexual reproduction involving two human-animal chimeras, it is theoretically possible that a human sperm could fertilize an animal egg or vice versa. Even if such a fertilization could lead to the development and birth of offspring, such offspring would be a hybrid rather than a chimera because every cell would contain 50% animal and 50% human genetic material whereas in chimeras, some cells contain only human genetic material and others contain only animal genetic material.

Footnote 231

Burk, supra note 207, at 1650.

Footnote 232

35 U.S.C § 101 (2000) (mandating that patentable inventions be "new and useful," among other things).

Footnote 233

See In re Brana, 51 F.3d 1560, 1564 (Fed. Cir. 1995); Mueller, supra note 45, at 208.

Footnote 234

Lowell v. Lewis, 15 F. Cas. 1018, 1019 (C.C.D. Mass. 1817).

Footnote 235

See Juicy Whip, Inc. v. Orange Bang, Inc., 185 F.3d 1364, 1366-68 (Fed. Cir. 1999).

Footnote 236

See Mueller, supra note 45, at 208.

Footnote 237

Juicy Whip, 185 F.3d at 1366; accord Brooktree Corp. v. Advanced Micro Devices, Inc., 977 F.2d 1555, 1571 (Fed. Cir. 1992) ("To violate § 101 the claimed device must be totally incapable of achieving a useful result.").

Footnote 238

See, e.g., Brana, 51 F.3d at 1566; see also Rohrbaugh, supra note 47, at 388 & n.80 (collecting cases).

Footnote 239

Brenner v. Manson, 383 U.S. 519, 534 (1966).

Footnote 240

See id. at 534-35.

Footnote 241

U.S. Patent & Trademark Office, Manual of Patent Examining Procedure § 2107.01(I)(B)(A) (8th ed., rev. 6 2007).

Footnote 242

Id. § 2107.01(I)(C).

Footnote 243

Callison v. Dean, 70 F.2d 55, 58 (10th Cir. 1934) ("[A] device which may be used for innocent amusement possesses utility."); see also U.S. Patent No. 5,523,741 (filed Aug. 19, 1994) (patenting a "Santa Claus detector" that is useful for "providing [children] reassurance that [their] good behavior has … been rewarded by Santa Claus" by "providing selective illumination to signal the arrival of Santa Claus").

Footnote 244

Utility Examination Guidelines, 66 Fed. Reg. 1092, 1098 (Jan. 5, 2001). Such a "throw away" use in the human-animal chimera context would be to claim a chimera to be used as snake food. See Merges & Duffy, supra note 39, at 249 (using this example in the transgenic animal context). This use is neither specific, because any animal of comparable size could be used as snake food, nor substantial, because using a human-animal chimera, which would be rare and expensive to produce and probably unethical to feed to animals, as snake food is not a real world context of use. See id.

Footnote 245

See Magnani, supra note 1, at 456.

Footnote 246

See id.

Footnote 247

See id.

Footnote 248

U.S. Patent & Trademark Office, supra note 241, § 2107.01(I)(C).

Footnote 249

See Kopinski, supra note 126, at 629-30; Magnani, supra note 1, at 456. While the use of a human-animal chimera appears as a means of assessing the effects of drugs and/or other compounds on humans likely satisfies the utility requirement, a use for investigating the properties of the chimera itself, including its responsiveness to drugs, does not. See supra notes 238-39 and accompanying text.

Footnote 250

Of course, the operator of such attractions could employ chimeras considered human to appear in the attraction, much as carnival operators have employed people with physical deformities. People might consider it unseemly to hold that such a human-animal chimera classified as human is "useful" for amusement purposes. Furthermore, a chimera considered human (or those charged as its guardians) cannot be forced to appear in an attraction for amusement purposes. However, for an invention to be unpatentable for want of utility, it "must be totally incapable of achieving a useful result." Brooktree Corp. v. Advanced Micro Devices, Inc., 977 F.2d 1555, 1571 (Fed. Cir. 1992). Thus, since a chimera considered human can choose to partake in an attraction, it is not totally incapable of achieving the useful result of amusement. Thus, such chimeras appear to meet the practical or specific utility requirement, although people might be uneasy about the result. Likewise, a chimera considered human could not be impressed as an organ donor or research subject without the type of consent required from humans, but could choose to engage in such activities. Thus, the chimera is not totally incapable of achieving a useful result in these contexts.

Footnote 251

Bedford v. Hunt, 3 F. Cas. 37, 37 (C.C.D. Mass. 1817); see Lowell v, Lewis, 15 F. Cas 1018, 1019 (C.C.D. Mass. 1817).

Footnote 252

See, e.g., Scott & Williams, Inc. v. Aristo Hosiery Co., 7 F.2d 1003, 1004 (2d Cir. 1925). See Brewer v. Lichtenstein, 278 F. 512, 512-14 (7th Cir. 1922); see Rickard v. Du Bon, 103 F. 868, 871-73 (2d Cir. 1900).

Footnote 253

Juicy Whip, Inc. v. Orange Bang, Inc., 185 F.3d 1364, 1366-67 (Fed. Cir. 1999).

Footnote 254

Ex parte Murphy, 200 U.S.P.Q. 801, 802 (B.P.A.I. 1977).

Footnote 255

Juicy Whip, 185 F.3d at 1367.

Footnote 256

See id.; Mueller, supra note 45, at 208.

Footnote 257

See Juicy Whip, 185 F.3d at 1368.

Footnote 258

See id. (citing 42 U.S.C. § 2181(a) (2000)).

Footnote 259

See Whistler Corp. v. Autotronics, Inc., 14 U.S.P.Q.2d 1885,1886 (N.D. Tex. 1988).

Footnote 260

See Geneva Pharms., Inc. v. Glaxosmithkline PLC, 213 F. Supp. 2d 597, 610 (E.D. Va. 2002).

Footnote 261

See Kopinski, supra note 126, at 629. There are numerous general policy objections to patenting living things such as animals that could equally apply to chimeras. However, these objections were rejected by the Supreme Court in Chakrabarty. See supra notes 75-76 and accompanying text. Thus, this discussion focuses on policy arguments specific to the chimera context.

Footnote 262

See Kopinski, supra note 126, at 629; Magnani, supra note 1, at 457.

Footnote 263

See Magnani, supra note 1, at 457.

Footnote 264

See id.

Footnote 265

See id.

Footnote 266

See id. at 457-58.

Footnote 267

See Kopinski, supra note 126, at 629.

Footnote 268

See Stankovic, supra note 19, at 31.

Footnote 269

Fortunately, such chimeric creatures would be unable to naturally reproduce as chimeras. See supra notes 227-28 and accompanying text. However, chimeras with human reasoning ability could conceivably generate other chimeras themselves using the chimera technology used by humans to originally create them.

Footnote 270

See Kopinski, supra note 126, at 629.

Footnote 271

See id. at 629-30; Magnani, supra note 1, at 456; supra note 247 and accompanying text. Although a court would likely find that use of a chimera for investigation of the effects of drugs on humans satisfies the utility requirement, see supra notes 246-47 and accompanying text, pure use of an invention like a human animal-chimera as a research tool, such as to discover its properties and characteristics, is insufficient to confer practical utility on an invention, see supra notes 238-40 and accompanying text. However, the character of such uses is germane in evaluation of the morality of an invention and thus figures into analysis of moral utility.

Footnote 272

See Magnani, supra note 1, at 455.

Footnote 273

See id. at 457.

Footnote 274

See id.

Footnote 275

See id.

Footnote 276

See id.

Footnote 277

The argument that the moral utility doctrine should be resurrected in the human-animal chimera context is strongest in regard to human-animal chimeras that qualify as human. Indeed, any moral uneasiness about permitting the inventor of a human-animal chimera to obtain intellectual property rights in it is heightened when the chimera is considered human. However, even human-animal

chimeras that are considered human have some beneficial uses to society and thus meet the standard for moral utility. See supra note 248. Furthermore, as discussed above, rewarding the inventor of a human-animal chimera legally constituting a human being does not infringe on the rights that the Constitution affords human beings under the Thirteenth and Fourteenth Amendments possessed by such a chimera. See supra Part II(C)-(D). Thus, a court would not likely find that allowing patent rights for the inventor of such a chimera would violate the moral utility doctrine on the grounds of infringement on the rights of the chimeric creature itself.

Footnote 278

35 U.S.C. § 102 (2000).

Footnote 279

§ 102(a), (g)(2).

Footnote 280

§ 103(a).

Footnote 281

Graham v. John Deere Co., 383 U.S. 1 (1966).

Footnote 282

Id. at 17-18; accord KSR Int'l Co. v. Teleflex Inc., 127 S. Ct. 1727, 1734 (2007).

Footnote 283

Graham, 383 U.S. at 17-18; accord KSR, 127 S. Ct. at 1734.

Footnote 284

KSR Int'l Co. v. Teleflex Inc., 127 S. Ct. at 1734.

Footnote 285

See, e.g., Hybritech, Inc. v. Monoclonal Antibodies, Inc., 802 F.2d 1367, 1383 & n.6 (Fed. Cir. 1986); McNeil-PPC, Inc. v. Perrigo Co., 516 F. Supp. 2d 238, 248 (S.D.N.Y. 2007) (decided after KSR) ("The claimed invention as a whole must be compared to the prior art as a whole.").

Footnote 286

McNeil-PPC, 516 F. Supp. at 248 (following KSR); see also KSR, 127 S. Ct. at 1742; Graham, 383 U.S. at 36; L & A Prods., Inc. v. Britt Tech Corp., 365 F.2d 83, 87 (8th Cir. 1966); cf. Interconnect Planning Corp. v. Feil, 774 F.2d 1132, 1143 (Fed. Cir. 1985).

Footnote 287

See In re Brouwer, 77 F.3d 422, 425 (Fed. Cir. 1996); cf. KSR, 127 S. Ct. at 1739.

Footnote 288

In re Vaeck, 947 F.2d 488, 493 (Fed. Cir. 1991) (citing In re Dow Chem. Co., 837 F.2d 469, 473 (Fed. Cir. 1988) ("The consistent criterion for determination of obviousness is whether the prior art would have suggested to one of ordinary skill in the art that this process should be carried out and would have a reasonable likelihood of success, viewed in light of the prior art.")); see Sanofi-Synthelabo v. Apotex Inc., 492 F. Supp. 2d 353, 388 (S.D.N.Y. 2007) ("To make [the obviousness] determination, the Court must assess, without the benefit of hindsight, whether the prior art would have suggested to a person of ordinary skill in the art that the invention should be made and that it would have a "reasonable likelihood of success.'") (quoting Dow, 837 F.2d at 473).

Footnote 289

See Hybritech, 802 F.2d at 1380 (holding an invention nonobvious when the prior art suggested it but "did not suggest how that end might be accomplished"); Rohrbaugh, supra note 47, at 409-10.

Footnote 290

Ryan Hagglund, Patentability of Cloned Extinct Animals, 15 Geo. Mason L. Rev. 381, 422 (2008); Rohrbaugh, supra note 47, at 409-10 (citing Hybritech, 802 F.2d at 1380). Nothing in the Supreme Court's KSR opinion alters this analysis. Hagglund, supra note 290, at 422 n.279; cf. Sanofi-Synthelabo, 492 F. Supp. 2d at 388 (applying this analysis after KSR was decided). In KSR, the Supreme Court rejected the Federal Circuit's "teaching, suggestion or motivation test," under which a claimed invention was nonobvious in the face of a combination of prior art references unless "some motivation or suggestion to combine the prior art teachings can be found in the prior art, the nature of the problem, or the knowledge of a person having ordinary skill in the art." 127 S. Ct. at 1734 (internal quotation marks omitted); see also id. at 1739, 1741. Therefore, "even in the absence of a suggestion that prior art teachings should be combined, the combination is not necessarily nonobvious." Hagglund, supra note 290, at 422 n.279. Nothing in KSR suggested that it modified or in any way repudiated the Federal Circuit's analysis of obviousness in situations beyond the combination of elements in the prior art. Id. The analysis articulated in the cases cited in this discussion does not involve whether the prior art suggested or taught that prior art references be combined but rather "whether there was a suggestion in the prior art that the invention should be made and whether the prior art revealed that one making it would have a reasonable expectation of success." Id. Indeed, after KSR was decided, a district court applied the analysis discussed here in determining whether an invention was obvious, and quoted Dow for the proposition that the obviousness inquiry turned on "whether the prior art would have suggested to a person of ordinary skill in the art that the invention should be made and that it would have a "reasonable likelihood of success.'" Sanofi-Synthelabo, 492 F. Supp. 2d at 388 (quoting Dow, 837 F.2d at 473). Although the KSR Court did state that it was error to conclude that a patent claim cannot be proved obvious merely by showing that a combination of prior art elements was obvious to try, 127 S. Ct. at 1742, the Court in no way indicated that this pronouncement applied outside of the situation where a person of ordinary skill in the art achieves anticipated success as a result of pursuing known options from a finite universe of particular solutions, Hagglund, supra note 290, at 422-23 n.279. The Court noted that the fact that a combination was obvious to try might render it obvious under circumstances where a person of ordinary skill in the art pursues known options from a finite universe of identified predictable solutions to a problem, for which there was a design need or market pressure for a solution, and achieves anticipated success reasoning that it is likely that such actions are the product of ordinary skill and common sense as opposed to innovation. 127 S. Ct. at 1742. Therefore, "KSR does not prescribe that the fact that it was obvious to try a combination is sufficient to render it obvious when the combination is not a known option drawn from a finite universe of predictable solutions or its success is surprising rather than anticipated." Hagglund, supra note 290, at 422 n.279. Indeed, "in the run-of-the-mine case, a suggestion to build an invention is insufficient to render the invention obvious because it is merely an invitation to construct the invention, but does not create a reasonable expectation of doing so." Id. Also, such a suggestion "provides no evidence that the invention can be

constructed successfully and thus is defective." Id. (citing Hybritech, 802 F.2d at 1380; Rohrbaugh, supra note 47, at 409-10). Accordingly, "[a] suggestion to try is insufficient to enable one of ordinary skill in the art to construct the invention." Hagglund, supra note 290, at 422 n.279. This stands in contrast to "the situation contemplated by KSR, where the prior art or common sense suggests a combination and a person of ordinary skill in the art pursues known options from a finite universe of identified predictable solutions to achieve anticipated success in addressing a known problem." Id. In this context, "the suggestion to try the combination [itself] is enabling because the suggestion alone allows the inventor to create the invention by using common sense and ordinary skill without innovation." Id. Therefore, even if the KSR Court's pronouncement that an invitation to try to construct an invention might be sufficient to render the invention obvious under some circumstances "were held to apply beyond the combination of prior art context, this rule would not alter the analysis in situations, such as the [human-animal chimera] context, where the mere suggestion to build an invention does not enable one skilled in the art to construct the invention without further innovation." Id.; see infra notes 293-95 and accompanying text.

Footnote 291

See Stankovic, supra note 19, at 6-8.

Footnote 292

See id.; supra note 25 and accompanying text.

Footnote 293

See Vicki Brower, The Ethics of Innovation, 4 EMBO Rep. 338 (2003).

Footnote 294

One is naturally uneasy about patenting a human transplant patient, even one who has received an animal xenotransplant. Such a patient is human by any standard employed. See supra Part II.A. However, a xenotransplant patient constitutes a chimera that does not occur in nature. Thus, a xenotransplant patient appears to be patentable subject matter. As discussed above, such a patent would not infringe on the individual's autonomy and would only preclude others from engaging in the same transplant technique. However, such a patent would be of limited value because Congress excludes health care practitioners and health care facilities from liability for infringement when they infringe patents while engaging in medical activity. See 35 U.S.C. § 287(c)(1) (2000). Medical activity is defined as "performance of a medical or surgical procedure on a body." § 287(c)(2)(A). Thus, an individual who patents a xenotransplant patient would never be able to recover damages for infringement because a surgical transplant is clearly a medical or surgical procedure on a body. However, § 287(c) does not foreclose infringement of a patent on a human-animal chimera created by modern molecular biological methods such as embryo fusion. The term "body" is defined as a "body, organ, or cadaver" of human or nonhuman animal used for research purposes. § 287(c)(2)(E). Procedures creating chimeras from embryos and probably fetuses as well do not involve bodies, organs, or cadavers. Although one might argue that a body includes an embryo, an embryo cannot fairly be said to have a body.

As mentioned in the preceding paragraph, a xenotransplant patient is a chimera that does not exist but for the intervention of man inasmuch as a xenotransplant patient is a human being which has at least one animal organ. However, man's application of labor to a natural article, such as a human being or animal, is not sufficient to render it patentable subject matter. See Am. Fruit Growers, Inc. v. Brogdex Co., 283 U.S. 1, 12 (1931). Under the product of nature doctrine, which excludes products of nature from the realm of patentable subject matter, for an invention derived from nature to be patentable, the inventor's work must result in a transformation giving rise to a "new and different article … having a distinctive name, character, or use." Id. at 13 (internal quotation

omitted); see Diamond v. Chakrabarty, 447 U.S. 303, 310 (1980). Thus, although cotton cannot be cleaned and ginned without the handiwork of man, the cleaned and ginned cotton is not patentable. See Am. Fruit, 283 U.S. at 12. A court would probably find that a xenotransplant patient containing animal vital organs, at least the types of organs that would render the xenotransplant nonobvious, met this standard. Such a xenotransplant patient would still be called "human" and thus would have the same name as natural humans and presumably would not have additional abilities and thus would have the same "uses." However, a xenotransplant patient has "characteristics different from those given by nature" inasmuch as it contains animal organs, which are different from any organs that naturally occur in humans. Gen. Elec. Co. v. De Forest Radio Co., 28 F.2d 641, 642 (3d Cir. 1928). Furthermore, animal organs may have different sizes than human organs and might have important physiologic differences. Thus, a xenotransplant patient has a distinctive character from natural humans and animals as it is a human being that contains an organ of a different species. One might attempt to take this argument to an absurd conclusion and argue that a normal transplant patient who received a human transplant is patentable subject matter because humans do not contain organs from other individuals save for the intervention of man. The product of nature doctrine proscribes the patentability of such transplant patients. Unlike xenotransplant patients, normal transplant patients have the same characteristics and properties as natural humans. Indeed, a normal transplant patient's transplanted organ is a human organ, identical to those that appear in natural humans. Even though a transplant patient contains an organ derived from another individual, that organ is a normal human organ and has the properties of one. Such a transplant patient does not contain an organ that does not naturally occur in humans. Manufacture of an article that is a product of nature by humans independent of nature or repair of a naturally-occurring article by the hand of man does not render the article patentable because it does appear in nature. Thus as humans exist in nature and transplanting a human organ from one human into another does not give the recipient characteristics other than those of a natural human or the transplanted organ different properties, a normal transplant patient constitutes a product of nature and cannot be subject to a product patent.

Footnote 295

See supra note 290 and accompanying text.

Footnote 296

See Rohrbaugh, supra note 47, at 411.

Footnote 297

See, e.g., Jose B. Cibelli, et al., The First Human Cloned Embryo, Sci. Am., Nov. 24, 2001.

Footnote 298

See, e.g., Eli Lilly & Co. v. Premo Pharm. Labs., Inc., 630 F.2d 120, 137 (3d Cir. 1980).

Footnote 299

Footnote 300

See supra, Part I.B-C and cases cited therein.

Footnote 301

See supra Part II.B.1.

Terms: **25 SANTA CLARA COMP. & HIGH TECH. L. J. 51**
Search Type: **Citation**
Narrow by: **None**
Date and Time: **Monday, November 25, 2013 – 03:22 PM**

Bennett,

Comment: Chimera and the Continuum of Humanity,

55 EMORY L. J. 347 (2008)

55 EMORY L. J. 347

COMMENT: CHIMERA AND THE CONTINUUM OF HUMANITY: ERASING THE LINE OF CONSTITUTIONAL PERSONHOOD, 55 Emory L.J. 347 (Copy citation)

2006

Reporter: 55 Emory L.J. 347

Length: 19928 words

Author: D. Scott Bennett*

LexisNexis Summary

… The above quotation may sound like an excerpt from a science fiction novel, but it refers to real creatures known as "chimera," which scientists are creating with increasing frequency. … The resulting creature can be a truly unpredictable mixture of species. … Chimera and the PTO: Attempting to Patent Monsters This Part will explain how the United States Patent and Trademark Office (PTO), rather than the courts or the legislature, has made the most noteworthy attempts to define chimera personhood. … The controversial analysis of personhood from Roe v. Wade provides support for the proposition that no organism, be it a human fetus or a developing human-animal chimera embryo, can qualify for personhood prior to viability. … " While analogies to the legal definition of death can help clarify what is necessary for chimera personhood, namely a continuing capacity for higher-level cognitive function, it cannot fully resolve what is sufficient for personhood in a creature that is not entirely human. … This approach guides the following examination of the constitutional personhood of chimera. … The first approach to chimera personhood focuses on biological material because "it cannot reasonably be disputed that an essential part of the definition of Homo sapiens is genetically determined. … However, human-animal chimera technology is straining the dichotomous constitutional personhood construct beyond the breaking point. …

Highlight

In Minnesota, pigs are being born with human blood in their veins. In Nevada, there are sheep whose livers and hearts are largely human. In California, mice peer from their cages with human brain cells firing inside their skulls. [1]

Text

[347]

Introduction

The above quotation may sound like an excerpt from a science fiction novel, but it refers to real creatures known as "chimera," which scientists are creating with increasing frequency. [2] The term chimera has its origins in Greek mythology. The mythological chimera was a fire-breathing monster - with the head of a lion, the body of a goat, and the tail of a dragon - that terrorized the kingdom of Lycia. [3] In contemporary times, chimera have been the subject of science fiction novels, including H.G. Wells's The Island of Doctor Moreau, in which a renegade doctor surgically creates part-human and part-animal creatures. [4]

In modern biotechnology, [5] the term chimera describes an organism comprised of at least two genetically distinct populations of cells originating **[348]** from independent embryos. [6] Under this biotechnological definition, any particular cell in the chimera derives from one of the parent organisms but is not a mix of the two parents as in sexual reproduction. [7] Thus, each cell in a human-mouse

chimera would be either completely human or completely mouse. Chimera technology has rapidly left the realm of the hypothetical, and this technology opens up a Pandora's Box of legal and ethical questions by intimately mixing human and animal.

As an example of the types of creatures being produced by chimera technology, in early 2004 researchers at the Mayo Clinic produced chimera by injecting human stem cells [8] into forty-day-old fetal pigs. [9] Because the human cells were introduced well into fetal development, the organisms outwardly look like pigs. [10] However, closer examination reveals that these creatures have porcine cells and human cells mixed throughout their bodies. [11] Unlike a human receiving a transplanted piece of animal tissue or an animal with a few human genes inserted into its genome, these chimera represent a genetic and structural mix of human and animal. [12]

Presently, Irving Weissman, the director of Stanford University's Institute of Cancer/Stem Cell Biology and Medicine, is contemplating pushing the envelope of chimera research even further by producing human-mouse chimera whose brains would be composed of one hundred percent human cells. [13] **[349]** Weissman notes that the mice would be carefully watched: if they developed a mouse brain architecture, they would be used for research, but if they developed a human brain architecture or any hint of humanness, they would be killed. [14] This solution hardly resolves all moral and legal issues. These biotechnology creations highlight a significant and unresolved question: Do human-animal chimera deserve constitutional protection as "persons"?

This Comment does not take a stance on whether chimera research is inherently morally, ethically, or constitutionally wrong. Instead, this Comment provides a framework for determining if and when the U.S. Constitution and the rights it confers should even be applicable to chimera and chimera research. While Congress may ban the production of constitutionally uncertain chimera in the future, it has not done so yet, and such creatures may be created in the interim. [15]

Personhood is the necessary threshold requirement to the application of specific constitutional rights and therefore the personhood of various types of chimera is crucial. [16] Given the current state of chimera technology, the division between human and animal has become a continuum, not a bright line. [17] Scientists can create chimera with just a few human cells, chimera with primarily human cells, and everything in between. [18] At some point along the spectrum between human and animal, chimera must be afforded protection under the Constitution as constitutional persons. [19] To do this properly, a fundamental change in the interpretation of the seemingly unambiguous constitutional term "person" is required.

Since chimera potentially erase the line between human and animal, it is doctrinally unsound to rely on a strict person/nonperson dichotomous approach to constitutional personhood. [20] Therefore, to adequately reflect the realities of **[350]** the new personhood continuum, varying levels of constitutional protection should be afforded to chimera based on a sliding scale approach to personhood. [21] The application of these varying levels of protection should be guided by the fundamental characteristics of personhood: (1) higher-level human cognitive traits and (2) the possession of crucial human biological tissues. [22] Moral and ethical questions remain even under this approach; however, if human-animal chimera are to be produced, a more adaptive legal framework is needed.

Part I of this Comment provides the scientific background of chimera necessary to understand the legal and ethical issues surrounding chimera research. [23] Part II describes the current state of the law on chimera, which, problematically, has thus far been largely confined to the patent arena. Part III examines what it means to be a legal and constitutional "person" at the ends of the human life span. Part IV analyzes and coalesces the various moral and ethical theories that are applicable to chimera. Part V argues that it is possible to create chimeric "persons" and proposes a more flexible and inclusive approach to constitutional personhood.

I. The Science of Chimera

To appreciate the issues surrounding human-animal chimera, it is important to understand the biotechnology that creates them. While differentiating what is and what is not a chimera can be complicated, [24] the scientific definition is relatively straightforward: a chimera is an organism with two or more distinct populations of cells derived from separately fertilized embryos. [25] The resulting creature can be a truly unpredictable mixture of species. [26] Chimera are distinct from hybrids, [27]

clones, [28] and organisms created by recombinant **[351]** DNA technology. [29] Although it is possible to create animal-animal chimera, human-human chimera, or even chimera of more than two species, [30] this Comment will focus on human-animal chimera.

A. Chimera Production

Presently, the most common method of producing human-animal chimera is to inject stem cells from one species into an early embryo of another species. [31] Because stem cells are able to differentiate inside the embryo and integrate themselves into all tissue types, the resulting organism is a hodge-podge of the two species. [32] In recent years, experimenters have used the stem cell method to produce, for example, human-sheep and human-pig chimera. [33] So far, scientists conducting these experiments have used only animal embryos, not human ones, and have delayed the injection of the human stem cells in order to assure the chimera is essentially still a sheep or pig. [34] There are no guarantees, however, that researchers will not cross these boundaries in the future.

While most of the recent chimera research involves the stem-cell injection method, other methods of producing chimera are available. A technique that has proved quite successful in producing animal-animal chimera involves mixing embryos of two organisms at a very early stage. [35] In 1984, this embryonic mixing technique was used to create goat-sheep chimera, which **[352]** were fittingly named "geep." [36] The geep were successfully raised to adulthood and possessed traits of both species. The legs and skull of the geep were goat-like, the frame was that of a sheep, and the skin was covered in both the curly wool of a sheep and patches of the short, coarse hair of a goat. [37] Given the relative ease with which the geep were produced, it should be comparatively simple to use this method to produce a human-ape chimera given the vast advances in biotechnology since 1984 and the fact that apes are more closely related to humans than sheep are to goats. [38] Technically, chimera can also be produced by transplanting or engrafting tissues, such as an organ or heart valve, from one organism into another. [39]

A biotechnology technique that raises many of the same issues as chimera technology is human-nonhuman nuclear transfer. [40] Nuclear transfer utilizes cloning technology to transfer the cell nuclei from one species into the de-nucleated cells of another species. [41] The denucleated cells act as biological shells for the introduced nuclei, which take over control of the cells. In 2003, scientists in China used the nuclear transfer method to insert human DNA into denucleated rabbit eggs and allowed the embryos to develop for fourteen days. [42] These organisms, like chimera, are morally, ethically, and legally troubling because they have a very similar biological makeup to humans. [43]

[353]

B. The Use and Misuse of Chimera

Scientists are examining several medical and pharmaceutical uses of human-animal chimera. Because biotechnology is a rapidly changing field, the following list of three major uses is meant to be illustrative, not exhaustive. First, chimera may allow for improved testing of the benefits, side effects, and interactions of pharmaceutical drugs. [44] This is because entire human cells, tissues, and organs can be present in a fully-formed organism, allowing for more direct and effective testing than can be achieved in animal studies or cell-line research. [45] For example, a human-animal chimera with a fully human liver could be more effective at testing a drug's effect on the human liver than traditional testing using cultured human liver cells. Second, it may be possible to use chimera to grow organs for transplantation into humans. [46] Organs with all or nearly all human cells grown in a human-animal chimera are less likely to be rejected by the recipient's immune system than traditional xenotransplants, [47] and scientists have already produced chimeric sheep whose livers are eighty percent human. [48] Third, chimera are useful in studies of human development. [49] This is because developmental studies that are not possible with human embryos could be carried out on living chimera embryos. [50]

Despite these promising uses of chimera, there are worries of other, much less benevolent uses. Chimera might be created for artistic purposes, out of simple curiosity, or for commercial exploitation as servants or even as "freaks." [51] One colorful description of the possible, and troubling, uses of human-animal chimera was provided by environmental journalist Mark Dowie:

The technology could be used to manufacture soldiers with armadillolike shielding, quasi-human astronauts engineered for long-range space travel, and altered primates with enough cognitive ability **[354]** to ride a bus, follow basic instructions, pick crops in 119 degrees, or descend into a mine shaft without worrying their silly little heads about inalienable human rights and the resulting laws and customs that demand safe working conditions. [52]

Even when chimera technology is used for legitimate research purposes, serious concerns remain. [53] Outspoken biotechnology critic Jeremy Rifkin believes that the production of chimera violates the sanctity of "species integrity." [54] Religious attacks on chimera research cite the inviolability of the human form [55] and inappropriateness of "playing God" by manipulating life. [56] Others argue that the manipulation of life diminishes its "significance and mystery." [57] Still, others criticize the research for its potentially harmful effects on the environment and its exploitation of animals. [58] In addition, the mixing of human and animal cells may make it easier for animal diseases to cross over into humans. [59]

[355] Unfortunately, the best way to increase the scientific utility of human-animal chimera is to increase the amount of human tissue in the chimera. [60] This leads to a corresponding increase in the legal and moral concern about allowing this research. [61] Therefore, chimera technology offers both great promise and peril, and the surrounding legal issues are of substantial importance.

II. Chimera and the PTO: Attempting to Patent Monsters

This Part will explain how the United States Patent and Trademark Office (PTO), rather than the courts or the legislature, has made the most noteworthy attempts to define chimera personhood. Because there is a substantial chance that chimera research will lead to economically valuable inventions, it is not surprising that the majority of the legal discourse on chimera has taken place in the patent law arena. [62] Due to the economic incentives patents provide, the resolution of chimera patentability is of considerable importance. [63] The applicability of the Constitution, which is dependant on the initial resolution of chimera personhood, will be vital in resolving chimera patentability. While the PTO is not the proper body to ultimately decide this constitutional issue, the PTO's positions on chimera are important because they have led to much of the current chimera discourse. [64] This Part will therefore introduce and contextualize the battles that have been fought over chimera patents, including whether chimera are entitled to constitutional protection.

A. The Chakrabarty Revolution

Prior to 1980, the PTO generally did not issue patents on any living organisms. [65] While patents had been granted on cell lines, it was PTO policy that living organisms themselves, even those selectively bred or scientifically **[356]** altered, would not be afforded protection under the Patent Act. [66] The reason typically given to justify the blanket rejection of such applications was that "products of nature" are not patentable subject matter. [67]

Fortunately for the biotechnology industry, the U.S. Supreme Court soundly rejected the PTO's stance against patenting life in Diamond v. Chakrabarty in 1980. [68] The Chakrabarty Court held that a modified oil-digesting bacterium qualified as patentable subject matter. [69] In the key language of the opinion, the Court concluded that Congress intended patentable subject matter to "include anything under the sun that is made by man." [70] The Chakrabarty decision does not allow for the patenting of all living things; rather, it applies only to those created or significantly modified by the hand of man. [71] This represents the distinction between generally patentable inventions and generally nonpatentable discoveries. [72] Still, the language of Chakrabarty is very broad and puts no explicit restrictions on the patentability of living organisms, human or otherwise, provided the patent is for a "human-made invention[]." [73]

In 1987, the patentability of multicellular organisms under Chakrabarty was tested in Ex parte Allen, which involved a disputed application for a polyploid oyster. [74] The Board of Patent Appeals and Interferences determined that the oyster represented patentable subject matter under the Patent Act, although the patent was ultimately rejected on other grounds. [75] Thus, Ex parte Allen interpreted Chakrabarty as applicable to both single and multicellular **[357]** organisms. [76] Soon after Ex parte Allen, the first patent on an animal, the Harvard Onco-Mouse, was issued. [77] The Onco-Mouse was also significant because it was the first patent on an animal with an introduced human gene. [78]

B. The PTO's Attempts to Limit the Patentability of "Human Beings"

In response to Ex parte Allen, the PTO issued a notice regarding its stance on the patentability of life on April 21, 1987. [79] The notice stated that the PTO considered "nonnaturally occurring non-human multicellular living organisms, including animals, to be patentable subject matter" under 35 U.S.C. 101 of the Patent Act. [80] However, the PTO limited its position by stating that a "claim directed to or including within its scope a human being" would not be considered patentable subject matter under the Act because "the grant of a limited, but exclusive property right in a human being is prohibited by the Constitution." [81] It is widely believed by legal commentators that the PTO was invoking the Thirteenth Amendment ban on slavery and indentured servitude as the constitutional provision precluding patentability of "human beings." [82] The apparent rationale is that the grant of a property right in a human being would be a form of slavery or indentured servitude. [83] It was not clear, however, whether the 1987 notice's ban on claims directed to or including "human beings" would affect the patentability of human-animal chimera.

C. The Rejection of the Newman Application

On December 18, 1997, Jeremy Rifkin and Dr. Stuart Newman filed a patent application (Newman Application) seeking protection for both a method **[358]** of producing human-animal chimera and for the chimera themselves. [84] The Newman Application does not cover all controversial human-animal chimera; it covers only chimera containing less than fifty percent human DNA. [85] Newman and Rifkin did not intend to actually produce chimera if a patent was granted to them. [86] Instead, they had a no-lose plan to discourage chimera research. First, if the patent were granted, they intended to prevent anyone else from producing chimera for the term of the patent. [87] Second, even if the patent was denied, Newman and Rifkin hoped to take away some of the economic incentives of engaging in chimera research by setting a precedent that chimera are unpatentable. [88] Regardless of the result, they sought to spark a public debate on the commercialization and commodification of life. [89]

While the PTO has rejected the Newman Application several times, its reasoning is ambiguous and may ultimately be driven more by emotion than sound legal principles. [90] It initially appeared that the PTO would invoke the April 21, 1987 notice and reject the Newman Application as a "claim[] directed to, or including within [its] scope, a human being," presumably barred by the Thirteenth Amendment. [91] However, in an April 2, 1998 media advisory, the PTO invoked a new rationale in apparent opposition to the Newman Application. [92] The media advisory stated that it was the PTO's position that "inventions directed to human/non-human chimera could, under certain circumstances, not be patentable because, among other things, they **[359]** would fail to meet the public policy and morality aspects of the utility requirement" of the Patent Act. [93]

To further add to the confusion, the PTO ultimately did not rely on the moral utility argument of the media advisory in rejecting the Newman Application. [94] Instead, in March 1999, the PTO first rejected the Newman Application on more traditional patent law grounds including insufficient disclosures and for failing the nonobviousness requirement. [95] Newman and Rifkin continued to fight the rejection for seven years. [96] In rejecting later reapplications, the PTO has offered additional rationales in opposition to chimera patentability such as the constitutional rights to privacy and procreative liberty. [97] It now appears that the Newman Application has been rejected for the final time, but the precise legal basis of that rejection remains unclear. [98]

As legal commentators have noted, several of the arguments invoked by the PTO against patenting humans or chimera have serious doctrinal flaws. [99] The 1987 notice stated that a "claim directed to or including within its scope a human being" would not be considered patentable subject matter under the Patent Act because "the grant of a limited, but exclusive property right in a human being is prohibited by the Constitution." [100] This subject matter argument appears to depend on an implicit exclusion of human beings for patentability under the Patent Act. [101] However, this is in conflict with Chakrabarty, in which the Supreme Court strongly discouraged implying subject matter limitations in the Patent Act. [102] After Chakrabarty, the law **[360]** seems to impose no subject matter restriction on the patentability of human beings under the Patent Act, and the PTO cannot unilaterally insert one. [103] In addition, the PTO's more recent reliance on the moral utility doctrine in its 1998 media advisory to attack the usefulness of a chimera patent is legally unsound because the doctrine has been largely rejected in recent years. [104] If asserted by the PTO as a limitation on patentability, any application of the moral utility doctrine "is bound to be overturned in court." [105] Even if there were still an established moral utility test in U.S. patent law, [106] there is a strong argument that the moral utility would favor allowing patents on chimera because of the possible benefits to medical research. [107]

Given the expansive scope of the Patent Act, the most substantial arguments against chimera patentability rest on other constitutional provisions that may independently bar chimera patentability. [108] These arguments require an interpretation of the Constitution to determine first if the Constitution and its protections even apply to human-animal chimera and second, if the threshold question is satisfied, if other constitutional provisions do in fact bar chimera patents. [109] The PTO, however, is not the proper body to make these ultimate determinations. The role of the PTO is to administer the law, not to **[361]** create the law. [110] It may even be a violation of the separation of powers doctrine for the PTO to make such constitutional interpretations. [111] In deciding if constitutional protections apply to chimera, it is "emphatically the province and duty of the judicial department to say what the law is." [112]

D. Why Chimera Patents Are Unique

In recent years, the PTO has routinely granted patents on organisms that contain human genetic material while rejecting chimera patents. [113] Many patents have been granted for claims to recombinant organisms in which human genes have been inserted into the genome of another organism. [114] The reason for the PTO's differential treatment of these recombinant organisms and human-animal chimera seems to relate to the very nature of chimera: they contain entire, unaltered human cells, and by changing the method of production, the percentage of human tissue could be very high. In describing the difference between chimera and recombinant animals, Thomas Murray, the director of the Center for Biomedical Ethics at Case Western Reserve University, stated:

If we put one human gene in an animal, or two or three, some people may get nervous but you're clearly not making a person yet. But when you talk about a hefty percentage of the cells being human … this really is problematic. Then you have to ask these very hard questions about what it means to be human. [115]

Thus, chimera toe the line of humanity more conspicuously than traditional recombinant organisms. [116]

[362]

E. Has Congress Taken a Stance on the Patentability of Chimera?

In 2004, Congress passed and President Bush signed into law a provision of the federal budget that prohibits the PTO from issuing patents on "human organisms." [117] The provision has become widely known as the "Weldon Amendment," after its author, Representative Dave Weldon of Florida. [118] The Weldon Amendment states that "none of the funds appropriated or otherwise made available under this Act may be used to issue patents on claims directed to or encompassing a human organism." [119] The Amendment does not directly alter the scope of the Patent Act, however, since it is only a temporary budget provision. [120] According to Representative Weldon, the intent of the provision is, on one hand, to codify the PTO's policy that genetically-engineered adult, fetal, and embryonic human organisms are not patentable, but on the other hand, not to affect the patentability of human DNA sequences, cell lines, stem cells, and other biological products. [121] Commenting specifically on chimera, Representative Weldon made it clear that in his view the provision does not affect the PTO's policy on the patentability of human-animal chimera:

What about an animal that is modified to include a few human genes so it can produce a human protein or antibody? What about a human/animal "chimera" (an embryo that is half human, half animal)? … The USPTO has already granted patents on the former. It has also thus far rejected patents on the latter, the half-human embryo, because the latter can broadly but reasonably be construed as a human organism. The Weldon amendment does nothing to change this, but leaves the USPTO free to address new or borderline issues on the same case-by-case basis as it already does. [122]

While Representative Weldon has stated that the provision does not directly affect the present status of chimera patentability, the American Bar Association has argued that the language of the enacted provision "confuses the situation" **[363]** because it is unclear how broadly the term "human organism"

in the provision will be interpreted. [123]

The Weldon Amendment arguably prohibits the patentability of chimera produced by injecting animal stem cells into human embryos or by using human embryos in an embryonic mixing technique. This is because a chimera produced in this way would utilize a human embryo and therefore may "encompass[] a human organism" under the provision. This line of reasoning would not apply to chimera produced by injecting human stem cells into animal embryos because human stem cells do not appear to be covered by the provision. [124] The Amendment may also provide implicit congressional support for current PTO policies in opposition to human-animal chimera patents. [125]

Regardless of the Weldon Amendment's effect on chimera patentability, broader constitutional issues remain. The Weldon Amendment does not attempt to ban the production of human-animal chimera altogether because it is limited to PTO funding. While patents offer an important economic incentive to pursue questionable research, [126] the major objections to chimera technology focus on preventing this type of research entirely, and this cannot be done through patent law. [127] Assuming certain chimera are entitled to legal protection from exploitation and abuse, patent law cannot fully shelter them, so a broader constitutional answer is needed. Therefore, the remainder of this Comment will address an unresolved and underdeveloped question: Is it possible for chimera to be "persons" under the U.S. Constitution?

[364]

III. Legal and Constitutional Personhood on the Margins of Life

Constitutional rights and protections are afforded only to "persons." [128] Constitutional personhood has been described as "a magic incantation that opens the door to the powerful spirit of the U.S. Constitution." [129] The crucial term "person," however, is not explicitly defined in the Constitution. [130] While there may be no one unifying concept of constitutional personhood, [131] personhood has generally been synonymous with humanness: any and all humans are constitutional persons. [132] While some nonhuman legal entities such as corporations do qualify as persons, animals clearly do not. [133] **[365]** Therefore, chimera exist within the fissure between human persons and animal nonpersons.

Determining when, if ever, a human-animal chimera should be treated as a constitutional person is a difficult task. [134] This determination is significantly more complex in the chimera context than other areas of biotechnology. A human clone would be as much a person as an identical twin, [135] and most recombinant animals have only a few human genes and, typically, are unmistakably nonhuman. [136] Chimera represent the murky middle ground that pushes the limits of humanity. [137] If the constitutional term "person" is defined too narrowly, there is a risk of subjecting very human-like, intelligent creatures to suffering and servitude, violating fundamental constitutional ideals. Yet, if person is defined too broadly, it may deliver a deathblow to a valuable field of medical research. [138] Thus, declaring chimera persons potentially trades life-saving research in favor of respect for human dignity and freedom. [139]

A. The Vocabulary

To reduce uncertainty caused by imprecise terminology, the remainder of this Comment will utilize a defined set of terms. The term "human" will be used quasi-technically to refer to those organisms that are composed of one hundred percent Homo sapiens cells. "Humanity" and "humanness" will refer to the set of cognitive and biological characteristics that are the hallmarks of "humans." The slightly more ambiguous term "human being" will be avoided. [140] The terms "person," "personhood," "constitutional person," and "constitutional personhood" will all refer to the legal and constitutional **[366]** construct of who or what is and should be entitled to constitutional protections. [141]

B. Legal Personhood on the Boundaries of Life: A Doctrine Frayed at the Ends

This Comment seeks to reinterpret constitutional personhood in a way that reconciles traditional constitutional concepts of personhood with the modern reality of chimera biotechnology. As an interpretive matter, the Framers of the Constitution could not have anticipated or addressed the blurring of the lines of personhood caused by chimera technology, so an originalist approach to constitutional interpretation is likely futile. [142] There are also presently no cases or statutes addressing the personhood of human-animal chimera under the Constitution. The one place where the personhood of chimera has arisen thus far is in patent law via the Newman Application. [143] However, the PTO has

little authority or competency to make constitutional proclamations on the personhood of chimera, so positions taken by the PTO are of limited legal significance. [144]

Thus, traditional legal sources are of reduced value, and important guidance in resolving chimera personhood will be derived from the moral and ethical discourse presented in Part IV of this Comment. Nevertheless, some insight into the fundamental elements of personhood can be gleaned by examining the history of what it means to be a legal person in other contexts. The personhood debate has had the greatest, and most controversial, legal significance on the edges of the human life cycle: fetal personhood and brain death. [145]

[367]

1. Abortion and Human Embryos

In upholding the constitutionality of abortion, the U.S. Supreme Court in Roe v. Wade noted that if the personhood of a fetus "is established, the appellant's case, of course, collapses, for the fetus' right to life would then be guaranteed specifically by the [Fourteenth] Amendment." [146] After examining the legal history of the Constitution and abortion, the Court decided that "the word "person' … does not include the unborn." [147] The Court came to this conclusion because the use of "person" in various constitutional provisions did not presuppose prenatal application. [148] The controversial analysis of personhood from Roe v. Wade provides support for the proposition that no organism, be it a human fetus or a developing human-animal chimera embryo, can qualify for personhood prior to viability. [149]

Despite a superficial similarity, personhood in the abortion context is quite different from human-animal chimera personhood. This is principally because the privacy and reproductive autonomy rights of the mother play a fundamental role in the abortion calculus while this concern is absent in laboratory-based chimera research. [150] Maternal rights may render fetal personhood in the context of abortion sui generis. [151] The Roe Court's examination of constitutional personhood might therefore be "cabined" to abortion and largely inapplicable to the problems of chimera. [152]

While the legal status of human embryos produced or stored outside of the uterus - and thus outside the maternal interest - might provide a more fitting **[368]** analogy to chimera, the legal status of such embryos is presently unresolved. [153] In addition, it is unquestioned that fetuses and embryos achieve personhood upon full gestation, so the personhood of fetuses and embryos is fundamentally a question of development and timing, whereas the personhood of chimera relates to their inherent nature. In order to separate the chimera debate, the focus of this Comment is on the personhood of fully gestated human-animal chimera.

2. Defining Legal Death

Knowing when the law has deemed a person no longer legally living provides some insight into those attributes that are necessary for legal personhood. This is because "when the crucial aspects of "personhood' are irretrievably lost, we feel that an individual has died." [154] The common law definition of death was an "absence of spontaneous respiratory and cardiac functions." [155] This approach did not look to brain activity as the defining characteristic of legal life but due to advances in modern technology and medicine, the common law rule is outdated. [156] This common law definition of death would include individuals whose brains are still active but whose respiratory and cardiac systems require life support and exclude individuals with total, irreparable loss of higher-level brain function but with some continuing respiratory and cardiac functions. [157]

As a result, the medical community and most state legislatures have attempted to redefine the meaning of legal death. [158] Modern statutes and proposals typically add to the common law definition and provide that a human with irreversible, total, or whole "brain death" will be considered legally dead. [159] The Uniform Determination of Death Act states that "an individual who has sustained … irreversible cessation of all functions of the entire brain, **[369]** including the brain stem, is dead." [160] The ability of other parts of the body to function via life support is not relevant if the brain has completely failed.

Still, commentators argue that the now widely accepted total or whole brain death definition remains inadequate. [161] This is because "a person may suffer an irreversible loss of consciousness and cognition, the earmarks of higher brain activity, without losing brain stem functions." [162] The capacity for higher-level brain activity is central to legal life, not the vegetative function of the lower brain. [163]

The difficulty of defining brain death also arises with respect to anencephalic [164] newborns lacking the physical capability to ever achieve higher brain function. [165] Because these newborns can never display human intellectual traits, several authors have argued that they should not be considered legal "persons." [166] This tragic condition is more analogous to chimera than other humans who have suffered higher brain death because, like a human-animal chimera without human brain tissue, anencephalic newborns are incapable from inception of ever achieving higher-level human brain function. [167]

Humans who lack the capacity for brain function, either because of congenital defect or subsequent brain death, illustrate that a capacity for brain function is necessary for legal life and therefore legal personhood. [168] Further, in line with the modern trend criticizing a strict whole brain approach, the relevant brain functions for defining life are higher-level consciousness and **[370]** cognition. Consequently, these higher-level brain functions are crucial in defining constitutional personhood. [169] Some commentators argue that "all rights enumerated in the Constitution and the Bill of Rights are predicated on consciousness, or the capacity for consciousness, except for the right to life itself, which becomes meaningless when consciousness can never exist." [170] While analogies to the legal definition of death can help clarify what is necessary for chimera personhood, namely a continuing capacity for higher-level cognitive function, it cannot fully resolve what is sufficient for personhood in a creature that is not entirely human.

IV. The Morality and Bioethics of Chimera

Traditional legal sources alone are insufficient to resolve the thorny question of human-animal chimera personhood. [171] Therefore, the constitutional proposal presented in Part V must also make use of the moral and bioethical sources presented in this Part. [172] This reliance on non-legal sources is not an analytical deficiency because defining "personhood" should be an exercise in morality and natural law, [173] as opposed to a sterile syllogistic analysis. [174] Moral and ethical considerations, a bedrock of natural law, play a vital role in analyzing this type of constitutional issue. [175]

[371]

A. Moral and Ethical Attempts to Crystallize Humanity [176]

Traditional moral philosophies generally operate on the assumption that only humans are entitled to moral rights. Owing to that initial position, they then attempt to crystallize either the biological or the cognitive factors that define humanness in order to separate and morally elevate humans over all other beings. [177] Even accepting the legitimacy of such an anthropocentric view, [178] these moral approaches based on separating humans from nonhumans break down when applied to human-animal chimera. [179]

1. The Biological Approach to Applying Human Morality

The traditional view of human morality is simple: biological humans are moral people and biological nonhumans are not. [180] Thus, under this view, membership in the Homo sapiens species alone leads directly to moral humanness and its corollary, personhood: "that possession of the genetic material of Homo sapiens is a necessary and sufficient condition for personhood." [181] Therefore, moral rights theory collapses into the biological question of humanness. [182] However, this purely biological analysis takes insufficient account of cognition in determining morality and personhood. [183] The commentary surrounding the legal death of clearly biological humans presented in Part III.B.2 demonstrates that cognition has a major role to play in the human moral framework. A biological approach may elevate the form of humanness over the moral substance of humanness. [184] This simple human- **[372]** animal moral distinction based on biology is greatly complicated when the biological material is both human and animal, as in a chimera.

Still, the biological approach, if applied as a guide rather than a rule, has an important contribution to the personhood debate. [185] Contemporary commentators recognize that the defining traits of humanity are inseparably connected with the organ responsible for producing them, the human brain. [186] Therefore, chimera that present the most difficult moral and ethical problems are those that contain human brain cells. [187] Chimera do not necessarily become morally uncertain simply because they contain a trivial number of human brain cells; this "would have to involve the introduction of substantial numbers of human neural cells into a nonhuman embryo." [188] Taking guidance from the brain death debate presented in Part III.B.2, a further distinction can be made between the neural tissues of the

higher brain, responsible for consciousness and cognition, and the lower brain, responsible for vegetative function. [189] It is the higher brain functions, and therefore the higher brain cells and tissues, that factor most heavily into the personhood question. [190] Regardless, while this biological approach alerts us that a moral danger might arise when human neural cells are incorporated into the higher brain structures of chimera, it does not provide a firm rule for defining chimera as either human or animal. [191]

2. The Cognitive Approaches: Separating Humanity Via Intellect

Many modern theorists argue that the defining characteristics of humanity are intellectual attributes not physical human form, as is the case for biologically-based theories. [192] Modern bioethicists note that all creatures share **[373]** similar genetic components and that biology alone may provide insufficient grounds for the moral uniqueness of humanity. [193] These theorists adopt more fluid tests for humanity that look to the psychological properties that differentiate humans from animals. [194] Among the properties that have been proposed as quintessentially human by modern theorists are the capacities to: reason; act for normative, including moral, reasons; act autonomously; engage in complex social relationships; display empathy and sympathy; and have faith in a higher being. [195]

Even before the biological bases of human cognition were understood, philosophers and theorists searched for the essential intellectual attributes that define humanity in order to apply moral rights. These theories tend to be inflexible as they draw sharp distinctions between human and nonhuman based intellectual traits once thought to be unique to the human species but now shown to be possessed to various degrees by other animal species. [196] For example, natural law theorists focus on the ability to reason as the defining characteristic of humanity. [197] Moral rights are only applied after defining and separating humans from other creatures based on this ability to reason. [198] Prior to modern animal research, the ability to communicate was also often given as a distinguishing factor of humans. [199]

Immanuel Kant's influential moral theory also attempts to separate man from beast based on a particular cognitive trait: rationality. According to Kant, **[374]** a being has full moral standing if and only if it is rational. [200] Kant believed that only humans are rational and, therefore, rationality was the morally defining characteristic of humanity. [201] Animals, which lack the capacity to reason, are thus not morally relevant. [202] The most familiar application of Kant's moral theory is his categorical imperative, which states that a human should "act in such a way that you always treat humanity, whether in your own person or in the person of any other, never simply as a means, but always at the same time as an end." [203] According to the categorical imperative, the killing of a human is always morally prohibited, regardless of any countervailing considerations. [204] It is not clear, however, if and how Kant's moral theory applies to chimera that are part human (and thus owed total, inviolable moral respect) and part animal (and thus owed none).

Despite numerous attempts over several centuries, no one has established one fixed set of characteristics such as communicative ability or rationality that includes all humans and excludes all nonhuman organisms. [205] Modern research has demonstrated that animals can communicate, exhibit intelligence, and experience emotion. [206] Some intelligent animals exhibit these traits more strongly than certain humans, such as the very young, the severally mentally handicapped, or the comatose. [207] Similar to Justice Stewart's famous statement on defining pornography, [208] maybe in the era of biotechnology we cannot define humanity, but we know it when we see it. [209] Nevertheless, the **[375]** possession of the cluster of high-level cognitive traits recognized as characteristically human by ethicists and philosophers is of vital importance to the application of moral rights. [210]

B. Weakening the Moral Divisions between Humans, Animals, and Chimera

Despite being too restrictive by only applying moral rights to a rigid concept of humanity, the biological and cognitive moral approaches presented above demonstrate the importance of human neural cells and certain cognitive traits. The following analysis first examines additional problems that may be caused by relying on a restrictive, definitional, humans-only approach to moral rights and then examines the possible application of a more flexible moral framework.

1. The Species Construct and Speciesism

The traditional view is that humans are superior and distinct from other species and that it is

inappropriate to mix biological humans and animals. [211] This notion that humans are inherently superior to animals has been compared to racism and labeled "speciesism" [212] by animal rights activists. [213] While a certain degree of speciesism is accepted in our society, this type of thinking could be dangerous if applied to human-animal chimera that are not simply animals. [214] Much as early notions of racial superiority lead to subjugation and oppression in the United States, a distinction between "pure" humans and chimera that are very closely aligned to humanity could lead to morally troubling results. [215]

[376] Beyond speciesism, the "species" concept itself has been criticized as being inherently flawed as a scientific distinction. [216] Even putting aside modern biotechnology, the species concept is merely a convenient mechanism by which to group life forms that are evolutionarily related to varying degrees. [217] Nevertheless, the line between humans and animals has importance as a moral and social construct; "we rely on the notion of fixed species identities and boundaries in the way we live our lives and treat other creatures, whether in decisions about what we eat or what we patent." [218] Chimera technology strongly challenges these notions of "fixed species identities." [219] The gap between our moral concept of a fixed, unique human species and the modern scientific reality of chimera technology has left us with an inadequate moral framework. [220] This decoupling of the moral concept of personhood from the biological concept of humanness will, according to some commentators, lead to moral ambiguity. [221] Thus, we must be open to a more flexible moral framework, one that does not rely only on drawing sharp distinctions between humans and nonhumans.

[377]

2. More Flexible Approaches to Morality

An initially promising approach to morality in the age of biotechnology is utilitarianism. A moral utilitarian approach asserts that an action is morally proper if it will produce the best overall societal result relative to all other possible actions, thereby maximizing the overall good or utility of society. [222] Utilitarianism rejects the concept of inherent rights, natural law, and the Kantian categorical imperative. [223] While many theories attempt to establish the scope of morality by defining and separating humans, utilitarianism can take into account the interests of any being that has capacity to recognize and appreciate those interests. [224] Utilitarianism can draw distinctions between creatures, including nonhumans, with different levels of cognitive ability by using cognitive interests as the utility interest or "good" to be maximized. [225] This type of moral flexibility is important in a world where the dichotomy between human and animal is falling away. For example, Jeremy Bentham would classify the rights of a being based on whether it could suffer and feel pain regardless of species. [226] Therefore, it could be immoral to subject nonhuman beings, such as chimera, to pain and suffering if it reduces overall utility. [227]

Moral utilitarianism, however, has significant shortcomings in the real world. Under a strict utilitarian model, moral and legal rights need only be granted to creatures that can recognize and appreciate them. [228] This is because "with respect to … unperceived, unrecognized interests, a species is not morally considerable and, as to these interests, its members may be ignored." [229] Yet it is often imperative that society be inclusive in granting rights, even when those rights and interests might not be fully recognized. For example, our society does not limit the moral and legal status of young children, the mentally handicapped, or the infirm simply because they are not able to fully recognize certain interests. [230] Therefore, society attaches extra **[378]** significance and sanctity to human life beyond what a strict interest based form of utilitarianism would predict. [231] Likewise, society should not simply apply a pure moral utilitarian calculus to human-animal chimera because this may not afford sufficient respect and protection to chimera with substantial human characteristics. [232]

3. A Moral Resolution

Two interesting approaches limit and hybridize utilitarianism in order to reflect the inviolability of certain moral principles. The first, as discussed by Robert Nozick, combines utilitarianism and Kantianism. [233] Under this approach, the utilitarian concept of utility maximization applies to all moral questions involving the use of living beings, human and animal alike. [234] However, in the case of humans, the stricter Kantian moral imperative is also applied so that it is never morally proper to do certain things to humans, such as murder or sacrifice. [235] A similar approach, rule-utilitarianism, limits the applicability of the utilitarian calculus by requiring compliance with certain fundamental moral and behavioral rules of society. [236]

While these two approaches do not contemplate the morally murky territory of human-animal chimera, they recognize that moral utility, while useful, should also take into account the sanctity of certain long established moral principles, such as the inherent value of human life and Kant's categorical imperative. [237] When this hybrid moral utility approach is combined with the biological and cognitive moral theories presented in Part IV, a flexible, a two-part approach to applying moral rights to chimera takes shape. This approach first looks for the capacity for higher-level cognitive abilities that are recognized as crucial by both cognition based moral theories and interest maximizing utilitarianism. The second part of this approach requires the presences of crucial human tissues, in accordance with the biological moral **[379]** theories and in recognition of the inherent value of human life. [238] A chimera's moral status can thus be determined by examining (1) its cognitive capacity and (2) how much crucial human tissue it possesses. This approach guides the following examination of the constitutional personhood of chimera.

V. A Proposal for Determining When a Human-Animal Chimera Is a Constitutional Person

The production of morally questionable human-animal chimera is becoming a reality, and a legal framework is needed that could grant constitutional protections to chimera by overcoming the personhood obstacle. [239] While several legal commentators have flatly rejected the concept that chimera could qualify as persons under the Constitution, this view is overly formalistic and shortsighted. [240] It is now scientifically possible to create chimera in which the majority of the cells, including the brain cells, are human rather than animal. [241] It is also possible to create chimera in which the vast majority of the nervous tissue is human, even when the total number of human cells is less than fifty percent. [242] Researchers could also create chimera by combining humans with closely related, intelligent apes such as chimpanzees. [243] Fears that such chimera will exhibit human cognitive abilities are not unfounded; previous animal-animal chimera research has shown that complex behaviors can be transferred across species lines. [244] Thus, a chimera with a significant amount of human nervous cells may well exhibit human intellectual and behavioral traits. [245]

[380] As an extreme example of the error in totally rejecting chimera personhood, the technical definition of chimera includes humans who have received medical implants derived from animals, such as pig heart valves. [246] Regardless of their opinions of his politics, most people would unquestionably consider Senator Jesse Helms a "person," but strictly speaking Helms is a human-animal chimera because he has a surgically implanted pig heart valve. [247] A drop of animal does not an animal make. [248] At some point, chimera must qualify as persons under the Constitution. [249] To conclude otherwise would necessitate an overly formalistic definition of person. Therefore, it is important to have a flexible analytical framework based on essential human biological and cognitive traits in order to decipher the personhood of questionable human-animal chimera. [250] The development of such a framework is desirable so that courts will not be wholly unprepared to address the issues of chimera. [251]

A. The Essential Factors of Constitutional Personhood

Paralleling the moral rights discussion in Part IV, two distinct but interrelated approaches to chimera personhood have been suggested. The first approach to chimera personhood focuses on biological material because "it cannot reasonably be disputed that an essential part of the definition of Homo **[381]** sapiens is genetically determined." [252] This approach might lead to a definition of personhood based on percentages; for example, a chimera with more than fifty percent human cells qualifies as a person. [253] However, this approach overlooks the moral and legal significance of cognitive factors and runs the risk of being overly formalistic. [254] The second approach is based on cognitive traits such as intelligence, rationality, and emotional capacity. [255] From the discussion of legal death in Part III.B.2 and cognitive morality in Part IV.A.2, it is clear that cognitive function is critical in defining legal personhood. The major theoretical difficulty with such a cognitive approach to personhood is that, if this were the sole test for constitutional personhood, it might exclude newborns, certain mentally handicapped individuals, and the comatose, while including intelligent animals or computers. [256] The cognitive approach also has practical difficulties when applied to chimera because it may not be clear how sentient or intelligent a human-animal chimera will be until it has been produced and raised.

By combining cognitive approaches with a human biological requirement, it becomes much easier to include all humans, exclude animals and artificial intelligence, and focus the inquiry on chimera. [257] The foremost indicators of constitutional personhood should be the capacity for higher-level human cognition combined with a significant percentage of human tissue. Human neural cells represent the most crucial human tissue because they affect cognitive capacity; thus the percentage of these cells

is of primary importance. [258] Addressing moral rights theories, the focus on cognitive ability responds to cognitive rights philosophies and utilitarian principles of interest **[382]** maximization, [259] while the biological requirement recognizes biological morality and the Kantian principle that there is inherent value in human life. [260]

B. The Flaw in a Dichotomous Approach to Constitutional Personhood

While it is incorrect to assert that chimera are never persons under the Constitution, it is also incorrect to claim, as most commentators do, that there is a distinct, identifiable point at which a chimera shifts from being a nonperson to being a person. [261] Regardless of their view on chimera personhood, most commentators presume that personhood must be an either/or proposition. [262] As an example of a dichotomous approach to personhood in the context of biotechnology, it has been proposed that "all and only species that are characterized by a capacity for [self-awareness] must be considered constitutional persons." [263] This is an example of a reasonably flexible, cognitive approach to personhood that could include chimera exhibiting a fundamental trait of human cognition, self-awareness, or sentience. [264] However, this analysis presupposes an identifiable point at which constitutional personhood kicks in. [265]

The line of demarcation between human and animal is being erased by chimera technology; what remains is a continuum with pure humans on one end, pure animals on the other, and various forms of chimera in between. Scientists can alter where their creations fall. [266] Thus, a bright line rule of personhood is no longer appropriate. [267] The goal should not be to draw an **[383]** arbitrary line between person and nonperson, but instead to grant constitutional protections proportionate to the critical human characteristics of a human-animal chimera. Therefore, this Comment proposes a reconceptualization of personhood that more accurately reflects the realties of modern biotechnology: a sliding scale of constitutional personhood. Under this approach, chimera with different critical human characteristics will qualify for different categories of constitutional protection. The fundamental characteristics of personhood are the higher-level human cognitive traits and the possession of crucial human biological tissue. [268]

The granting of partial constitutional rights is not unheard of; the Supreme Court already grants less than full constitutional protection to certain types of humans, including children, [269] prisoners, [270] and noncitizen aliens. [271] An analogous break from the traditionally rigid concept of constitutional personhood has also been alluded to in the human embryo context. [272]

[384]

C. The Categories of Human-Animal Chimera

This section will suggest four loose categories of chimera personhood to guide in the application of chimera personhood. [273] A sliding scale approach should not limit the constitutional rights of chimera that are fundamentally human simply because they have a "drop of animal" in them. [274] Therefore, the first category of chimera includes nominal chimera that are so clearly human that no further inquiry is warranted. Persons with xenotransplants, such as Senator Helms, exemplify this category. [275]

The second category falls at the other end of the human-animal chimera continuum and includes chimera with only small percentages of human cells and no human nervous tissue. [276] Such chimera should not be defined as constitutional persons. An example of this category would be a chimera in which the only human cells are limited to one organ, such as a kidney or liver, or tissue to be used for human transplantation or research. The utility of using chimera with small amounts of human cells and no potential for human cognitive traits such as intelligence, sentience, or emotions in research substantially outweighs any moral arguments in favor of granting them constitutional rights. [277] This second category of chimera may still be protected by legislative action, but they should not be covered by the Constitution.

The third category of chimera includes those with a substantial percentage of human neural cells that have the capacity for higher-level human cognition. Chimera in this category should initially be granted full constitutional rights as persons, and the category should be interpreted broadly. [278] Examples of a category three organism would be a human-pig chimera with a significant amount of pig tissue but a one hundred percent human nervous system or a human-chimpanzee chimera. These differ from category one in that the protections offered these chimera may be limited if it becomes clear that they

[385] do not and will not actually exhibit significant human cognitive traits. [279] A limitation based on such a showing could place such chimera in category four.

The fourth category includes two types of constitutionally ambiguous chimera: (1) chimera with a non-insignificant percentage of human neural cells that may demonstrate limited human cognitive ability and (2) chimera with insignificant human neural cells but a high total percentage of human cells. [280] Because a bright line of personhood is inadequate, this category of chimera should be afforded limited constitutional personhood in proportion to their place along the human-animal continuum. [281] This category includes creatures that do not have a significant potential for human intelligence, sentience, or emotions but are too biologically similar to humanity to be written off as mere animals. [282] These creatures would have only a limited capacity to appreciate constitutional rights so, taking guidance from the hybrid moral utility theories presented in Part IV.B.3, the extent of the rights afforded them may be limited but not eliminated. [283] If chimera in this category were to exhibit higher-level human cognitive traits, they could be moved into the third category and granted full constitutional protection.

The goal of this Comment is to address the threshold question of personhood in order to open the door for the application of the Constitution to chimera. This Comment does not attempt to analyze exactly how various constitutional provisions apply to the patentability, production, or research use **[386]** of chimera. [284] This is left to the courts and Congress to determine. [285] As a cursory overview, however, it is clear that the Reconstruction amendments would play a central role. Various levels of protection against undue pain and frivolous use of chimera in research seem likely under both the Thirteenth [286] and Fourteenth Amendments. [287] The Constitution may also bar the patenting of chimera. [288] In addition, it has been argued that the mere act of producing chimera may be unconstitutional. [289]

Conclusion

For the protections of the Constitution to apply, an organism must be a "constitutional person." However, human-animal chimera technology is straining the dichotomous constitutional personhood construct beyond the breaking point. This is because the line between humans and nonhumans, the bedrock of constitutional personhood, is being rendered obsolete. Scientists will soon be able to create chimera possessing any ratio of human to animal they please, leaving only a continuum of humanity. We must be open to some form of chimera personhood. Because it is too arbitrary to simply draw a line in the sand dividing chimeric persons from nonperson chimera, the traditional dichotomous concept of constitutional personhood should be replaced with a more flexible approach, bringing more beings under the constitutional **[387]** umbrella. To do this, different categories of chimera should be afforded differing levels of protection in relation to the fundamental characteristics of humanity that they possess. Those fundamental characteristics are higher-level human cognitive traits and the possession of crucial human biological tissues.

Footnote *

J.D., Emory University School of Law, Atlanta, Georgia (2006); B.A., Duke University, Durham, North Carolina (2002). I would like to thank Professor Sara Stadler for her insight and guidance throughout the drafting process and Professor Margo Bagley for her assistance in selecting my Comment topic. I would also like to thank my friends and family for their support, encouragement, wisdom, and patience.

Footnote 1

Rick Weiss, Of Mice, Men and In-Between: Scientists Debate Blending of Human, Animal Forms, Wash. Post, Nov. 20, 2004, at A1.

Footnote 2

Human-animal chimera have been raised to adulthood in recent years. See, e.g., Brenda M. Ogle et al., Spontaneous Fusion of Cells Between Species Yields Transdifferentiation and Retroviral Transfer in Vivo, 18 Fed'n Am. Societies For Experimental Biology J. 548, 548-50 (2004) (describing the production of human-pig chimera).

Footnote 3

Thomas Bulfinch, Bulfinch's Mythology 117 (1993).

Footnote 4

H.G. Wells, The Island of Doctor Moreau (1896). A recent science fiction book features human-animal chimera that are bred to serve humans. Will Shetterly, Chimera (2000).

Footnote 5

Biotechnology is defined as "any technique that uses living organisms or substances from those organisms to make or modify a product, to improve plants or animals, or to develop microorganisms for specific uses." Jasemine Chambers, Patent Eligibility of Biotechnological Inventions in the United States, Europe, and Japan: How Much Patent Policy Is Public Policy?, 34 Geo. Wash. Int'l L. Rev. 223, 223 (2002).

Footnote 6

Nicole E. Kopinski, Human-Nonhuman Chimeras: A Regulatory Proposal on the Blurring of Species Lines, 45 B.C. L. Rev. 619, 624-25 (2004). In accordance with most commentators, the term chimera will be used as both the singular and plural in this Comment.

Footnote 7

Id. at 625.

Footnote 8

Stem cells are undifferentiated cells that have the potential to develop into specialized cells. Consuelo G. Erwin, Embryonic Stem Cell Research: One Small Step for Science or One Giant Leap Back for Mankind?, 2003 U. Ill. L. Rev. 211, 213.

Footnote 9

Ogle et al., supra note 2, at 548-50.

Footnote 10

See Gaia Vince, Pig-Human Chimeras Contain Cell Surprise, NewScientist.com, Jan. 13, 2004, http://www.newscientist.com/news/news.jsp?id=ns99994558 ("The injections must be given after the body plan of the fetus has developed, but before the immune system is active. The former ensures the animals look like normal pigs and sheep. The latter prevents the human stem cells being rejected.").

Footnote 11

Id. Surprisingly, these chimera also contain hybrid cells with a mixture of human and porcine genetic material. This was the first time researchers witnessed such hybrid cells in a chimera. Id.

Footnote 12

Researchers discovered human cell lines integrated into the tissue structures in the thymus, kidney, and skin of the chimeric animals. Ogle et al., supra note 2, at 548-50. These particular pig-human chimera are much more pig than human because the total percentage of human cells present at one year and beyond was approximately .1%. Id. As a result, it is difficult to imagine granting these particular chimera being viewed as persons. However, there does not seem to be a scientific barrier to creating a human-animal chimera with a much higher percentage of human cells by, for example, injecting the human stem cells at a much earlier time. See Vince, supra note 10.

Footnote 13

Weiss, supra note 1.

Footnote 14

Id.

Footnote 15

In 2005, Senator Brownback of Kansas introduced a bill that would prohibit any person from creating or attempting to create a "human chimera." The Human Chimera Prohibition Act of 2005, S. 659, 109th Cong. According to the Library of Congress' website, the Bill has been referred to the Committee on the Judiciary. Library of Congress, Bill Summary of S. 659, http://thomas.loc.gov/cgi-bin/bdquery/z?d109:s.00659: (last visited Jan. 21, 2006). Canada already bans chimera research via the Assisted Human Reproduction Act. Maryann Mott, Animal-Human Hybrids Spark Controversy, Nat'l Geographic News, Jan. 25, 2005, http://news.nationalgeographic.com/news/2005/01/0125_050125_chimeras.html.

Footnote 16

See infra notes 128-33 and accompanying text.

Footnote 17

See infra notes 24-43 and accompanying text.

Footnote 18

See Rick Weiss, U.S. Denies Patent for a Too-Human Hybrid, Wash. Post, Feb. 13, 2005, at A3.

Footnote 19

See infra Part V.

Footnote 20

See infra Part V.B.

Footnote 21

See infra Part V.B-C.

Footnote 22

See infra Part V.

Footnote 23

Other controversial areas of biotechnology, such as cloning and human stem cell research, are beyond the scope of this Comment.

Footnote 24

Henry T. Greely, Defining Chimera … and Chimeric Concerns, Am. J. Bioethics, Summer 2003, at 17, 18 (noting that under a loose definition of chimera, all humans might be considered human-bacteria chimera due to the huge numbers of bacteria living within our intestines).

Footnote 25

Kopinski, supra note 6, at 624.

Footnote 26

See Carole B. Fehilly et al., Interspecific Chimaerism Between Sheep and Goat, 307 Nature 634 (1984) (describing a goat-sheep chimera).

Footnote 27

Hybrids result from the sexual reproduction of different species. For example, a mule is a hybrid of a male donkey and a female horse. Kopinski, supra note 6, at 623 n.32.

Footnote 28

Clones are organisms produced with identical genetic material to the "parent" organism. The best known example of a clone is Dolly the sheep. See Ian Wilmut, Cloning for Medicine, Sci. Am., Dec. 1998, at 58, 58.

Footnote 29

While chimera technology mixes cells of two different species into one organism, recombinant DNA technology is used to transfer isolated genes from one organism to another. Recombinant technology is frequently used to insert specific human genes into the genome of another organism for research or to produce human biological products. Eileen Morin, Of Mice and Men: The Ethics of Patenting Animals, 5 Health L.J. 147, 149 (1997).

Footnote 30

Greely, supra note 24, at 17.

Footnote 31

Kopinski, supra note 6, at 624-25.

Footnote 32

Id. at 625. For a description of the production of such an organism, see infra notes 36-37 and accompanying text.

Footnote 33

Weiss, supra note 1.

Footnote 34

See Vince, supra note 10. There may be substantial public opposition to even this limited chimera research if any part of the chimera's brain cells were found to be human. Sylvia Pagan Westphal, Growing Human Organs on the Farm, NewScientist, Dec. 20, 2003-Jan. 9, 2004, at 4, 4-5, available at http://newscientist.com/news/news.jsp?id=ns99994492. A lead scientist conducting human-sheep chimera research, Esmail Zanjani of the University of Nevada, stated that "there is no way for us to know" if human cells might enter into the chimera's brain tissue, "but at the level we're working with the animal, it's still a sheep." Id. at 5.

Footnote 35

Thomas A. Magnani, The Patentability of Human-Animal Chimeras, 14 Berkley Tech. L.J. 443, 445-46 (1999).

Footnote 36

See Fehilly et al., supra note 26, at 634-36. For a startling picture of a geep, see Colo. State Univ., Cytogenetics and Chromosomal Disorders: Mosaicism and Chimerism, http://arbl.cvmbs.colostate.edu/hbooks/genetics/medgen/chromo/mosaics.html (last visited Jan. 21, 2006).

Footnote 37

Fehilly et al., supra note 26, at 635-36.

Footnote 38

Magnani, supra note 35, at 446.

Footnote 39

Hilary Bok, What's Wrong with Confusion?, Am. J. Bioethics, Summer 2003, at 25, 25.

Footnote 40

See Kopinski, supra note 6, at 626-27. Although some authors have described human-nonhuman nuclear transfer as a chimeric technology, it does not technically produce chimera because each cell

of the resulting organism is a mixture of the parent organisms at the cellular level. Transcript, Nat'l Bioethics Advisory Comm'n, 25th Meeting 102-03 (Nov. 17, 1998).

Footnote 41

Nuclear transfer involves "transferring the nucleus with its chromosomal DNA from one (donor) cell to another (recipient) cell. In cloning, the recipient is a human egg cell and the donor cell can be any one of a number of different adult tissue cells." President's Council on Bioethics, Scientific Aspects of Human and Animal Cloning (2002) (staff working paper), http://www.bioethics.gov/background/workpaper2.html.

Footnote 42

See, e.g., Ying Chen et. al., Embryonic Stem Cells Generated by Nuclear Transfer of Human Somatic Nuclei into Rabbit Oocytes, 13 Cell Res. 251 (2003). These cells developed into embryos that contained both the nuclear human DNA and mitochondrial rabbit DNA. Rick Weiss, Cloning Yields Human-Rabbit Hybrid Embryo, Wash. Post, Aug. 14, 2003, at A4.

Footnote 43

The DNA is not completely human because while human chromosomal DNA is transferred in the animal cell, mitochondrial DNA is not. Jason Scott Robert & Francoise Baylis, Crossing Species Boundaries, Am. J. Bioethics, Summer 2003, at 1, 2, 8.

Footnote 44

Magnani, supra note 35, at 456.

Footnote 45

Id.

Footnote 46

Weiss, supra note 1.

Footnote 47

See Margaret A. Clark, This Little Piggy Went to Market: The Xenotransplantation and Xenozoonose Debate, 27 J.L. Med. & Ethics 137, 139 (1999). Xenotransplantation is the term for interspecies transplantation, such as transplanting a baboon heart into a human. Id. at 138.

Footnote 48

Weiss, supra note 1.

Footnote 49

Magnani, supra note 35, at 456.

Footnote 50

Weiss, supra note 1.

Footnote 51

Julian Savulescu, Human-Animal Transgenesis and Chimeras Might Be an Expression of Our Humanity, Am. J. Bioethics, Summer 2003, at 22, 22; see Greely, supra note 24, at 19 (stating that "making a chimera of a human and a nonhuman is much less controversial when done for medical purposes than if such a creature were made for entertainment or "art'").

Footnote 52

Mark Dowie, Gods and Monsters, Mother Jones, Jan.-Feb. 2004, at 48, 50, available at http://online.sfsu.edu/<diff>rone/GEessays/chimerapatent.htm.

Footnote 53

In April of 2005, the National Academies of Sciences - a private, nonprofit group that advises the federal government - issued guidelines for human embryonic stem cell research that address some of the ethical issues related to chimera. Comm. on Guidelines for Human Embryonic Stem Cell Res., Nat'l Res. Council, Guidelines for Human Embryonic Stem Cell Research (2005) [hereinafter Guidelines], available at http://www.nap.edu/books/0309096537/html/. Of particular note here are the nonbinding guidelines: (1) recognize the importance of chimera research, (2) restrain the use of human embryonic stem cells to "circumstances where no other experiment can provide the information needed," (3) provide that human embryonic stem cells should not be inserted into nonhuman primates, (4) state that animals into which human embryonic stem cells have been inserted should not be allowed to breed, and (5) caution that no animal embryonic stem cells should ever be inserted into a human blastocyst. New Federal Guidelines Will Allow Creation of Human-Animals Chimeras, Env't News Service (Apr. 27, 2005), available at http://www.organicconsumers.org/patent/chimeras042805.cfm.

Footnote 54

Robert P. Merges, Intellectual Property in Higher Life Forms: The Patent System and Controversial Technologies, 47 Md. L. Rev. 1051, 1060 (1988). In Rifkin's view, each species should be allowed to have its genetic composition unaltered and so biotechnology should never be used to cross species lines. Morin, supra note 29, at 170.

Footnote 55

Tara Seyfer, The Ethics of Chimeras and Hybrids, 29 Ethics & Medics 8 (Aug. 2004) ("Jesus Christ did not come as an animal, but specifically as a human being, in a human body. This bespeaks the dignity which God accords human beings and their bodies… . It thus seems to lead towards the blasphemous to purposefully combine the genetic or bodily material of a human being and an animal."), available at http://www.lifeissues.net/writers/sey/sey_02ethicschimeras.html.

Footnote 56

Dan L. Burk, Patenting Transgenic Human Embryos: A Nonuse Cost Perspective, 30 Hous. L. Rev. 1597, 1637 (1993).

Footnote 57

Rebecca Dresser, Ethical and Legal Issues in Patenting New Animal Life, 28 Jurimetrics J. 399, 410-11 (1988).

Footnote 58

Burk, supra note 56, at 1637-38.

Footnote 59

John Rennie, Human-Animal Chimeras: Some Experiments Can Disquietly Blur the Line Between Species, Sci. Am.com (June 27, 2005), http://www.sciam.com/article.cfm?articleID=000BCF6C-7AF0-12B8-BAF083414B7FFE9F.

Footnote 60

Jenna Greene, He's Not Just Monkeying Around, Legal Times, Aug. 16, 1999, at 16 (quoting Jeremy Rifkin, who stated that "the more humanized you make your animal models, the better").

Footnote 61

Id. Perhaps the initial discomfort felt by many at thought of human-animal chimera should not be completely disregarded. It has been stated that "repugnance is the emotional expression of deep wisdom, beyond reason's power fully to articulate it." Leon R. Kass, The Wisdom of Repugnance, in Leon R. Kass & James Q. Wilson, The Ethics of Human Cloning 3, 18 (1998).

Footnote 62

See supra notes 44-50 and accompanying text.

Footnote 63

Margo A. Bagley, Patent First, Ask Questions Later: Morality and Biotechnology in Patent Law, 45 Wm. & Mary L. Rev. 469, 534-45 (2003).

Footnote 64

See infra Part II.C.

Footnote 65

See James P. Daniel, Of Mice and "Manimal": The Patent & Trademark Office's Latest Stance Against Patent Protection for Human-Based Inventions, 7 J. Intell. Prop. L. 99, 104 (1999).

Footnote 66

Id. at 104-05.

Footnote 67

Magnani, supra note 35, at 447.

Footnote 68

Diamond v. Chakrabarty, 447 U.S. 303 (1980).

Footnote 69

Id. at 310.

Footnote 70

Id. at 309. However, this was not a forgone conclusion: Section 101 permits patents on machines, manufactures, or compositions of matter. A living organism is, properly speaking, none of these. Thus, Chakrabarty is particularly controversial and important because the Court read into the Patent Act and its legislative history support for extending patents to living organisms. Linda J. Demaine & Aaron Xavier Fellmeth, Reinventing the Double Helix: A Novel and Nonobvious Reconceptualization of the Biotechnology Patent, 55 Stan. L. Rev. 303, 317 (2002).

Footnote 71

Chakrabarty, 447 U.S. at 310 ("Here … the patentee has produced a new bacterium with markedly different characteristics from any found in nature… . His discovery is not nature's handiwork, but his own; accordingly it is patentable subject matter under 101.").

Footnote 72

See Chakrabarty, 447 U.S. at 309 (noting that "the laws of nature, physical phenomena, and abstract ideas have been held not patentable"); Daniel, supra note 65, at 105.

Footnote 73

Chakrabarty, 447 U.S. at 313.

Footnote 74

Ex parte Allen, 2 U.S.P.Q.2d (BNA) 1425 (PTO B.P.A.I. 1987). A polyploid organism has one or more extra sets of chromosomes. Id.

Footnote 75

See id. at 1425-27. The patent failed the requirement that an application be non-obvious. Id. at 1428-29.

Footnote 76

Michael B. Landau, Multicellular Vertebrate Mammals as "Patentable Subject Matter" Under 35 U.S.C. 101: Promotion of Science and the Useful Arts or an Open Invitation for Abuse?, 97 Dick. L. Rev. 203, 213 (1993).

Footnote 77

U.S. Pat. No. 4,736,866 (filed Apr. 12, 1988). The Onco-Mouse was a strain of mice that had been

genetically altered to be highly susceptible to cancer when exposed to cancer-causing agents. Mark Jagels, Notes and Comments, Dr. Moreau Has Left the Island: Dealing with Human-Animal Patents in the 21st Century, 23 T. Jefferson L. Rev. 115, 132 (2000).

Footnote 78

Jagels, supra note 77, at 132.

Footnote 79

Morality' Aspect of Utility Requirement Can Bar Patent for Part-Human Inventions, 55 Pat. Trademark & Copyright J. (BNA) 556 (1998).

Footnote 80

Patent and Trademark Office Notice: Animals-Patentability, 1077 Official Gazette U.S. Pat. & Trademark Off. 24 (1987).

Footnote 81

Id.

Footnote 82

Cynthia M. Ho, Splicing Morality and Patent Law: Issues Arising from Mixing Mice and Men, 2 Wash. U. J.L. & Pol'y 247, 251 (2000); Magnani, supra note 35, at 448.

Footnote 83

Bagley, supra note 63, at 502.

Footnote 84

Rick Weiss, Patent Sought on Making of Part-Human Creatures, Wash. Post, Apr. 2, 1998, at A12. Newman is a cellular biologist at New York Medical College and Rifkin is a prominent opponent of biotechnology. Id.

Footnote 85

Magnani, supra note 35, at 450.

Footnote 86

Weiss, supra note 84.

Footnote 87

Greene, supra note 60.

Footnote 88

Id.

Footnote 89

Weiss, supra note 84.

Footnote 90

On April fools' day 1998, within hours of reading U.S. patent application No. 08/993,564, the Honorable Bruce Lehman did something no other commissioner of patents had done in the 200-year history of America's oldest government agency. He stepped before a cluster of microphones and announced that the patent would never be approved. No half-human "monsters" would be patented, Lehman declared angrily, or any other "immoral inventions."

Dowie, supra note 52, at 49.

Footnote 91

Daniel, supra note 65, at 117-20.

Footnote 92

Id. at 118-19. Due to confidentiality concerns, the PTO was not commenting directly on the Newman Application but it was clearly the impetus for the media advisory. See Media Advisory, U.S. Patent & Trademark Office, Facts on Patenting Life Forms Having a Relationship to Humans (Apr. 1, 1998), http://www.uspto.gov/web/offices/com/speeches/98-06.htm [hereinafter Media Advisory].

Footnote 93

Media Advisory, supra note 92. In attempting to reinvigorate the moral utility doctrine in reference to chimera, the PTO went on to state that:

the PTO will not, therefore, issue a patent for an invention of incredible or specious utility or for inventions whose utilization is not adequately disclosed in the application. Additionally, the courts have interpreted the utility requirement to exclude inventions deemed to be "injurious to the well being, good policy, or good morals of society." Id. (citations omitted).

Footnote 94

Jagels, supra note 77, at 133.

Footnote 95

Id.

Footnote 96

Weiss, supra note 18.

Footnote 97

Id.

Footnote 98

Id.

Footnote 99

Id.

Footnote 100

Patent and Trademark Office Notice, supra note 80.

Footnote 101

Daniel, supra note 65, at 118-19.

Footnote 102

The Patent Act is to be construed without implied restrictions. Diamond v. Chakrabarty, 447 U.S. 303, 315-16 (1980); Ho, supra note 82, at 252. The Chakrabarty Court noted that "a statute is not to be confined to the "particular applications … contemplated by the legislators.' This is especially true in the field of patent law… . Congress employed broad general language in drafting 101 precisely because such inventions are often unforeseeable." Chakrabarty, 447 U.S. at 315-16 (citations omitted).

Footnote 103

See Bagley, supra note 63, at 498 (stating that "section 101 of the Patent Act, as interpreted, encompasses "anything under the sun that is made by man,' including, apparently, animals and even other men").

Footnote 104

No court has relied on the moral utility doctrine in rejecting a patent since the PTO Board of Appeals held that a gambling device was patentable in 1977. Ho, supra note 82, at 249; see Lauren Cirlin, Comment, Human or Animal: A Resolution to the Biotechnological Blurring of the Lines, 32 Sw. U. L. Rev. 501, 514-15 (2003) (noting that moral questions are highly subjective and allowing the PTO to make such judgments would inject damaging uncertainty into patent law). But see Tol-O-Matic, Inc. v. Proma Produkt-und Mktg. Gesellschaft, 945 F.2d 1546, 1553 (Fed. Cir. 1991).

Footnote 105

Bagley, supra note 63, at 492. Professor Bagley goes on to note that "it would be difficult in the extreme to resurrect a rule which … does not exist under the current patent statute." Id. at 493.

Footnote 106

Europe does have a moral utility requirement in patent law. Young-Gyoo Shim, Intellectual Property Protection of Biotechnology and Sustainable Development in International Law, 29 N.C. J. Int'l L. & Com. Reg. 157, 220-21 (2003).

Footnote 107

Kopinski, supra note 6, at 657 (stating that "the moral utility test in patent law is not particularly useful in a patent application for human-nonhuman chimeras, because the utilitarian arguments would inevitably outweigh the deontological arguments"); see Magnani, supra note 35, at 456.

Footnote 108

See Weiss, supra note 18 (listing constitutional privacy rights, the Thirteenth Amendment, and the right to travel as barriers asserted by the PTO against chimera patentability).

Footnote 109

While several commentators have addressed the patentability of chimera, the threshold question of the constitutional personhood of chimera has not been well addressed in the current literature and is therefore the focus of this Comment.

Footnote 110

Article I of the Constitution gives Congress the power to legislate "to promote the Progress of … useful Arts, by securing for limited Times to … Inventors the exclusive Right to their … Discoveries." U.S. Const. art. I, 8, cl. 8. In contrast, the PTO has only the statutory authority to apply the legislative scheme that Congress has enacted. Graham v. John Deere Co., 383 U.S. 1, 6 (1966).

Footnote 111

Daniel, supra note 65, at 117.

Footnote 112

Marbury v. Madison, 5 U.S. 137, 177 (1803). Further, if the PTO's constitutional proclamations against chimera patentability are in fact wrong and overturned by the courts, it will have needlessly stifled valuable medical research by eliminating the economic incentives of patentability.

Footnote 113

Jagels, supra note 77, at 117.

Footnote 114

Id.

Footnote 115

Weiss, supra note 84.

Footnote 116

One author has remarked that the reason the Newman Application was rejected is that it caught the attention of the PTO by being explicit about the potential scope and applications of its claims: "Newman and Rifkin's application is different only in that it calls a pig a pig, so to speak." Clark, supra note 47, at 143.

Footnote 117

Consolidated Appropriations Act of 2004, Pub. L. No. 108-199, 634, 118 Stat. 101; Section of Intellectual Prop. Law, Am. Bar Ass'n, Report to the House of Delegates 2 (2004) [hereinafter ABA Report], http://www.abanet.org/leadership/2004/annual/104.doc.

Footnote 118

See generally ABA Report, supra note 117.

Footnote 119

634, 118 Stat. 101. The language of the provision may not prohibit patents on the methods used to create human organisms, just the organisms themselves. On August 24, 2004, the PTO issued a methods patent for cloning mammals that did not exclude human beings from its claims. See U.S. Patent No. 6,781,030 (filed Nov. 2, 1999).

Footnote 120

See generally ABA Report, supra note 117.

Footnote 121

Kopinski, supra note 6, at 635; Rick Weiss, Hill Negotiators Agree to Bar Patents for Human Organisms, Wash. Post, Nov. 25, 2003, at A19.

Footnote 122

149 Cong. Rec. E2235 (daily ed. Nov. 5, 2003) (statement of Rep. Weldon).

Footnote 123

ABA Report, supra note 117, at 4. The ABA also expressed concern that, despite Weldon's statements, the "human organism" language of the provision "could be interpreted as broadening the current USPTO prohibition to prescribe also the patenting of human cells or human cell lines, such as embryonic stem cell lines." Id.

Footnote 124

Kopinski, supra note 6, at 635; Weiss, supra note 121.

Footnote 125

Mikyung Kim, An Overview of the Regulation and Patentability of Human Cloning and Embryonic Stem Cell Research in the United States and Anti-Cloning Legislation in South Korea, 21 Santa Clara Computer & High Tech. L.J. 645, 665 (2005). PTO Director James Rogan has stated that the provision gives "unequivocal congressional backing" for the USPTO's refusal "to grant any patent containing a claim that encompasses any member of the species Homo sapiens at any stage of development." Letter from James E. Rogan, Dir., U.S. Patent and Trademark Office, to Sen. Ted Stevens, Chairman, Comm. on Appropriations (Nov. 20, 2003), available at http://www.nrlc.org/Killing_Embryos/Human_Patenting/WeldonamendUSPTO.pdf.

Footnote 126

Bagley, supra note 63, at 474; Magnani, supra note 35, at 459.

Footnote 127

Kopinski, supra note 6, at 656; see Leon R. Kass, Patenting Life, 63 J. Pat. & Trademark Off. Soc'y 571, 583 (1981) ("Denial of individual patent applications seems a poor way for society to decide questions about allegedly dangerous research and technology.").

Footnote 128

Roe v. Wade, 410 U.S. 113, 156-57 (1973); Magnani, supra note 35, 449-50. The term "person" is quite different from "citizen." Under the Fourteenth Amendment, for example, "citizen" is a subcategory of "person," one "born or naturalized in the United States, and subject to the jurisdiction thereof." U.S. Const. amend. XIV, 1. Thus, noncitizen persons are protected by most constitutional rights but could still be denied some constitutional protections that are reserved only for citizens, such as the privileges and immunities clause of the Fourteenth Amendment. Id.

Footnote 129

Richard M. Lebovitz, The Accordion of the Thirteenth Amendment: Quasi-Persons and the Right of Self-Interest, 14 St. Thomas L. Rev. 561, 563 (2002).

Footnote 130

Roe, 410 U.S. at 157. After noting the lack of specific definition, the Roe Court then described the various places in which the term "person" is used in the document:

Section 1 of the Fourteenth Amendment contains three references to "person." The first, in defining "citizens," speaks of "persons born or naturalized in the United States." The word also appears both in the Due Process Clause and in the Equal Protection Clause. "Person" is used in other places in the Constitution: in the listing of qualifications for Representatives and Senators, Art. I, 2, cl. 2, and 3, cl. 3; in the Apportionment Clause, Art. I, 2, cl. 3; in the Migration and Importation provision, Art. I, 9, cl. 1; in the Emolument Clause, Art. I, 9, cl. 8; in the Electors provisions, Art. II, 1, cl. 2, and the superseded cl. 3; in the provision outlining qualifications for the office of President, Art. II, 1, cl. 5; in the Extradition provisions, Art. IV, 2, cl. 2, and the superseded Fugitive Slave Clause 3; and in the Fifth, Twelfth, and Twenty-second Amendments, as well as in 2 and 3 of the Fourteenth Amendment. Id.

Footnote 131

The Supreme Court has used only pragmatic concerns to derive a legal conclusion of constitutional personhood… . This lack of theory plagues the law of personhood for both natural persons and corporations… . Such decisions appear to be made on a case-by-case basis, probably with an eye toward practical effects, without consideration for developing a coherent doctrine.

Michael D. Rivard, Comment, Toward a General Theory of Constitutional Personhood: A Theory of Constitutional Personhood for Transgenic Humanoid Species, 39 UCLA L. Rev. 1425, 1465-66 (1992).

Footnote 132

Note, What We Talk About When We Talk About Persons: The Language of a Legal Fiction, 114 Harv. L. Rev. 1745, 1747 (2001).

Footnote 133

David Favre, Integrating Animal Interests into Our Legal System, 10 Animal L. 87, 92 (2004). For a description of the "schizophrenic" corporate personhood doctrine, see generally Note, supra note 132.

Footnote 134

See generally Greely, supra note 24 (recommending an exhaustive taxonomy to aid in analyzing the ethical concerns presented by different types of chimera); Weiss, supra note 84 (noting that Congress failed in its attempt to pass a law restricting patents on humans in 1989, in part because of the difficulty in defining "human").

Footnote 135

Jagels, supra note 77, at 122.

Footnote 136

However, it is possible to transfer a large portion of the human genome into host animals by recombinant technology or by nuclear transfer. Id. at 124-25.

Footnote 137

See Weiss, supra note 84 (statement of Thomas Murray).

Footnote 138

See supra notes 44-50 and accompanying text.

Footnote 139

In describing human biotechnology patents, Jagels notes that "the greatest challenge lies in limiting the transfer of human character without overly restricting beneficial uses of human gene products." Jagels, supra note 77, at 126.

Footnote 140

This term that has been used frequently by the PTO and its precise legal meaning is not clear. See supra Part II.C.

Footnote 141

As John Chipman Gray stated, "the technical legal meaning of "person' is a subject of legal rights and duties." Lawrence B. Solum, Legal Personhood for Artificial Intelligences, 70 N.C. L. Rev. 1231, 1238-39 (1992) (citing John Chipman Gray, The Nature and Sources of the Law 27 (1909)). A similar taxonomic distinction between "human" and "person" is mentioned by Robert & Baylis, supra note 43, at 9.

Footnote 142

In addition, this country's history of slavery cautions strongly against an orginalist approach to personhood in many contexts. Kayhan Parsi, Metaphorical Imagination: The Moral and Legal Status of Fetuses and Embryos, 2 DePaul J. Health Care L. 703, 779 (1999). For general discussions of originalist debate, see generally Daniel A. Farber, The Originalism Debate: A Guide for the Perplexed, 49 Ohio St. L.J. 1085 (1989), and Paul Finkelman, The Constitution and the Intentions of the Framers: The Limits of Historical Analysis, 50 U. Pitt. L. Rev. 349 (1989).

Footnote 143

See supra Part II.C.

Footnote 144

See supra notes 99-112 and accompanying text.

Footnote 145

Douglas O. Linder, The Other Right-to-Life Debate: When Does Fourteenth Amendment "Life" End?, 37 Ariz. L. Rev. 1183, 1183 n.1 (1995) ("Interestingly, the slavery, abortion, and end-of-life debates all relate to the meaning of constitutional personhood."); Solum, supra note 141, at 1285.

Footnote 146

Roe, 410 U.S. at 156-57.

Footnote 147

Id. at 158.

Footnote 148

Id. But see Gerard V. Bradley, Life's Dominion: A Review Essay, 69 Notre Dame L. Rev. 329, 338-47 (1993) (arguing for fetal personhood); Charles I. Lugosi, Respecting Human Life in the 21st Century: A Model Perspective to Extend Civil Rights to the Unborn from Creation to Natural Death, 48 St. Louis U. L.J. 425, 447 (2004) (arguing that legal personhood should attach at the moment of conception).

Footnote 149

Bagley, supra note 63, at 503. Nevertheless, Roe does not cut off all rights possessed by nonperson fetuses since "the decision postulates a sliding scale that allows the state to recognize powerful fetal rights that, at viability, even trump maternal rights." John B. Attanasio, The Constitutionality of Regulating Human Genetic Engineering: Where Procreative Liberty and Equal Opportunity Collide, 53 U. Chi. L. Rev. 1274, 1294 (1986).

Footnote 150

Burk, supra note 56, at 1652-53.

Footnote 151

See id.

Footnote 152

William L. Saunders, Jr., The Current State of Abortion Law and Reproductive Rights: Lethal Experimentation on Human Beings: Roe's Effect on Bioethics, 31 Fordham Urb. L.J. 817, 829 (2004). However, Saunders argues that the cabining argument has proven to be false because the Roe "principle" has been extended to the debates over stem cell research and cloning. Id. at 830.

Footnote 153

See Nathan A. Adams, Creating Clones, Kids & Chimera: Liberal Democratic Compromise at the Crossroads, 17 Notre Dame J.L. Ethics & Pub. Pol'y 71, 106-08 (2003); Erwin, supra note 8, at 221-37.

Footnote 154

Steven Goldberg, The Changing Face of Death: Computers, Consciousness, and Nancy Cruzan, 43 Stan. L. Rev. 659, 659 (1991) (arguing that self-awareness should be the central issue in defining death).

Footnote 155

Joseph N. Harden, The "Gift" of Life: Should Anencephalic Infants Die to Serve Noble Goals?, 27 Cumb. L. Rev. 1279, 1290 (1997); see Kathleen L. Paliokas, Anencephalic Newborn as Organ Donors: An Assessment of "Death" and Legislative Policy, 31 Wm. & Mary L. Rev. 197, 201-03 (1989).

Footnote 156

Harden, supra note 155, at 1290.

Footnote 157

Id. at 1291-92.

Footnote 158

Goldberg, supra note 154, at 667-68.

Footnote 159

See Unif. Determination of Death Act, 1, 12A U.L.A. 386 (1980 & Supp. 1994); Alexander Morgan Capron & Leon R. Kass, A Statutory Definition of the Standards for Determining Human Death: An Appraisal and a Proposal, 121 U. Pa. L. Rev. 87, 118 (1972) (proposing an early model brain death statute).

Footnote 160

12A U.L.A. 386, 1(2); see President's Comm'n for the Study of Ethical Problems in Medicine and Biomedical and Behavioral Res., Defining Death 109-34 (1981) (compiling statutes and showing that most states recognize some form of "brain death").

Footnote 161

David Randolph Smith, Legal Recognition of Neocortical Death, 71 Cornell L. Rev. 850, 857 (1986).

Footnote 162

Id.

Footnote 163

Goldberg, supra note 154, at 667-69.

Footnote 164

Anencephaly is a congenital defect where brain development is incomplete resulting in an absence of the major brain structures responsible for cognition. Paliokas, supra note 155, at 197. The brain death statutes generally do not apply to this condition because the residual lower brain stem often remains and still operates after birth. Id.

Footnote 165

Id. at 226-29.

Footnote 166

Gary B. Gertler, Brain Birth: A Proposal for Defining When a Fetus is Entitled to Human Life Status, 59 S. Cal. L. Rev. 1061, 1069-70 (1986) (proposing that fetal personhood begin when neocortical brain activity begins because higher-level intellectual functioning characterizes personhood); Paliokas, supra note 155, at 226-29. These newborns should still be treated with a high level of moral respect and need not be left to die simply because they qualify as brain dead or are not defined as legal "persons." See Paliokas, supra note 155, at 228.

Footnote 167

Paliokas, supra note 155, at 226-27.

Footnote 168

Ronald E. Cranford & David Randolph Smith, Consciousness: The Most Critical Moral (Constitutional) Standard For Human Personhood, 13 Am. J.L. & Med. 233, 247 (1987).

Footnote 169

See id. ("Personhood encompass an inner-directed and an outer-directed will. An individual who is permanently unconscious has no will. In the absence of will, thought, expression, or consciousness, legal rights and liberties have no reference and thus no meaning.").

Footnote 170

Id.

Footnote 171

See supra Part III.

Footnote 172

See Attanasio, supra note 149, at 1328 (noting that moral theory can help overcome inadequacies in constitutional theory in the biotechnology field).

Footnote 173

Natural law is defined as: "[a] philosophical system of legal and moral principles purportedly deriving from a universalized conception of human nature or divine justice rather than from legislative or judicial action; moral law embodied in principles of right and wrong." Black's Law Dictionary 1049 (7th ed. 1999).

Footnote 174

Paul E. Sigmund, Natural Law in Political Thought 109 (1971) (discussing the use of natural law to interpret the Fifth and Fourteenth Amendments).

Footnote 175

As Justice Cardozo stated, a form of natural law provides "the main rule of judgment to the judge when precedent and custom fail." Id. at 169 (citing Benjamin Cardozo, The Nature of the Judicial Process 142 (1921)). Unfortunately, "judges not only fail to invoke philosophical support for their ideas of personality, but also inconsistently apply jurisprudential theory in resolving problems of legal personhood, approaching it more as a legal conclusion than as an open question." Note, supra note 132, at 1747.

Footnote 176

These sources do not generally attempt to define constitutional personhood. Instead, they seek to find the traits that separate humans from animals on moral and ethical grounds and the insights from these discussions are useful in resolving the ultimate constitutional issue.

Footnote 177

See infra Part IV.A.1-2.

Footnote 178

Anthropocentrism is defined as "1. Regarding humans as the central element of the universe; 2. Interpreting reality exclusively in terms of human values and experience." The American Heritage College Dictionary 60 (4th ed. 2002).

Footnote 179

See infra Part IV.A.1-2.

Footnote 180

See Lugosi, supra note 148, at 447 (arguing in the abortion context that personhood should be granted to all biological Homo sapiens from the moment of conception forward).

Footnote 181

Solum, supra note 141, at 1284.

Footnote 182

Id.

Footnote 183

Charles M. Kester, Is There a Person in That Body? An Argument for the Priority of Persons and the Need for a New Legal Paradigm, 82 Geo. L.J. 1643, 1652-58 (1994) (arguing that a purely biological approach to legal standing (used by Kester as a substitute for personhood) creates doctrinal anomalies when applied to fetuses and patients in a persistent-vegetative-state); Solum, supra note 141, at 1284.

Footnote 184

See Bok, supra note 39, at 26 (stating that "it is not at all obvious why membership in a species (as opposed to possession of properties that members of that species commonly have) should entail radically different moral status").

Footnote 185

Greely notes that "the "importance' of the parts - brains and gametes are more important than heart valves or skin - and the number of parts moved - transplanting five visceral organs would be more troubling than transplanting one - seem significant" to determining the humanity of human-animal chimera. Greely, supra note 24, at 19.

Footnote 186

Gertler, supra note 166, at 1069-70; Paliokas, supra note 155, at 226-29.

Footnote 187

Westphal, supra note 34.

Footnote 188

Bok, supra note 39, at 26.

Footnote 189

See supra notes 161-67 and accompanying text.

Footnote 190

See Smith, supra note 161, at 860 (arguing that "if neocortical functions - the capacity to think, feel, communicate, or experience our environment - are the key to human life, then the loss of neocortical functions should be the key to human death").

Footnote 191

The "moral threshold of human neural development we might set as the limit" on chimera research is presently undefined. Mott, supra note 15 (quoting William Cheshire, associate professor of neurology at the Mayo Clinic).

Footnote 192

Savulescu, supra note 51, at 23 ("Any attempt to base moral status on biology is fundamentally flawed. Genes, cells, organs, or bodies are not what matter intrinsically… . What is special about Homo sapiens compared to all other animals? The answer is not to be found in biology but in certain psychological characteristics."); see Joseph Fletcher, Humanness in Humanhood: Essays in Biomedical Ethics, in Barry R. Furrow et al., Bioethics: Health Care Law and Ethics 37, 37 (3d ed. 1997).

Footnote 193

See Robert & Baylis, supra note 43, at 4 (noting that when a large portion of human DNA is shared with other animals, "there is little (if any) uniquely human DNA").

Footnote 194

Savulescu, supra note 51, at 23-24.

Footnote 195

Id. at 23. Savulescu asserts that the hallmark of humanity may lie in our "practical rationality," the "capacity to make normative judgments, including moral judgments, and act on these" judgments. Id. at 24.

Footnote 196

Notions that humans are the sole possessors of many intellectual traits has been proven wrong by modern animal research. Eugene Linden, Can Animals Think?, Time, Mar. 22, 1993, at 54, 56. Linden

notes:

Since antiquity, philosophers have argued that higher mental abilities - in short, thinking and language - are the great divide separating humans from other species. The lesser creatures, Rene Descartes contended in 1637, are little more than automatons, sleepwalking through life without a mote of self-awareness… . Darwinism raised a series of tantalizing questions for future generations: If other vertebrates are similar to humans in blood and bone, should they not share other characteristics, including intelligence? Id.

Footnote 197

Sigmund, supra note 174, at 206.

Footnote 198

See id.

Footnote 199

Linden, supra note 196.

Footnote 200

Andrew W. Siegel, The Moral Insignificance of Crossing Species Boundaries, Am. J. Bioethics, Summer 2003, at 33, 34.

Footnote 201

Margaret MacDonald, Natural Rights, in Theories of Rights 21, 28 (Jeremy Waldon ed., 1984) (for Kant, "men share all other characteristics with the brutes … but reason was alike in all men, it was man's defining characteristic").

Footnote 202

Margit Livingston, Desecrating the Ark: Animal Abuse and the Law's Role in Prevention, 87 Iowa L. Rev. 1, 15 (2001). Under this view, the value of any nonhuman things resides only in its value to humans. Gregory Vlastos, Justice and Equality, in Theories of Rights, supra note 201, at 41, 55-56.

Footnote 203

Robert Nozick, Anarchy, State, and Utopia 32 (1974) (quoting Immanuel Kant, Groundwork of the Metaphysics of Morals 96 (H.J. Paton trans., 1956)).

Footnote 204

Emanuel Gross, Thwarting Terrorist Acts by Attacking the Perpetrators or Their Commanders as an Act of Self-Defense: Human Rights Versus the State's Duty to Protect Its Citizens, 15 Temp. Int'l & Comp. L.J. 195, 230 (2001).

Footnote 205

Robert & Baylis, supra note 43, at 5.

Footnote 206

See generally Linden, supra note 196.

Footnote 207

Robert & Baylis, supra note 43, at 5.

Footnote 208

Jacobellis v. Ohio, 378 U.S. 184, 197 (1964) (Stewart, J., concurring) ("I shall not today attempt further to define the kinds of material I understand to be embraced within that shorthand description [of hard-core pornography]; and perhaps I could never succeed in intelligibly doing so. But I know it when I see it.").

Footnote 209

See Robert & Baylis, supra note 43, at 5 (stating that "we all know a human when we see one, but, really, that is all that is known about our identity as a species").

Footnote 210

See Savulescu, supra note 51, at 23-24.

Footnote 211

See supra Part IV.A. See generally Robert & Baylis, supra note 43, at 5.

Footnote 212

The term speciesism refers to discrimination between species. Tom Regan, The Case For Animal Rights 155 n.3 (1983).

Footnote 213

Robert & Baylis, supra note 43, at 9. In describing the parallels between speciesism and racism, philosopher Rosalind Hursthouse states:

Racists think that, for instance, the death or enslavement of someone of their own race matters … . Similarly, it is said, a speciesist would be one who thought that the death or enslavement of a member of their own species mattered, but that the death or enslavement of a member of a different species, say an extraterrestrial person, did not, despite the (imagined) fact that the difference in species does not make for a difference in how much the two beings want to live or be free, or how worthwhile their lives might be. Rosalind Hursthouse, Beginning Lives 102-03 (1987).

Footnote 214

Clearly, humans routinely use animals in ways that would not be conscionable if animals possessed the same moral standing as humans. Robert & Baylis, supra note 43, at 6.

Footnote 215

See Dred Scott v. Sandford, 60 U.S. (19 How.) 393, 404-05 (1856) (noting that African slaves were considered a "subordinate and inferior class of beings" at the time that the Constitution was framed); Richard Kluger, Simple Justice 38 (1975) (stating that "north and south, he [the black man] was classified as a lower form of human life and therefore fair game for continual debasement"); Rachel E. Fishman, Patenting Human Beings: Do Sub-Human Creatures Deserve Constitutional Protection?, 15 Am. J.L. & Med. 461, 468-69 (1989) (stating that "early geneticists believed that qualities such as intelligence, industry and righteousness were linked to genetic endowment and would predict race, ethnicity, physical handicap and social class. The risks of racism have thus long been associated with genetic research"). But see David Wasserman, Species and Races, Chimeras, and Multiracial People, Am. J. Bioethics, Summer 2003, at 13, 13 (distinguishing racial classifications and arguing that moral lines between humans and chimera should be maintained).

Footnote 216

David Castle, Hopes Against Hopeful Monsters, Am. J. Bioethics, Summer 2003, at 28, 28; see Robert & Baylis, supra note 43, at 3 ("At present, there are somewhere between nine and twenty-two definitions of species in the biological literature. Of these, there is no one species concept that is universally compelling.").

Footnote 217

Robert & Baylis, supra note 43, at 2-4.

Footnote 218

Id. at 6. While animals are not afforded constitutional rights, society has recognized the special moral status of certain intelligent animals. For instance, there are strict limitations and requirements for primate research. Castle, supra note 216, at 29.

Footnote 219

Robert & Baylis, supra note 43, at 2.

Footnote 220

Id.

Footnote 221

Id. However, it is not clear that Robert and Baylis believe that this threat of moral ambiguity is sufficient to make it immoral or unethical to produce chimera. In response to Robert and Baylis, others have argued that affording special moral standing to certain chimera would not actually threaten society's moral structure. Siegel, supra note 200, at 34. This is because in the "extraordinary instance" where a chimera might exhibit high-level cognitive abilities, we would be able to accord it special respect without changing the firmly entrenched idea that human life is sacred and distinct from other animals. Id. The extent of this "special respect," however, is undetermined

because while humans do possess a framework for understanding our moral obligations to chimera, society still needs to determine which "properties are relevant to moral status" and "whether chimeras possess the relevant moral properties." Id.

Footnote 222

Gross, supra note 204, at 229.

Footnote 223

According to Kant, the morality of actions derives from an inherent moral duty, not a calculation of alternative consequences. Sigmund, supra note 174, at 161-62. Jeremy Bentham, a strict utilitarian, stated that the concept of "natural and imprescriptible rights" is "nonsense on stilts." Id. at 146.

Footnote 224

Siegel, supra note 200, at 34.

Footnote 225

Rivard, supra note 131, at 1477.

Footnote 226

Nozick, supra note 203, at 337 n.9 (citing Jeremy Bentham, An Introduction to the Principles of Morals and Legislation ch. 17, 1 n.1 (Haffner 1948) (1789)).

Footnote 227

Livingston, supra note 202, at 17.

Footnote 228

Rivard, supra note 131, at 1473-74.

Footnote 229

Id. at 1478-79.

Footnote 230

Robert & Baylis, supra note 43, at 5.

Footnote 231

It has been argued that a major problem with the utilitarian calculus is that is it relies on a "too narrow conception of good," downplaying the importance of fundamental rights. Nozick, supra note 203, at 28.

Footnote 232

For illustration, since human-animal chimera have the potential to be quite useful in medical research, there is a high utility in exploiting them in research. This increase in overall utility may swamp any utility loss suffered by individual chimera. This could thereby justify exploitation of these chimera from a strict utilitarian standpoint.

Footnote 233

Nozick, supra note 203, at 39.

Footnote 234

Id.

Footnote 235

Id.

Footnote 236

David Lyons, Utility and Rights, in Theory of Rights, supra note 201, at 110, 128.

Footnote 237

See Nozick, supra note 203, at 28.

Footnote 238

This second part of this approach uses the crucial tissues presented in Part IV.A.1 as a proxy for the inherent moral value of human life.

Footnote 239

See supra notes 128-39 and accompanying text.

Footnote 240

See Cirlin, supra note 104, at 508 (stating that "notwithstanding the controversy over the classification of chimera, the prospect of Constitutional protection for them is absurd. Chimera are not humans"); see also Alan R. Geraldi, Comment, In His Image: On Patenting Human-Based Bioproducts, 25 U.S.F. L. Rev. 583, 597 (1991) ("Because products of genetic research are changed from natural human tissue, then logically they can not be considered to be "human' for the purposes of the thirteenth amendment."). The hesitancy of commentators to accept the personhood of chimera may stem from the fact that "all of the persons we have met have been humans, and the overwhelming majority have been normal humans." Solum, supra note 141, at 1285.

Footnote 241

See supra notes 8-14 and accompanying text.

Footnote 242

See id.

Footnote 243

Magnani, supra note 35, at 446. Chimpanzees are the closest relative of humans. See, e.g., Roger Lewin, My Close Cousin the Chimpanzee, 238 Sci. 273 (1987).

Footnote 244

Weiss, supra note 1 (describing quail-chicken chimera research).

Footnote 245

The National Academies of Sciences report notes that the "idea that human neuronal cells might participate in "higher-order' brain functions in a nonhuman animal, however unlikely that may be, raises concerns that need to be considered." Guidelines, supra note 53, at 40.

Footnote 246

See supra note 39 and accompanying text.

Footnote 247

See Bok, supra note 39, at 25.

Footnote 248

See Wasserman, supra note 215, at 13 (discussing the one drop concept of human-animal chimera in comparison to racial classifications).

Footnote 249

See Rivard, supra note 131, at 1468 (arguing that "if members of another species are like humans in relevant ways, then it would be wrong to deny them constitutional personhood on the basis of irrelevant criteria such as genetic composition or appearance").

Footnote 250

It maybe irresponsible to leave this research unexamined and unchecked by the legal system, solely to whims of individual researchers. While human-animal chimera research certainly has promise, "endorsing scientific research simply because it is interesting and it might prove useful is a dangerous path … . Much "useful' information can be derived from experiments that are objectively evil. The ends, no matter how noble, cannot justify any and all possible means." Maureen L. Condic & Samuel B. Condic, The Appropriate Limits of Science in the Formation of Public Policy, 17 Notre Dame J.L. Ethics & Pub. Pol'y 157, 167-68 (2003) (emphasis removed).

Footnote 251

See Elizabeth L. DeCoux, In the Valley of the Dry Bones: Reuniting the Word "Standing" with Its

Meaning in Animal Cases, 29 Wm. & Mary Envtl. L & Pol'y Rev. 681, 761 (2005) ("Courts could suddenly find themselves having to scramble, unprepared, to weigh a scientist's assertion of the right to "head off' any pesky claims an human/animal chimera might make, against the claims of the chimera himself. In such a situation, courts would also have to contend with the multitude of amici that may ask to express their views, while the court tries to avoid such embarrassing computations as those that were used many decades ago to determine who was mulatto and who was not.").

Footnote 252

Demaine & Fellmeth, supra note 70, at 441 n.598.

Footnote 253

Magnani, supra note 35, at 443-45.

Footnote 254

See supra Part IV.A.1.

Footnote 255

See supra Part IV.A.2.

Footnote 256

Robert & Baylis, supra note 43, at 5.

Footnote 257

This Comment does not address whether animals or artificial intelligence computers should be granted constitutional personhood. At present they are not, and therefore the analytical framework for chimera personhood presented here attempts to maintain this status quo. For a discussion of computers and artificial intelligence, see Solum, supra note 141, and Goldberg, supra note 154. A purely cognitive approach does have the advantage of being able to better address future technology that might allow scientists to enhance the intelligence of animals or create artificial intelligence computers. Presently, however, higher-level human cognitive ability is only produced by the human brain tissue.

Footnote 258

The inclusion of human reproductive cells in human-animal chimera also raises serious moral and ethical concern. In a human-animal chimera, every cell is either human or animal. Therefore, the sperm or eggs produced by such an organism might be human. If two human-animal chimera were to breed, they might both produce human gametes and therefore potentially a fertilized human embryo. Weiss, supra note 1.

Footnote 259

See supra Part IV.

Footnote 260

See supra Part IV.

Footnote 261

See Fishman, supra note 215, at 478 ("To prevent the loss of legal rights of an altered human being who may no longer be found to be a member of the human species, it is imperative that the definition of "human being' be expanded … it is better to err on the side of generosity rather than parsimony when depriving a being of his or her legal rights."); Rivard, supra note 131, at 1509 ("If the average, mature member of a species has the capacity for self-awareness, then all members of that species are entitled to a rebuttable presumption of personhood. This theory may be used to solve the problem of constitutional personhood for nonhuman species.").

Footnote 262

A human-animal chimera is clearly partially biologically human. This highlights the fact that the personhood question has been divorced from biological reality. Robert & Baylis, supra note 43, at 7-9.

Footnote 263

Rivard, supra note 131, at 1488.

Footnote 264

Id.

Footnote 265

This analysis also presupposes identifiable species lines, a concept that loses meaning when different species are combined in a chimera. This problem could be avoided however by replacing a species by species approach with a chimera by chimera approach.

Footnote 266

Weiss, supra note 18.

Footnote 267

See Magnani, supra note 35, at 449-50 ("It is probably safe to say that any organism composed of over 50% human genetic material would be considered human. From a common sense standpoint, this standard seems reasonable enough, but it is somewhat simplistic and artificial.").

Footnote 268

See supra Part V.A.

Footnote 269

The Court has held that children are entitled to due process rights in juvenile proceedings, but implied that children are not offered the full panoply of due process protections. In re Gault, 387 U.S. 1, 13 (1967).

Footnote 270

In deciding that prisoners are not stripped of all constitutional protection by virtue of imprisonment, the Court stated:

Lawful imprisonment necessarily makes unavailable many rights and privileges of the ordinary citizen, a retraction justified by the considerations underlying our penal system. But though his rights may be diminished by the needs and exigencies of the institutional environment, a prisoner is not wholly stripped of constitutional protections when he is imprisoned for crime. Wolff v. McDonnell, 418 U.S. 539, 555 (1974) (citations and internal quotations omitted).

Footnote 271

See Mathews v. Diaz, 426 U.S. 67, 78 (1976) (stating that "the fact that all persons, aliens and citizens alike, are protected by the due process clause does not mean that all aliens are entitled to enjoy all the advantages of citizenship or that all aliens must be placed in a single homogenous legal classification … a legitimate distinction between citizens and aliens may justify attributes and benefits for one class not accorded to the other"); Johnson v. Eisentrager, 339 U.S. 763, 770 (1950) (stating that "mere lawful presence in the country creates an implied assurance of safe conduct and gives [the alien] certain rights; they become more extensive and secure when he makes [a] preliminary declaration of intention to become a citizen, and they expand to those of full citizenship upon naturalization").

Footnote 272

Burk, supra note 56, at 1655 ("Perhaps the most sensible observation that has been made … is that the dichotomy … that embryos are either property or persons, is a false choice. Embryos fit neither of these categories, but are something quite new, entitled to a category of their own… . Neither our present evaluation of biology nor our present categories should determine the status the law assigns to embryos.").

Footnote 273

This Comment acknowledges that any type of rights classification is suspect. However, the first and third categories, which grant full rights, collectively are broad enough to encompass all intelligent, sentient beings that have a substantial amount of crucial human tissue. The classifications are not based on superficial traits like skin color or the presence of a few animal cells; they derive from fundamental differences in cognitive capacity and the presence of human neural tissue. See Wasserman, supra note 215, at 13-14.

Footnote 274

See supra notes 246-51 and accompanying text.

Footnote 275

See id.

Footnote 276

It is assumed that such chimera cannot exhibit human cognitive traits.

Footnote 277

See supra Part IV.B.2 (discussing moral utility theory).

Footnote 278

See Fishman, supra note 215, at 478 ("It is preferable that the definition [of human being] be broad rather than narrow, as it is better to err on the side of generosity rather than parsimony when depriving a being of his or her legal rights.").

Footnote 279

Some commentators would strip a chimera of any constitutional protection if they "cannot perceive the interests underlying the Constitution." Rivard, supra note 131, at 1477. Such an approach does not give sufficient respect to creatures that are biologically very closely aligned to humans and deserve some degree of constitutional protection.

Footnote 280

This middle ground avoids a troubling application of a pure intelligence/sentience based cognitive definition of person under which scientists could create chimera with no human brain or nervous cells but with a very high total percentage of human cells without those creautures qualifying as persons. The fear that such creatures might be produced has led to calls to ban chimera research outright. See Cirlin, supra note 104, at 509-10.

Footnote 281

Such chimera might be referred to as "quasi-persons" or "organisms of special constitutional concern." Lebovitz applies the term "quasi-person" to frozen embryos and animals that cannot be properly classified as either persons or property. Lebovitz, supra note 129, at 563-64.

Footnote 282

See supra notes 228-32 and accompanying text discussing the shortcomings of pure moral utilitarianism. Animal rights theorists have argued for an analogous intermediate position in which animals would no longer be viewed as mere property. See Gary Francione, Animal Rights Theory and Utilitarianism: Relative Normative Guidance, 3 Animal L. 75, 100-01 (1997).

Footnote 283

See Harrison, The Anencephalic Newborn as Organ Donor, Hastings Center Rep., Apr. 1986, at 22 ("The possession of rights exists along a spectrum; while the comatose person may experience pain or even hunger, and may have rights based on such capacities, he may not have other capacities necessary to having other rights.").

Footnote 284

For a partial listing of the possible constitutional issues, see generally Adams, supra note 153.

Footnote 285

Due to the nature of such chimera, it is highly unlikely that they would have the ability to lobby

Congress or bring suit to enforce their constitutional rights. However, the same guardian mechanisms that protect young children and the mentally handicap could provide these chimera legal redress. Further, to avoid standing problems, Congress and the courts could allow private citizens to bring suits on behalf of such chimera to enforce their constitutional rights as has been done in other areas of the law. See, e.g., Am. Soc'y. for Prevention of Cruelty to Animals v. Ringling Bros. & Barnum & Bailey Circus, 317 F.3d 334, 335 (D.C. Cir. 2003) (holding that the plaintiff had standing due to his concern for the well-being of an elephant that he had seen abused by the defendant, and therefore could bring suit under the Endangered Species Act).

Footnote 286

See Cirlin, supra note 104, at 507 (noting that forcing human chimera to undergo a life of experimentation would be a form of slavery or indentured servitude in violation of the Thirteenth Amendment).

Footnote 287

As James P. Daniel stated, "the use of a person, in the form of a hybrid human, for medical research or any other use not voluntarily chosen by the person could violate that person's rights under substantive due process and the Equal Protection clause of the Constitution." Daniel, supra note 65, at 123.

Footnote 288

Weiss, supra note 18.

Footnote 289

Arguments have been made the mere production of genetically engineered persons might subject them to unconstitutional "genetic bondage." Kevin D. DeBre, Patents on People and the U.S. Constitution: Creating Slaves or Enslaving Science, 16 Hastings Const. L.Q. 221, 233 (1989). This is because the genetic engineer could preprogram the altered person and this would represent a type of subjection that would violate the Thirteenth Amendment. Id.; see also Andrea Wang, Regulating Human Cloning Within an Environmental Human Rights Framework, 12 Colo. J. Int'l Envtl. L. & Pol'y 165, 166-67 (2001).

Terms: **55 EMORY L. J. 347**
Search Type: **Citation**
Narrow by: **None**
Date and Time: **Monday, November 25, 2013 – 03:22 PM**

Robert and Baylis,

Crossing Species Boundaries,

THE AM. JOURNAL OF BIOETHICS, vol. 3, no. 3 (Summer 2003)

Target Article

Crossing Species Boundaries

Jason Scott Robert, Dalhousie University
Françoise Baylis, Dalhousie University

This paper critically examines the biology of species identity and the morality of crossing species boundaries in the context of emerging research that involves combining human and nonhuman animals at the genetic or cellular level. We begin with the notion of species identity, particularly focusing on the ostensible fixity of species boundaries, and we explore the general biological and philosophical problem of defining *species.* Against this backdrop, we survey and criticize earlier attempts to forbid crossing species boundaries in the creation of novel beings. We do not attempt to establish the immorality of crossing species boundaries, but we conclude with some thoughts about such crossings, alluding to the notion of moral confusion regarding social and ethical obligations to novel interspecies beings.

Keywords

genomics
chimeras
species
stem cells
hybrids
embryos
taboo
abomination

Open Peer Commentaries

Paul B. Thompson, p. 14
Bernard E. Rollin, p. 15
Henry T. Greely, p. 17
A. M. Chakrabarty, p. 20
Julian Savulescu, p. 22
Hilary Bok, p. 25
Linda MacDonald Glenn, p. 27
David Castle, p. 28
Mark Sagoff, p. 30
Rachel A. Ankeny, p. 31
Andrew W. Siegel, p. 33
David B. Resnik, p. 35
Robert Streiffer, p. 37
Louis C. Charland, ✉ 1
Cynthia B. Cohen, ✉ 3
Josephine Johnston and Christopher Eliot, ✉ 6
Lee L. Zwanziger, ✉ 9
Daniel B. McGee, ✉ 11
David Wasserman, ✉ 13
Nao R. Kobayashi, ✉ 15
Phillip Karpowicz, ✉ 17
Kimberly A. Urie, Alison Stanley, and Jerold D. Friedman, ✉ 20
Sarah K. Brem and Karen Z. Anijar, ✉ 22
Sarah Franklin, ✉ 25

Introduction

Crossing species boundaries in weird and wondrous ways has long interested the scientific community but has only recently captured the popular imagination beyond the realm of science fiction. Consider, for instance, the print and pictorial publicity surrounding the growth of a human ear on the back of a mouse;[1] the plight of Alba, artist Eduardo Kac's green-fluorescent-protein bunny stranded in Paris;[2] the birth announcement in *Nature* of ANDi, the first transgenic primate;[3] and, most recently, the growth of pigs' teeth in rat intestines[4] and miniature human kidneys in mice.[5]

But, bizarrely, these innovations that focus on discrete functions and organs are almost passé. As part of the project of harnessing the therapeutic potential of human stem cell research, researchers are now involved in creating novel interspecies whole organisms that are unique cellular and genetic admixtures (DeWitt 2002). A human-to-animal embryonic chimera is a being produced through the addition of human cellular material (such as pluripotent or restricted stem cells) to a nonhuman blastocyst or embryo. To give but four examples of relevant works in progress, Snyder and colleagues at Harvard have transplanted human neural stem cells into the forebrain of a developing bonnet monkey in order to assess stem cell function in development (Ourednik et al. 2001); human embryonic stem cells have been inserted into young chick embryos by Benvenisty and colleagues at the Hebrew University of Jerusalem (Goldstein et al. 2002); and most recently it has been reported that human genetic material has been transferred into rabbit eggs by Sheng (Dennis 2002), while Weissman and colleagues at Stanford University and StemCells, Inc., have created a mouse with a significant proportion of human stem cells in its brain (Krieger 2002).

Human-to-animal embryonic chimeras are only one sort of novel creature currently being produced or contemplated. Others include: *human-to-animal fetal or adult chimeras* created by grafting human cellular material to late-stage nonhuman fetuses or to postnatal nonhuman creatures; *human-to-human embryonic, fetal, or adult chimeras* created by inserting or grafting exogenous human cellular material to human embryos, fetuses, or adults (e.g., the human recipient of a human organ transplant, or human stem cell therapy); *animal-to-human embryonic, fetal, or adult chimeras* created by inserting or grafting nonhuman cellular material to human embryos, fetuses, or adults (e.g., the recipient of a xenotransplant); *animal-to-animal embryonic, fetal, or adult chimeras* generated from nonhuman cellular material whether within or between species (excepting hu-

1. See, e.g., Mooney and Mikos (1999); and the *Scientific American Frontiers* coverage of "The Bionic Body," available from: http://www.pbs.org/saf/1107/features/body.htm. See also Bianco and Robey (2001).

2. See the bibliography of media coverage at http://www.ekac.org/transartbiblio.html.

3. A sample headline from *The Independent* (London): "How a Glowing Monkey Will Help Cure Disease" (12 January 2001; available from: http://www.independent.co.uk/story.jsp?story=49841). See also Chan et al. (2001); and Harris (2001).

4. "Scientists Grow Pig Teeth in Rat Intestines" is available from: http://www.laurushealth.com/HealthNews/reuters/NewsStory0926200224.htm. See also Young et al. (2002).

5. "Human Kidneys Grown in Mice" is available from: http://news.bbc.co.uk/2/hi/health/2595397.stm. See also Dekel et al. (2003).

man beings); *nuclear-cytoplasmic hybrids,* the offspring of two animals of different species, created by inserting a nucleus into an enucleated ovum (these might be intraspecies, such as sheep-sheep; or interspecies, such as sheep-goat; and, if interspecies, might be created with human or nonhuman material); *interspecies hybrids* created by fertilizing an ovum from an animal of one species with a sperm from an animal of another (e.g., a mule, the offspring of a he-ass and a mare); and *transgenic organisms* created by otherwise combining genetic material across species boundaries.

For this paper, in which we elucidate and explore the concept of species identity and the ethics of crossing species boundaries, we focus narrowly on the creation of interspecies chimeras involving human cellular material—the most recent of the transgressive interspecies creations. Our primary focus is on human-to-animal *embryonic* chimeras, about which there is scant ethical literature, though the scientific literature is burgeoning.

Is there anything ethically wrong with research that involves the creation of human-to-animal embryonic chimeras? A number of scientists answer this question with a resounding "no." They argue, plausibly, that human stem cell proliferation, (trans)differentiation, and tumorigenicity must be studied in early embryonic environments. For obvious ethical reasons, such research cannot be carried out in human embryos. Thus, assuming the research must be done, it must be done in nonhuman embryos—thereby creating human-to-animal embryonic chimeras. Other scientists are less sanguine about the merits of such research. Along with numerous commentators, they are quite sensitive to the ethical conundrum posed by the creation of certain novel beings from human cellular material, and their reaction to such research tends to be ethically and emotionally charged. But what grounds this response to the creation of certain kinds of part-human beings? In this paper we make a first pass at answering this question. We critically examine what we take to be the underlying worries about crossing species boundaries by referring to the creation of certain kinds of novel beings involving human cellular or genetic material. In turn, we highlight the limitations of each of these arguments. We then briefly hint at an alternative objection to the creation of certain novel beings that presumes a strong desire to avoid introducing moral confusion as regards the moral status of the novel being. In particular we explore the strong interest in avoiding any practice that would lead us to doubt the claim that humanness is a necessary (if not sufficient) condition for full moral standing.

Species Identity

Despite significant scientific unease with the notion of *species identity,* commonplace among biologists and commentators are the assumption that species have particular identities and the belief that the boundaries between species are fixed rather than fluid, established by nature rather than by social negotiation. Witness the ease with which biologists claim that a genome sequence of some organism—yeast, worm, human—represents the identity of that species, its blueprint or, alternatively, instruction set. As we argue below, such claims mask deep conceptual difficulties regarding the relationship between these putatively representative species-specific genomes and the individual members of a species.

The ideas that natural barriers exist between divergent species and that scientists might someday be able to cross such boundaries experimentally fuelled debates in the 1960s and 1970s about the use of recombinant DNA technology (e.g., Krimsky 1982). There were those who anticipated the possibility of research involving the crossing of species boundaries and who considered this a laudable scientific goal. They tried to show that fixed species identities and fixed boundaries between species are illusory. In contrast, those most critical of crossing species boundaries argued that there were fixed natural boundaries between species that should not be breached.

At present the prevailing view appears to be that species identity is fixed and that species boundaries are inappropriate objects of human transgression. The idea of fixed species identities and boundaries is an odd one, though, inasmuch as the creation of plant-to-plant[6] and animal-to-animal hybrids, either artificially or in nature, does not foster such a vehement response as the prospective creation of interspecies combinations involving human beings—no one sees rhododendrons or mules (or for that matter goat-sheep, or geep) as particularly monstrous (Dixon 1984). This suggests that the only species whose identity is gener-

6. A possible exception is the creation of genetically modified crops. But here the arguments are based on human health and safety concerns, as well as on political opposition to monopolistic business practices, rather than on concern for the essential identity of plant species.

ally deemed genuinely "fixed" is the human species. But, what is a *species* such that protecting its identity should be perceived by some to be a scientific, political, or moral imperative? This and similar questions about the nature of species and of species identities are important to address in the context of genetics and genomics research (Ereshefsky 1992; Claridge, Dawah, and Wilson 1997; Wilson 1999b).

Human beings (and perhaps other creatures) intuitively recognize species in the world, and cross-cultural comparative research suggests that people around the globe tend to carve up the natural world in significantly similar ways (Atran 1999). There is, however, no one authoritative definition of species. Biologists typically make do with a plurality of species concepts, invoking one or the other depending on the particular explanatory or investigative context.

One stock conception, propounded by Dobzhansky (1950) and Mayr (1940), among others, is the *biological species concept* according to which species are defined in terms of reproductive isolation, or lack of genetic exchange. On this view, if two populations of creatures do not successfully interbreed, then they belong to different species. But the apparent elegance and simplicity of this definition masks some important constraints: for instance, it applies only to those species that reproduce sexually (a tiny fraction of all species); moreover, its exclusive emphasis on interbreeding generates counterintuitive results, such as the suggestion that morphologically indistinguishable individuals who happen to live in neighboring regions but also happen never to interbreed should be deemed members of different species. (Imagine viewing populations of human beings "reproductively isolated" by religious intolerance as members of different species, and the biological species concept fails to pick out *Homo sapiens* as a discrete species comprising all human beings.)

Such results can be avoided by invoking other definitions of species, such as the *evolutionary species concept* advanced by G. G. Simpson and E. O. Wiley, which emphasizes continuity of populations over geological time: "a species is a single lineage of ancestral descendant populations of organisms which maintains its identity from other such lineages and which has its own evolutionary tendencies and historical fate" (Wiley 1978, 18; see also Simpson 1961). Unlike the biological species concept, this definition of species applies to both sexually and asexually reproducing creatures and also underscores shared ancestry and historical fate—and not merely capacity to interbreed—as what unifies a group of creatures as a species. The evolutionary species concept is by no means unproblematic, however, mainly because it is considerably more vague than the biological species concept, and so also considerably more difficult to operationalize.

A third approach to defining species has lately received considerable attention among philosophers of biology. This approach is known as the *homeostatic property cluster* view of species, advocated in different ways by Boyd (1999), Griffiths (1999), and Wilson (1999a). Following Wilson (1999a, 197–99) in particular, the homeostatic property cluster view of species is properly understood as a thesis about natural kinds, of which a species is an instance. The basic idea is that a species is characterized by a cluster of properties (traits, say) no one of which, and no specific set of which, must be exhibited by any individual member of that species, but some set of which must be possessed by all individual members of that species. To say that these property clusters are "homeostatic" is to say that their clustering together is a systematic function of some causal mechanism or process; that an individual possesses any one of the properties in the property cluster significantly increases the probability that this individual will also possess other properties in the cluster. So the list of distinguishing traits is a property cluster, wherein the properties cluster as a function of the causal structure of the biological world. Of course, an outstanding problem remains, namely that of establishing the list of traits that differentiate species one from the other. Presumably this would be achieved by focusing on reproductive, morphological, genealogical, genetic, behavioral, and ecological features, no one of which is necessarily a universal property of the species and no set of which constitutes a species essence. We return below to the homeostatic property cluster view of species when we consider how best to characterize *Homo sapiens.*

To these definitions of species many more can be added: at present, there are somewhere between nine and twenty-two definitions of species in the biological literature.[7] Of these, there is no one species concept that is universally compelling. Accordingly, rather than asking the generic question, "How is 'species' defined?" it might be useful to fo-

7. Kitcher (1984) and Hull (1999) each discuss nine concepts. Mayden (1997) discusses twenty-two.

cus instead on the narrower question "How is a species defined?" In response to the latter question Williams (1992) proposes that a species be characterized by a description comprising a set of traits differentiating that species from all others. It is no small task, however, to devise a satisfactory species description for any particular group of beings. Take, for example, *Homo sapiens.* Significantly, not even a complete sequence of *the* human genome can tell us what particular set of traits of *Homo sapiens* distinguishes human beings from all other species.

When molecular biologists first talked about mapping and sequencing the human genome, their goal was to construct the sequence of nucleotides in all the genes in all the chromosomes in the normal human body. The sequence was meant to serve as a reference point to which individual genomes could be compared in efforts to locate deviant genes implicated in phenotypic variation. As well, the sequence was meant to facilitate the study of gene function in development (often in comparison with the consensus genomes of organisms belonging to other species) and to establish historical relationships among organisms.

Two draft sequences of a "standard" or "typical" human genome were published in 2001, one produced under the auspices of the publicly-funded Human Genome Project (HGP), the other by Celera Genomics. The HGP's official genome is a composite of genetic information from tens or hundreds of human individuals, while Celera Genomics' official genome is a composite of genetic information from five individuals (but principally Craig Venter, Celera's former president; Wade 2002). The sequences are nonetheless supposed to be 99.9% identical to individual human genomes, and that 0.1% variation, in concert with environmental variations, is supposed to explain the immense diversity among human beings (for a recent statement of this position, see Plomin et al. 2002). But, excepting identical twins, every human genome is different from every other. Further, while one's maternal DNA may differ by 0.1% from one's paternal DNA, and one's own DNA may differ from that of any other individual by 0.1%, it is not the case that there is a certain part of an individual's genome that is 99.9% identical with every other human's genome. Although human beings might share 99.9% commonality at the genetic level, there is nothing as yet identifiable as *absolutely* common to all human beings. According to current biology, there is no genetic lowest common denominator, no genetic essence, "no single, standard, "normal" DNA sequence that we all share" (Lewontin 1992, 36). The only way to determine how common the standard sequences are is to compare them with the actual sequences of a large number of individuals in an effort to detect conserved portions and polymorphisms; no one, though, is proposing such an endeavor. Even so, there is no way in which a single genome—not even Craig Venter's—can *represent* the immense genetic variability characteristic of *Homo sapiens* (Tauber and Sarkar 1992; Lloyd 1994; Robert 1998).

Moreover, comparative genomic research has thus far been of no help in establishing the boundary of human species identity. Much of "our" DNA is shared with a huge variety of apparently distantly related creatures (e.g., yeast, worms, mice). Indeed, given the evidence that all living things share a common ancestor, there is little (if any) uniquely human DNA.[8] More strikingly perhaps, though human beings are morphologically and behaviorally vastly different from chimpanzees, we differ genomically from chimps by no more than 1.2–1.6% (Allen 1997; Marks 2002; Enard et al. 2002; Olson and Varki 2003). Further, the surprisingly small number of genes in the sequenced human genome, as compared to original estimates, offers a serious blow to the idea of human uniqueness at the genomic level (Claverie 2001). Finally, there is no comfort to be found in the assessment that a tiny number of physical, chemical, genetic, and developmental accidents made human history possible. In sum, even though biologists are able to identify a particular string of nucleotides as human (as distinct from, say, yeast or even chimpanzee), the unique identity of the human species cannot be established through genetic or genomic means.

What Is Homo sapiens?

What, then, is *Homo sapiens?* Though clearly there is no one authoritative definition of species, notions of "species essences" and "universal properties of species" persist, always in spirit if not always in name, in discussions about breaching species boundaries. For this reason, on occasion, attempts to define *Homo sapiens* are reduced to attempts to define *human nature.* This is a problem, however, insofar as the literature exhibits a wide range of

8. In fact, through studies in comparative genomics biologists have demonstrated horizontal transfer of genes between lineages, suggesting a remarkable fluidity of species "boundaries" at the genomic level. Some of this literature is reviewed in Doolittle (1999).

opinion on the nature of *human nature;* indeed, many of the competing conceptions of *human nature* are incommensurable (for a historical sampling of views, see Trigg 1988). On one view the claim that there is such a thing as human nature is meant to be interpreted as the claim that all members of *Homo sapiens* are essentially the same. But since everything about evolution points toward variability and not essential sameness, this would appear to be an inherently problematic claim about human nature (Hull 1986). One way of avoiding this result is to insist that talk of human nature is not about essential sameness but rather about universality and then to explain universality in terms of distinct biological attributes—a functional human nervous system, a human anatomical structure and physiological function, or a human genome (Campbell, Glass, and Charland 1998). A classic example of the latter strategy, explaining universality genetically, appears in an article on human nature by Eisenberg (1972), who writes that "one trait common to man everywhere is language; in the sense that only the human species displays it, the capacity to acquire language must be genetic" (126).[9] In this brief passage Eisenberg moves from the claim that language is a human universal, to the claim that the ability to have a language is unique and species specific, to the claim that this capacity is genetic (Hull 1986). But, of course, language is not a human universal—some human beings neither speak nor write a language, and some are born with no capacity whatsoever for language acquisition. Yet, in a contemporary context, no one would argue that these people, simply by virtue of being nonverbal and/or illiterate, are not members of the same species as the rest of us.[10]

And therein lies the rub. We all know a human when we see one, but, really, that is all that is known about our identity as a species. Of course we all know that human beings are intelligent, sentient, emotionally-complex creatures. We all know the same of dolphins, though. And, of course, not all human beings are intelligent, sentient, or emotionally complex (for instance, those who are comatose); nevertheless, most among us would still consider them human.

9. Other examples are everywhere to be found in commentaries on the human genome project.

10. And even were language a human universal *par excellence,* there is simply no basis for the assumption that invariability (universality) and genetics must be connected. See Hull (1986); and Oyama (2000).

The homeostatic property cluster approach to species avoids the problem of universality but at the possible expense of retaining an element of essentialism. Recall that, according to the homeostatic property cluster view, membership in a species is not determined by possession of *any particular* individual homeostatically clustered property (or *any particular sets* of them) but rather by possession of *some* set of homeostatically clustered properties. Nevertheless, although possession of property *x* (or of property set *x*-*y*-*z*) is not *necessary* for species membership, possession of *all* the identified homeostatically clustered properties is *sufficient* for membership, which suggests that a hint of essentialism persists (Wilson 1999a).

This is an ironic result, inasmuch as essentialism in biology is vanishingly rare. This is because essentialism—or at least stock conceptions of essentialism according to which a species is identified by essential intrinsic properties—is at odds with evolutionary biology.[11] Significantly, commentators of all stripes tend to revert to essentialist thinking when pondering the locus of humanity. This might be because of a persistent folk essentialism, reflecting "a way of thinking about living systems whose continuing grip on us is explained by the fact that it develops long before we are exposed to scientific biology" (Griffiths 2002, 77). It might also be because the very idea of a "locus of humanity" is always already an essentialist idea.

Moral Unrest with Crossing Species Boundaries[12]

As the above discussion of species identity makes clear, there is no consensus on what exactly is being

11. This case is usually made in terms of Mayr's distinction between (non-Darwinian) typological thinking and (Darwinian) population thinking (Mayr 1959). For a useful account of Mayr's distinction, see Sober (1980). Griffiths (1999) attempts to resurrect an alternative account of essentialism compatible with Darwinism, wherein he deals not with intrinsic essential properties but rather extrinsic (relational) ones. We will not discuss this effort here, nor will we address the view that typological thinking has an important role to play in contemporary evolutionary biology in approaching the evolution of form (Love 2003).

12. Given our suggestion that the notion of species boundaries is problematic, at least biologically speaking, it might seem odd for us to continue using the language of "crossing species boundaries." We offer two defenses: first, the language is commonly used, especially to capture some sort of moral demarcation line (see below); second, we intend the notion, biologically, in a limited sense. Consider any individual human. That individual human contains a genome, a specifically human genome; call this genome H. Next, con-

breached with the creation of interspecies beings. As against what was once commonly presumed, there would appear to be no such thing as fixed species identities. This fact of biology, however, in no way undermines the reality that fixed species exist independently as moral constructs. That is, notwithstanding the claim that biologically species are fluid, people believe that species identities and boundaries are indeed fixed and in fact make everyday moral decisions on the basis of this belief. (There is here an analogy to the recent debate around the concept of race. It is argued that race is a biologically meaningless category, and yet this in no way undermines the reality that fixed races exist independently as social constructs and they continue to function, for good or, more likely, ill, as a moral category.) This gap between science and morality requires critical attention.

Scientifically, there might be no such thing as fixed species identities or boundaries. Morally, however, we rely on the notion of fixed species identities and boundaries in the way we live our lives and treat other creatures, whether in decisions about what we eat or what we patent. Interestingly, there is dramatically little appreciation of this tension in the literature, leading us to suspect that (secular) concern over breaching species boundaries is in fact concern about something else, something that has been mistakenly characterized in the essentialist terms surveyed above. But, in a sense, this is to be expected. While a major impact of the human genome project has been to show us quite clearly how similar we human beings are to each other and to other species, the fact remains that human beings are much more than DNA and moreover, as we have witnessed throughout the ages, membership within the human community depends on more than DNA. Consider, for example, the not-so-distant past in which individual human beings of a certain race, creed, gender, or sexual orientation were denied moral standing as members of the human community. By appealing to our common humanity, ethical analysis and social activism helped to identify and redress what are now widely seen as past wrongs.

Although in our recent history we have been able to broaden our understanding of what counts as human, it would appear that the possible permeability of species boundaries is not open to public debate insofar as novel part-human beings are concerned. Indeed, the standard public-policy response to any possible breach of human species boundaries is to reflexively introduce moratoriums and prohibitions.[13]

But why should this be so? Indeed, why should there be *any* ethical debate about the prospect of crossing species boundaries between human and nonhuman animals? After all, hybrids occur naturally, and there is a significant amount of gene flow between species in nature.[14] Moreover, there is as yet no adequate biological (or moral) account of the distinctiveness of the species *Homo sapiens* serving to capture all and only those creatures of human beings born. As we have seen, neither essentialism (essential sameness, genetic or otherwise) nor universality can function as appropriate guides in establishing the unique identity of *Homo sapiens.* Consequently, no extant species concept justifies the erection of the fixed boundaries between human beings and nonhumans that are required to make breaching those boundaries morally problematic.[15] Despite this, belief in a fixed, unique,

sider some nonhuman animal, or even a plant; call this genome not-H. Next, consider the application of standard genetic manipulation techniques to isolate a particular functional stretch of DNA from this specific not-H genome. Finally, consider the application of standard gene transfer techniques to insert (across "species boundaries," as we here understand the term) the gene from not-H into H, via the germ line. Some of the offspring of the bearer of genome H would thereafter contain genomes in which the gene from not-H appears. The bearer of H and her/his offspring would thus be interspecies beings (in the limited biological sense intended).

13. See, for example, s6(2)(b) Infertility (Medical Procedures) Act 1984 (Victoria, Australia); s3(2)(a)–(b) and s3(3)(b) Human Fertilisation and Embryology Act 1990 (United Kingdom); and Article 25 Bill containing rules relating to the use of gametes and embryos (Embryo Bill), September 2000 (the Netherlands). See also Annas, Andrews, and Isasi (2002).

14. A particularly well-documented example of gene flow between species is Darwin's finches in the Galapagos Islands. For a recent account, see Grant and Grant (2002).

15. A possible objection is that the biological species concept could in fact do the required work: human beings do not successfully interbreed with mice or moose, and so the boundary is established. We do not find the objection compelling. Whether human beings can in fact successfully interbreed with mice or moose is an open empirical question; while it does not happen in nature, it might happen artificially in the ways noted at the outset of this paper. The artificiality of such reproduction does not render it of a different kind, though. Human beings requiring reproductive technologies in order to breed are nonetheless human, they nonetheless reproduce, and they nonetheless generate offspring who are unquestionably human. So, the biological species concept cannot be used to discount the potential artificial creation of hybrids or of chimeras as a matter of breaching fixed species boundaries.

human species identity persists, as do moral objections to any attempt to cross the human species boundary—whatever that might be.

According to some, crossing species boundaries is about human beings playing God and in so doing challenging the very existence of God as infallible, all-powerful, and all-knowing. There are, for instance, those who believe that God is perfect and so too are all His creations. This view, coupled with the religious doctrine that the world is complete, suggests that our world is perfect. In turn, perfection requires that our world already contains all possible creatures. The creation of new creatures—hybrids or chimeras—would confirm that there are possible creatures that are not currently found in the world, in which case "the world cannot be perfect; therefore God, who made the world, cannot be perfect; but God, by definition is perfect; therefore God could not exist" (Morriss 1997, 279).[16] This view of the world, as perfect and complete, grounds one sort of opposition to the creation of human-to-animal chimeras.

As it happens, however, many do not believe in such a God and so do not believe it is wrong to "play God." Indeed, some would argue further that not only is it *not* wrong to play God, but rather this is exactly what God enjoins us to do. Proponents of this view maintain that God "left the world in a state of imperfection so that we become His partners"—his co-creators (Breitowitz 2002, 327).

Others maintain that combining human genes or cells with those of nonhuman animals is not so much about challenging God's existence, knowledge, or power, as it is about recognizing this activity as inherently unnatural, perverse, and so offensive. Here the underlying philosophy is one of repugnance. To quote Kass (1998), repugnance

> revolts against the excesses of human wilfulness, warning us not to transgress what is unspeakably profound. Indeed in this age in which . . . our given human nature no longer commands respect . . . repugnance may be the only voice left that speaks up to defend the central core of humanity. (19)

For many, the mainstay of the argument against transgressing species "boundaries" is a widely felt reaction of "instinctive hostility" (Harris 1998, 177) commonly known as the "yuck factor." But in important respects repugnance is an inchoate emotive objection to the creation of novel beings that requires considerable defense. If claims about repugnance are to have any moral force, the intuitions captured by the "yuck" response must be clarified. In the debate about the ethics of creating novel beings that are part human, it is not enough to register one's intuitions. Rather, we need to be able to clearly identify and critically examine these intuitions, recognizing all the while that they derive "from antecedent commitment to categories that are themselves subject to dispute" (Stout 2001, 158).

A plausible "thin" explanation for the intuitive "yuck" response is that the creation of interspecies creatures from human materials evokes the idea of bestiality—an act widely regarded as a moral abomination because of its degrading character. Sexual intimacy between human and nonhuman animals typically is prohibited in law and custom, and some, no doubt, reason from the prohibition on the erotic mixing of human and nonhuman animals to a prohibition on the biotechnological mixing of human and nonhuman cellular or genetic material. There are important differences, however. In the first instance the revulsion is directed toward the shepherd who lusts after his flock and acts in a way that makes him seem (or actually be) less human (Stout 2001, 152). In the second instance the revulsion is with the purposeful creation of a being that is neither uncontroversially human nor uncontroversially nonhuman.

A more robust explanation for the instinctive and intense revulsion at the creation of human-to-animal beings (and perhaps some animal-to-human beings) can be drawn from Douglas's work on taboos (1966). Douglas suggests that taboos stem from conceptual boundaries. Human beings attach considerable symbolic importance to classificatory systems and actively shun anomalous practices that threaten cherished conceptual boundaries. This explains the existence of well-entrenched taboos, in a number of domains, against mixing things from distinct categories or having objects/actions fall outside any established classification system. Classic examples include the Western response to bisexuality (you can't be both heterosexual and homosexual) and intersexuality. Intersexuality falls outside the "legitimate" (and exclusive) categories of male and female, and for this reason intersex persons have been carved to fit into the existing categories (Dreger 2000). Human-to-animal chimeras, for instance, are neither clearly animal nor clearly human. They obscure the classification system (and concomitant social structure) in such a way as to constitute an unacceptable threat to valu-

16. Note that Morriss does not subscribe to such a position.

able and valued conceptual, social, and moral boundaries that set human beings apart from all other creatures. Following Stout, who follows Douglas, we might thus consider human-to-animal chimeras to be an abomination. They are anomalous in that they "combine characteristics uniquely identified with separate kinds of things, or at least fail to fall unambiguously into any recognized class." Moreover, the anomaly is loaded with social significance in that interspecies hybrids and chimeras made with human materials "straddle the line between *us* and *them*" (Stout 2001, 148). As such, these beings threaten our social identity, our unambiguous status as human beings.

But what makes for unambiguous humanness? Where is the sharp line that makes for the transgression, the abomination? According to Stout, the line must be both sharp and socially significant if trespassing across it is to generate a sense of abomination: "An abomination, then, is anomalous or ambiguous with respect to some system of concepts. And the repugnance it causes depends on such factors as the presence, sharpness, and social significance of conceptual distinctions" (Stout 2001, 148). As we have seen, though, there is no biological sharp line: we have no biological account of unambiguous humanness, whether in terms of necessary and sufficient conditions or of homeostatic property clusters. Thus it would appear that in this instance abomination is a social and moral construct.

Transformative technologies, such as those involved in creating interspecies beings from human material, threaten to break down the social dividing line between human beings and nonhumans. Any offspring generated through the pairing of two human beings is by natural necessity—reproductive, genetic, and developmental necessity—a human. But biology now offers the prospect of generating offspring through less usual means; for instance, by transferring nuclear DNA from one cell into an enucleated egg. Where the nuclear DNA and the enucleated egg (with its mitochondrial DNA) derive from organisms of different species, the potential emerges to create an interspecies nuclear-cytoplasmic hybrid.

In 1998 the American firm Advanced Cell Technology (ACT) disclosed that it had created a hybrid embryo by fusing human nuclei with enucleated cow oocytes. The goal of the research was to create and isolate human embryonic stem cells. But if the technology actually works (and there is some doubt about this) there would be the potential to create animal-human hybrids (ACT 1998; Marshall 1998; Wade 1998). Any being created in this way would have DNA 99% identical with that of the adult from whom the human nucleus was taken; the remaining 1% of DNA (i.e., mitochondrial DNA) would come from the enucleated animal oocyte. Is the hybrid thus created simply part-human and part-nonhuman animal? Or is it unequivocally human or unequivocally animal (see Loike and Tendler 2002)? These are neither spurious nor trivial questions. Consider, for example, the relatively recent practice in the United States of classifying octoroons (persons with one-eighth negro blood; the offspring of a quadroon and a white person) as black. By analogy, perhaps 1% animal DNA (i.e., mitochondrial DNA) makes for an animal.[17]

A more complicated creature to classify would be a human-to-animal chimera created by adding human stem cells to a nonhuman animal embryo. It has recently been suggested that human stem cells should be injected into mice embryos (blastocysts) to test their pluripotency (Dewitt 2002). If the cells were to survive and were indeed pluripotent, they could contribute to the formation of every tissue. Any animal born following this research would be a chimera—a being with a mixture of (at least) two kinds of cells. Or, according to others, it would be just a mouse with a few human cells. But what if those cells are in the brain, or the gonads (Weissman 2002)? What if the chimeric mouse has human sperm? And what if that mouse were to mate with a chimeric mouse with human eggs?

All of this to say that when faced with the prospect of not knowing whether a creature before us is human and therefore entitled to all of the rights typically conferred on human beings, we are, as a people, baffled.

One could argue further that we are not only baffled but indeed fearful. Hybrids and chimeras made from human beings represent a metaphysical threat to our self-image. This fear can be explained in both historical and contemporary terms. Until the end of the eighteenth century the dominant Western worldview rested on the idea of the Great Chain of Being. The world was believed to be an ordered and hierarchical place with God at the top, followed by angels, human beings, and various classes of animals on down through to plants and

17. Mitochondrial DNA is not insignificant DNA. Like nuclear DNA it codes for functions.

other lesser living matter (Lovejoy 1970; see also Morriss 1997). On this worldview human beings occupied a privileged place between the angels and all nonhuman animals. In more recent times, though the idea of the Great Chain of Being has crumbled, the reigning worldview is still that human beings are superior to animals by virtue of the human capacity for reason and language. Hybrids and chimeras made from human materials blur the fragile boundary between human beings and "unreasoning animals," particularly when one considers the possibility of creating "reasoning" nonhuman animals (Krieger 2002). But is protecting one's privileged place in the world solid grounds on which to claim that hybrid- or chimera-making is intrinsically or even instrumentally unethical?

Moral Confusion

Taking into consideration the conceptual morass of species-talk, the lack of consensus about the existence of God and His role in Creation, healthy skepticism about the "yuck" response, and confusion and fear about obscuring, blurring, or breaching boundaries, the question remains as to why there should be any ethical debate over crossing species boundaries. We offer the following musings as the beginnings of a plausible answer, the moral weight of which is yet to be assessed.

All things considered, the engineering of creatures that are part human and part nonhuman animal is objectionable because the existence of such beings would introduce inexorable moral confusion in our existing relationships with nonhuman animals and in our future relationships with part-human hybrids and chimeras. The moral status of nonhuman animals, unlike that of human beings, invariably depends in part on features other than species membership, such as the intention with which the animal came into being. With human beings the intention with which one is created is irrelevant to one's moral status. In principle it does not matter whether one is created as an heir, a future companion to an aging parent, a sibling for an only child, or a possible tissue donor for a family member. In the case of human beings, moral status is categorical insofar as humanness is generally considered a necessary condition for moral standing. In the case of nonhuman animals, though, moral status is contingent on the will of regnant human beings. There are different moral obligations, dependent on social convention, that govern our behavior toward individual nonhuman animals depending upon whether they are bred or captured for food (e.g., cattle), for labor (e.g., oxen for subsistence farming), for research (e.g., lab animals), for sport (e.g., hunting), for companionship (e.g., pets), for investment (e.g., breeding and racing), for education (e.g., zoo animals), or whether they are simply cohabitants of this planet. In addition, further moral distinctions are sometimes drawn between "higher" and "lower" animals, cute and ugly animals, useful animals and pests, all of which add to the complexity of human relationships with nonhuman animals.

These two frameworks for attributing moral status are clearly incommensurable. One framework relies almost exclusively on species membership in *Homo sapiens* as such, while the other relies primarily on the will and intention of powerful "others" who claim and exercise the right to confer moral status on themselves and other creatures. For example, though some (including ourselves) will argue that the biological term *human* should not be conflated with the moral term *person,* others will insist that all human beings have an inviolable moral right to life simply by virtue of being human. In sharp contrast, a nonhuman animal's "right to life" depends entirely upon the will of some or many human beings, and this determination typically will be informed by myriad considerations.

It follows that hybrids and chimeras made from human materials are threatening insofar as there is no clear way of understanding (or even imagining) our moral obligations to these beings—which is hardly surprising given that we are still debating our moral obligations to some among us who are undeniably biologically human, as well as our moral obligations to a range of nonhuman animals. If we breach the clear (but fragile) *moral* demarcation line between human and nonhuman animals, the ramifications are considerable, not only in terms of sorting out our obligations to these new beings but also in terms of having to revisit some of our current patterns of behavior toward certain human and nonhuman animals.[18] As others have observed (e.g., Thomas 1983), the separateness of humanity is precarious and easily lost; hence the need for tightly guarded boundaries.

18. Animal-rights advocates might object to the creation of part-human hybrids on the grounds that this constitutes inappropriate treatment of animals solely to further human interests. Obviously, proponents of such a perspective will not typically have a prior commitment to the uniqueness and "dignity" of human beings. For this reason we do not pursue this narrative here.

Indeed, asking—let alone answering—a question about the moral status of part-human interspecies hybrids and chimeras threatens the social fabric in untold ways; countless social institutions, structures, and practices depend upon the moral distinction drawn between human and nonhuman animals. Therefore, to protect the privileged place of human animals in the hierarchy of being, it is of value to embrace (folk) essentialism about species identities and thus effectively trump scientific quibbles over species and over the species status of novel beings. The notion that species identity can be a fluid construct is rejected, and instead a belief in fixed species boundaries that ought not to be transgressed is advocated.

An obvious objection to this hypothesis is that, at least in the West, there is already considerable confusion and lack of consensus about the moral status of human embryos and fetuses, patients in a persistent vegetative state, sociopaths, nonhuman primates, intelligent computers, and cyborgs. Given the already considerable confusion that exists concerning the moral status of this range of beings, there is little at risk in adding to the confusion by creating novel beings across species boundaries. Arguably, the current situation is already so morally confused that an argument about the need to "avoid muddying the waters further" hardly holds sway.[19]

From another tack, others might object that confusion about the moral status of beings is not new. There was a time when many whom we in the West now recognize as undeniably human—for example, women and blacks—were not accorded this moral status. We were able to resolve this moral "confusion" (ongoing social discrimination notwithstanding) and can be trusted to do the same with the novel beings we create.

Both of these points are accurate but in important respects irrelevant. Our point is not that the creation of interspecies hybrids and chimeras adds a huge increment of moral confusion, nor that there has never been confusion about the moral status of particular kinds of beings, but rather that the creation of novel beings that are part human and part nonhuman animal is sufficiently threatening to the social order that for many this is sufficient reason to prohibit any crossing of species boundaries involving human beings. To do otherwise is to have to confront the possibility that humanness is neither necessary nor sufficient for personhood (the term typically used to denote a being with full moral standing, for which many—if not most—believe that humanness is at least a necessary condition).

In the debate about the ethics of crossing species boundaries the pivotal question is: Do we shore up or challenge our current social and moral categories? Moreover, do we entertain or preclude the possibility that humanness is not a necessary condition for being granted full moral rights? How we resolve these questions will be important not only in determining the moral status and social identity of those beings with whom we currently coexist (about whom there is still confusion and debate), but also for those beings we are on the cusp of creating. Given the social significance of the transgression we contemplate embracing, it behooves us to do this conceptual work now, not when the issue is even more complex—that is, once novel part-human beings walk among us.

Conclusion

To this point we have not argued that the creation of interspecies hybrids or chimeras from human materials should be forbidden or embraced. We have taken no stance at all on this particular issue. Rather, we have sketched the complexity and indeterminacy of the moral and scientific terrain, and we have highlighted the fact that despite scientists' and philosophers' inability to precisely define *species,* and thereby to demarcate species identities and boundaries, the putative fixity of putative species boundaries remains firmly lodged in popular consciousness and informs the view that there is an obligation to protect and preserve the integrity of human beings and *the* human genome. We have also shown that the arguments against crossing species boundaries and creating novel part-human beings (including interspecies hybrids or chimeras from human materials), though many and varied, are largely unsatisfactory. Our own hypothesis is that the issue at the heart of the matter is the threat of inexorable moral confusion.

With all this said and done, in closing we offer the following more general critique of the debate about transgressing species boundaries in creating part-human beings. The argument, insofar as there is one, runs something like this: species identities are fixed, not fluid; but just in case, prohibiting the transgression of species boundaries is a scientific, political, and moral imperative. The scientific imperative is prudential, in recognition of the inability to anticipate the possibly dire consequences for

19. This objection was raised for us by Vaughan Black.

the species *Homo sapiens* of building these novel beings. The political imperative is also prudential, but here the concern is to preserve and protect valued social institutions that presume pragmatically clear boundaries between human and nonhuman animals. The moral imperative stems from a prior obligation to better delineate moral commitments to both human beings and animals before undertaking the creation of new creatures for whom there is no apparent a priori moral status.

As we have attempted to show, this argument against transgressing species boundaries is flawed. The first premise is not categorically true—there is every reason to doubt the view that species identity is fixed. Further, the scientific, political, and moral objections sketched above require substantial elaboration. In our view the most plausible objection to the creation of novel interspecies creatures rests on the notion of moral confusion—about which considerably more remains to be said. ■

Acknowledgments

An early version of this paper was presented at the 2001 meeting of the American Society for Bioethics and Humanities, Nashville, Tenn. A significantly revised version of this paper was presented at the 2002 meeting of the International Association of Bioethics, Brasilia, Brazil. We are grateful to both audiences for engaging our ideas. Additional thanks are owed to Brad Abernethy, Vaughn Black, Fern Brunger, Josephine Johnston, Jane Maienschein, Robert Perlman, and Janet Rossant for helpful comments on interim drafts. Research for this paper was funded in part by grants from the Canadian Institutes of Health Research (CIHR) made independently to JSR and FB, a grant from the CIHR Institute of Genetics to JSR, and a grant from the Stem Cell Network (a member of the Networks of Centers of Excellence program) to FB.

References

Advanced Cell Technology. 1998. Advanced Cell Technology announces use of nuclear replacement technology for successful generation of human embryonic stem cells. Press release, 12 November. Available from: http://www.advancedcell.com/pr_11–12–1998.html.

Allen, B. 1997. The chimpanzee's tool. *Common Knowledge* 6(2):34–54.

Annas, G. J., L. B. Andrews, and R. M. Isasi. 2002. Protecting the endangered human: Toward an international treaty prohibiting cloning and inheritable alterations. *American Journal of Law & Medicine* 28:151–78.

Atran, S. 1999. The universal primacy of generic species in folkbiological taxonomy: Implications for human biological, cultural, and scientific evolution. In *Species: New interdisciplinary essays,* ed. R. A. Wilson, 231–61. Cambridge: MIT Press.

Bianco, P., and P. G. Robey. 2001. Stem cells in tissue engineering. *Nature* 414:118–21.

Boyd, R. 1999. Homeostasis, species, and higher taxa. In *Species: New interdisciplinary essays,* ed. R. A. Wilson, 141–85. Cambridge: MIT Press.

Breitowitz, Y. 2002. What's so bad about human cloning? *Kennedy Institute of Ethics Journal* 12:325–41.

Campbell, A., K. G. Glass, and L. C. Charland. 1998. Describing our "humanness": Can genetic science alter what it means to be "human"? *Science and Engineering Ethics* 4:413–26.

Chan, A. W. S., K. Y. Chong, C. Martinovich, C. Simerly, and G. Schatten. 2001. Transgenic monkeys produced by retroviral gene transfer into mature oocytes. *Science* 291:309–12.

Claridge, M. F., H. A. Dawah, and M. R. Wilson, eds. 1997. *Species: The units of biodiversity.* London: Chapman and Hall.

Claverie, J. M. 2001. What if there are only 30,000 human genes? *Science* 291:1255–57.

Dekel, B., T. Burakova, F. D. Arditti et al. 2003. Human and porcine early kidney precursors as a new source for transplantation. *Nature Medicine* 9:53–60.

Dennis, C. 2002. China: Stem cells rise in the East. *Nature* 419:334–36.

DeWitt, N. 2002. Biologists divided over proposal to create human-mouse embryos. *Nature* 420:255.

Dixon, B. 1984. Engineering chimeras for Noah's ark. *Hastings Center Report* 10:10–12.

Dobzhansky, T. 1950. Mendelian populations and their evolution. *American Naturalist* 84:401–18.

Doolittle, W. F. 1999. Lateral genomics. *Trends in Genetics* 15(12): M5–M8.

Douglas, M. 1966. *Purity and danger.* London: Routledge and Kegan Paul.

Dreger, A. D. 2000. *Hermaphrodites and the medical invention of sex.* Cambridge: Harvard University Press.

Eisenberg, L. 1972. The *human* nature of human nature. *Science* 176:123–28.

Enard, W., P. Khaitovich, J. Klose et al. 2002. Intra- and interspecific variation in primate gene expression patterns. *Science* 296:340–43.

Ereshefsky, M., ed. 1992. *The units of evolution: Essays on the nature of species.* Cambridge: MIT Press.

Goldstein, R. S., M. Drukker, B. E. Reubinoff, and N. Benvenisty. 2002. Integration and differentiation of human embryonic stem cells transplanted to the chick embryo. *Developmental Dynamics* 225:80–86.

Grant, P. R., and B. R. Grant. 2002. Unpredictable evolu-

tion in a 30-year study of Darwin's finches. *Science* 296:633–35.

Griffiths, P. 1999. Squaring the circle: Natural kinds with historical essences. In *Species: New interdisciplinary essays,* ed. R. A. Wilson, 209–28. Cambridge: MIT Press.

———. 2002. What is innateness? *The Monist* 85:70–85.

Harris, J. 1998. *Clones, genes, and immortality: Ethics and the genetic revolution.* New York: Oxford University Press.

Harris, R. 2001. Little green primates. *Current Biology* 11:R78-R79.

Hull, D. L. 1986. On human nature. In *Proceedings of the Biennial Meeting of the Philosophy of Science Association* 2:3–13.

———. 1999. On the plurality of species: Questioning the party line. In *Species: New interdisciplinary essays,* ed. R. A. Wilson, 23–48. Cambridge: MIT Press.

Kass, L. J. 1998. The wisdom of repugnance. In *The ethics of human cloning,* by L. J. Kass and J. Q. Wilson, 3–59. Washington: AEI Press.

Kitcher, P. 1984. Species. *Philosophy of Science* 51:308–33.

Krieger, L. M. 2002. Scientists put a bit of man into a mouse. *Mercury News,* 8 December. Available from: http://www.bayarea.com/mld/mercurynews/4698610.htm.

Krimsky, S. 1982. *Genetic alchemy: The social history of the recombinant DNA controversy.* Cambridge: MIT Press.

Lewontin, R. C. 1992. The dream of the human genome. *New York Review of Books,* 28 May, pp. 31–40.

Lloyd, E. A. 1994. Normality and variation: The Human Genome Project and the ideal human type. In *Are genes us? The social consequences of the new genetics,* ed. C. F. Cranor, 99–112. New Brunswick, NJ: Rutgers University Press.

Loike, J. D., and M. D. Tendler. 2002. Revisiting the definition of *Homo sapiens. Kennedy Institute of Ethics Journal* 12:343–50.

Love, A. C. 2003. Evolutionary morphology, innovation, and the synthesis of evolutionary and developmental biology. *Biology & Philosophy,* 18:309–345.

Lovejoy, A. O. 1970. *The great chain of being: A Study of the history of an idea.* Cambridge: Harvard University Press.

Marks, J. 2002. *What it means to be 98% chimpanzee: Apes, human beings, and their genes.* Berkeley: University of California Press.

Marshall, E. 1998. Claim of human-cow embryo greeted with skepticism. *Science* 282:1390–91.

Mayden, R. L. 1997. A hierarchy of species concepts: The denouement in the saga of the species problem. In *Species: The units of biodiversity,* ed. M. F. Claridge, H. A. Dawah, and M. R. Wilson, 381–424. London: Chapman and Hall.

Mayr, E. 1940. Speciation phenomena in birds. *American Naturalist* 74:249–78.

———. 1959. Typological versus populational thinking. In *Evolution and the Diversity of Life,* E. Mayr, 26–29. Cambridge: Harvard University Press, 1976.

Mooney, D. J., and A. G. Mikos. 1999. Growing new organs. *Scientific American* 280:38–43.

Morriss, P. 1997. Blurred boundaries. *Inquiry* 40:259–90.

Olson, M. V., and A. Varki. 2003. Sequencing the chimpanzee genome: Insights into human evolution and disease. *Nature Reviews Genetics* 4:20–28.

Ourednik, V., J. Ourednik, J. D. Flax et al. 2001. Segregation of human neural stem cells in the developing primate forebrain. *Science* 293:1820–24.

Oyama, S. 2000. *The ontogeny of information: Developmental systems and evolution,* rev. ed. Durham: Duke University Press.

Plomin, R., J. C. Defries, I. W. Craig, P. McGuffin, and J. Kagan, eds. 2002. *Behavioral genetics in the postgenomic era.* Washington: American Psychological Association.

Robert, J. S. 1998. Illich, education, and the Human Genome Project: Reflections on paradoxical counterproductivity. *Bulletin of Science, Technology, and Society* 18:228–39.

Simpson, G. G. 1961. *Principles of animal taxonomy.* New York: Columbia University Press.

Sober, E. 1980. Evolution, population thinking, and essentialism. *Philosophy of Science* 47:350–83.

Stout, J. 2001. *Ethics after Babel: The languages of morals and their discontents.* Boston: Beacon Books, 1988. Reprint, in expanded form and with a new postscript, Princeton: Princeton University Press.

Tauber, A. I., and S. Sarkar. 1992. The Human Genome Project: Has blind reductionism gone too far? *Perspectives in Biology and Medicine* 35:220–35.

Thomas, K. 1983. *Man and the natural world: Changing attitudes in England, 1500–1800.* London: Allen Lane.

Trigg, R. 1988. *Ideas of human nature: An historical introduction.* Oxford, U.K.: Basil Blackwell.

Wade, N. 1998. Researchers claim embryonic cell mix of human and cow. *New York Times,* 12 November, p. A1. Available from: http://query.nytimes.com/search/article-page.html?res=9C04E3D71731F931A25752C1A96E958260.

———. 2002. Scientist reveals genome secret: It's his. *New York Times,* 27 April. Available from: http://www.nytimes.com/2002/04/27/science/27GENO.html.

Weissman, I. 2002. Stem cells: Scientific, medical, and political issues. *New England Journal of Medicine* 346:1576–79.

Wiley, E. O. 1978. The evolutionary species concept reconsidered. *Systematic Zoology* 27:17–26.

Williams, M. B. 1992. Species: Current usages. In *Keywords in evolutionary biology,* ed. E. F. Keller and E. A. Lloyd, 318–23. Cambridge: Harvard University Press.

Wilson, R. A. 1999a. Realism, essence, and kind: Resuscitating species essentialism? In *Species: New interdisciplinary essays,* ed. R. A. Wilson, 187–207. Cambridge: MIT Press.

———. 1999b. *Species: New interdisciplinary essays.* Cambridge: MIT Press.

Young, C. S., S. Terada, J. P. Vacanti et al. 2002. Tissue engineering of complex tooth structures on biodegradable polymer scaffolds. *Journal of Dental Research* 81:695–700.

Karpowicz et al.,

Developing Human-Nonhuman Chimeras in Human Stem Cell Research:

Ethical Issues and Boundaries,

KENNEDY INSTITUTE OF ETHICS JOURNAL, vol. 15, no. 2 (2005)

Phillip Karpowicz, Cynthia B. Cohen, and Derek van der Kooy

Developing Human-Nonhuman Chimeras in Human Stem Cell Research: Ethical Issues and Boundaries

ABSTRACT. The transplantation of adult human neural stem cells into prenatal non-humans offers an avenue for studying human neural cell development without direct use of human embryos. However, such experiments raise significant ethical concerns about mixing human and nonhuman materials in ways that could result in the development of human-nonhuman chimeras. This paper examines four arguments against such research, the moral taboo, species integrity, "unnaturalness," and human dignity arguments, and finds the last plausible. It argues that the transfer of human brain or retinal stem cells to nonhuman embryos would not result in the development of human-nonhuman chimeras that denigrate human dignity, provided such stem cells are dissociated. The article provides guidelines that set ethical boundaries for conducting such research that are consonant with the requirements of human dignity.

But still 'tmust not be thought that in all ways
All things can be conjoined; for then wouldest view
Portents begot about thee every side;
Hulks of mankind half brute astarting up, . . .

And Nature along the all-producing earth
Feeding those dire Chimaeras breathing flame
From hideous jaws—Of which 'tis simple fact
That none have been begot. Titus Lucretius Carus, 50 B.C.E.

The chimera, a mythological creature that was part lion, part snake, and part goat and breathed forth blazing fire, was a figure of both fascination and repulsion to the ancient Greeks (Bazopoulou-Kyrkanidou 2001; Graves 1960, pp. 252–56). It was portrayed at best as

Kennedy Institute of Ethics Journal Vol. 15, No. 2, 107–134 © 2005 by The Johns Hopkins University Press

an inhuman, capricious creature to be avoided and at worst as a brutish, evil monster to be slain. Its form was symbolic of its nature: a monstrous unnatural body signified a monstrous unnatural disposition. The chimera and several other creatures of mixed categories—e.g., the Minotaur, Gorgons, and Sirens—were seen as signs that some terrible disorder permeates the universe, threatening humanity. Some Western historical and cultural sources have continued to maintain that to merge living beings from sharply distinguished categories is to invite evil and chaos.

Medical researchers are assembling new sorts of chimeras today. Some have fused goat and sheep embryos, developing a creature known as the "Geep" that displays some of the characteristics of a goat in a sheep's body, or vice versa (Polzin et al. 1987). In another experiment, researchers have transplanted regions of the quail brain into chicks, producing a creature with features of both (Balaban, Teillet, and Le Douarin 1988). These experiments are intriguing just because they obscure the boundaries between two different animals. Yet they have not resurrected cosmological fears associated with ancient chimeras among research ethics boards or the public, perhaps, in part, because these studies did not involve human components.

Investigators also have inserted nonhuman materials into humans and human bodily materials into nonhumans. They have, for instance, transplanted pig heart valves into human beings in order to treat serious heart disease. Apparently, inserting small amounts of animal tissue into human beings has not been taken to impinge on the humanness of the recipients, and these therapies have not been rejected as unethical. The transfer of human material, such as embryonic stomachs, intestine, tracheas, and lungs, into the bodies of mice has failed to create an ethical stir (Angioi et al. 2002). Researchers also have inserted human blood and skin stem cells into postnatal mice (Kamel-Reid and Dick 1988; Kauffman et al. 1993; Dick et al. 2001; Raychaudhuri et al. 2001) and fetal sheep (Zanjani, Mackintosh, and Harrison 1991) without significant ethical resistance. The transfer of these sorts of stem cells, perhaps because they are not identified with what is essential to being human, does not appear to raise the specter of converting mice and sheep into human beings (Vince 2004).

It is when investigators have proposed transplanting certain adult human stem cells derived from the brain or eye—an outgrowth of the brain—into animal embryos to learn how these cells, whose tissues develop largely in the prenatal period, differentiate, proliferate and regenerate (DeWitt 2002; Krieger 2002) that ethical concerns have been raised. Stem cells as-

sociated with the brain seem to have a close association with what it is to be human. Such trans-species forays are disquieting because they would introduce human central nervous system stem cells into animals during their formative development when their future biological characteristics are beginning to emerge and before their body plans have been completed. Would the human neural stem cells overwhelm the host animals? If embryonic mice with human neural stem cells were subsequently brought to term, would they possess human brains and think like humans or would they remain mice? The creation of human-nonhuman chimeras, some maintain, would be an outcome "too horrible to contemplate" (Wade 2002). The ancient fear of creating a monstrous interspecies chimera still hovers over contemporary Western society.

At least four ethical arguments could be given against the development of such human-nonhuman chimeras: doing so would (1) be morally taboo, (2) be contrary to nature, (3) violate the integrity of the species involved, or (4) denigrate human dignity. These arguments are not wholly separate conceptually and could be combined into one massive and powerful argument against the development of these chimeras. This, however, would obscure the various ethical, logical, and social nuances of each argument. We therefore consider each argument in turn and conclude that only one, the human dignity argument, has sufficient ethical force to warrant prohibiting the creation of human-nonhuman chimeras.

We then query whether the insertion of human neural (retinal or brain) stem cells into prenatal nonhuman embryos and fetuses would, in fact, result in the creation of human-nonhuman chimeras, using an illustration drawn from a proposed retinal stem cell experiment, and explain why this would be of ethical concern. We argue that the proposed experiment would not result in the development of human-nonhuman chimeras of a sort that contravene human dignity but that the transfer of undissociated stem cells would run that danger. Finally, we detail limits beyond which research involving the transfer of human central nervous system stem cells into animal embryos and fetuses should not go.

ARGUMENTS AGAINST THE DEVELOPMENT OF HUMAN-NONHUMAN CHIMERAS

The term "chimera" has been used somewhat loosely in different branches of the biological sciences to describe inter- and intra-species combinations at many levels, from molecules, to cells, to whole organs. For instance, in molecular biology, the term "chimera" sometimes is used

to describe the combination of DNA sequences taken from two separate individuals into a single sequence. In genetics, the term "interspecies hybrid" refers to the result of mating two genetically dissimilar, and normally nonreproductive, individuals, such as the horse and the donkey. In cell biology, the terms "inter- and intra-species nucleo-cytoplasmic hybrids" are used to denote the use of nuclear transfers (cloning). In embryology, "chimeras" are both inter- and intra-species prenatal combinations of cells that originally were derived from two separate zygotes. Finally in transplantation research, "chimeras" sometimes describes the result of xenografting cells, tissues, or whole organs from human beings into animals. The techniques for creating such chimeras are very different, but in principle they all involve the combination of material from two different sources into one. Our present use of the term "human-nonhuman chimeras" refers to entities that might result from transplants of human stem cells into prenatal nonhuman hosts.

THE MORAL TABOO ARGUMENT

Some react to the very thought of creating human-nonhuman chimeras with repugnance. To bring such creatures into the world seems to them an abomination akin to incest or cannibalism. It is an act that they claim is prohibited by taboos found in many cultures that have served to promote human well-being and important social values. To violate them would have serious negative repercussions on those involved and their societies. We term this argument "the moral taboo argument."

Leon Kass (1997), for instance, claims that repugnance provides the basis for strictures in many societies against such practices as incest, bestiality, and cannibalism. He maintains that we have some rarely articulated and "perhaps not altogether articulable" sense that putting human stem cells and their derivatives into animals would evoke a similar response (President's Council on Bioethics 2003). "[R]epugnance may be the only voice left that speaks up to defend the central core of humanity" (Kass 1997, p. 20). That certain practices elicit repugnance is sufficient to indicate that they are wrong, on this view. Attempts can be made to support such emotional responses with argument, Kass acknowledges, but he asks, "Would anybody's failure to give full rational justification for his or her revulsion at these practices make that revulsion ethically suspect?" (Kass 2001, p. 6; 1997, p. 79). Sheer repugnance is epistemologically foundational to this approach and is said to lead inexorably to the intuition that these sorts of practices are ethically unacceptable.

The force that emotions and intuitions should be given in ethical decision making is a contested philosophical issue. Surely it is ethically sound to feel outrage at the wrongful killing of an innocent person or the rape of a child. What makes such outrage justifiable, however, is not the emotion in itself, but the reasons why one responds with this emotion. We would be reluctant to accept ethical judgments based solely on emotions such as anger, outrage, or revenge without further explanation, for these can occur by chance and may be misplaced. Moreover, at times emotions can obscure, rather than clarify, ethical reasoning. Even those who give emotions considerable weight in making ethical assessments maintain that one must find reasons for such assessments that are based on coherent and supportable ethical standards of judgment (Midgely 2000).

Intuitions, as distinct from emotions, traditionally have been viewed as a response to an authoritative inner voice (Kekes 1986). They are said to have direct epistemological force and to require no further justification. Thus, one straightforwardly intuits that it is right to assist an innocent person who is about to be killed or a child about to be raped. Although intuitions undoubtedly play a role in ethical thinking, it is problematic to view them as providing the foundation for ethical reasoning, for they are fallible and sometimes conflict. What one person, or even a society, regards as an intuitively known truth may be shown to be wrong or rejected as dubious by others. This works against the belief that we have an inborn moral sense through which we derive infallible moral intuitions. For such reasons, W. D. Ross (1930) maintained that intuitions are presumptive. That is, they establish a *prima facie* case for acting in certain ways but can be overruled if there is good reason to dismiss them. As a way of knowing, intuitions need to have the support of some form of reasoning that is intersubjectively available and can be followed by others. In short, the main advantage of intuitions over emotions is that they are less vulnerable to chance changes. Their chief disadvantages are that they can vary from person to person and may prove to be erroneous.

Even so, it is important to acknowledge that taboos based on repugnance and intuition play a significant role in preserving core social values within most societies. They serve to bring order out of cosmological and social chaos in that they establish lines of authority that perpetuate traditions and ways of thinking (Levi-Strauss 1978; Douglas 1966). The taboo against incest, for example, forces societies to expand, bringing in new members who can help them to survive and flourish. However, the same taboos are not held universally across all cultures and, within a culture, can outlive their social role and be displaced.

Are the moral taboos that Kass lists universally accepted? It seems not. A paradigm moral taboo that he maintains is universal, that against cannibalism, is not found in all cultures. Indeed, cannibalism still exists today in some societies as a socially sanctioned practice. Taboos against crossing humans and nonhumans, in particular, have not been held universally. The ancient Egyptians, for instance, depicted some of their revered gods with nonhuman animal heads or bodies, and Native Americans have made use of sacred figures that combine human and nonhuman features.

Moreover, some of the moral taboos that have been entertained in the past within Western culture now are considered wrong or are under ethical siege. Blood transfusions, organ donations, interracial marriage, and homosexuality all have been viewed as morally taboo. Yet society's attitude towards blood transfusions, for example, has undergone complete reversal; what was once morally abhorrent has now become a moral and civic responsibility. Similarly, ancient taboos that once prohibited mixing the human and nonhuman seem to be dissolving today, as is exhibited by the general acceptance of the insertion of pig heart valves into human beings. This variability in inter-categorical taboos indicates that moral taboos alone cannot provide a reliable basis for making ethical assessments about whether to create human-nonhuman chimeras.

Jeffrey Stout (1988, pp. 145–62), in presenting a theory about social taboos, distinguishes the repulsive from the repugnant, or what he terms the "abominable." That which is repulsive mixes categories that we keep sharply separate, but does so in ways that are not socially or cosmologically significant. The repugnant or abominable, in contrast, not only displays great anomalies, but does so in ways that create a major disturbance in the social or cosmological system of a culture. Stout probably would agree that the human ear, although uniquely identified with human beings, does not have major societal or global import and that its transfer to the back of a mouse (Cao et al. 1997) would be repulsive, but not repugnant. However, in cultures that sharply distinguish humans from animals, he might well find that such an experiment would be viewed as abominable just because it obscured that distinction. Violations of moral taboos pose a threat to the established conceptual order on which the social order depends, and members therefore respond to them with repugnance, labeling them as morally taboo.

Repugnance, on Stout's view, which strikes us as well-founded, is not a primitive emotion at the foundation of moral judgments about the creation of such beings as human-nonhuman chimeras. Instead, it arises

from an antecedent commitment to social and cosmological categories relative to which human-nonhuman beings seem anomalous. Thus, it is not the creation of such chimeras that needs defense but the system of social and cosmological categories that informs repugnance toward them and that grounds moral taboos against combining the human and the nonhuman.

Taboos are social conventions that emerge from diverse historical and cultural contexts. They are subject to alteration as the context in which they arise alters. Such change is occurring today as the context in which mixing human materials with those of animals moves away from fearsome chimeric creations of fantasy to human-nonhuman combinations initiated to study the development of human cells and, ultimately, to treat those with diseased tissue (Karpowicz, Cohen, and Van der Kooy 2004). Indeed, it is arguable that there is an ethical imperative today to resist taboos about human-nonhuman chimeras derived from an earlier historical era, since they do not take into account the reasons why such chimeras might rightly be pursued within the contemporary context (Franklin 2003). We conclude that the simple assertion that human-nonhuman chimeras are morally taboo, unvarnished by a rationale or justification, does not provide an adequate basis for rejecting studies using human-nonhuman chimeras or other experiments in which human and nonhuman bodily materials are merged.

THE "UNNATURALNESS" ARGUMENT

The "unnaturalness" argument does not appeal to an inarticulable sense of repugnance or abomination, but it can be seen as a way of explaining the connection between such repugnance and the structure of the universe. The argument objects to the creation of human-nonhuman chimeras on grounds that doing so would be contrary to the orderly way in which the natural world functions (President's Council on Bioethics 2003; Midgely 2000).

This argument maintains that the operations of nature are to be understood and valued in terms of their purposes (Kass 1985). It is indebted to Aristotelian thought, which asserts that every living thing has an inner tendency to reach its appropriate end or goal (*telos*) by exercising certain characteristic biological functions. According to traditional natural law theorists, the very fact that a living entity pursues a particular kind of life through certain biological processes is its own justification (Crowe 1977, pp. 192–245; d'Entreves 1970, *passim*). Moreover, a life that unfolds in accordance with its intrinsic principles of operation displays a kind of

goodness. Accordingly, for natural law theorists, it is a moral good for each kind of being to be aligned with its appropriate end and a moral wrong to alter its natural functioning in ways that distort or violate this end. For these thinkers, the naturalness of a practice provides *prima facie* justification for engaging in it.

There is a pervasive sense of the continuity between humans and nonhumans in much natural law thought (Porter 1999). Yet curiously natural law theorists also maintain that the proper end of human beings differs radically from that of, say, mice or monkeys (Kass 1985, p. 272). The very nature of each sort of living being sets moral limits on human action. To transfer human cells, tissues, and organs into nonhumans in ways that change their function, their progression toward their end or goal, would violate the natural teleology of these beings and therefore would be unnatural and wrong. Aspects of this natural law rationale have been held by some leading scholastic thinkers such as Aquinas (1968, 2a2ae, p. 154) and more recently by some moral theorists (Flanagan 1991; Kass 1985; Midgely 1978). Similar "unnaturalness" arguments have been given against emerging biotechnologies such as the use of xenotransplants, genetic engineering, and cloning.

The "unnaturalness" argument accepts an assumption that remains questionable, namely, that an organism's usual state of flourishing should be valued. However, having a certain mode of reproducing, for instance, is not, in itself, ethically significant (Savulescu 2003). Nature does not come with some sort of built-in ethical import that can be read from it, such that living beings' typical ways of functioning always must be kept intact. It is what one makes of natural functions and structures that is ethically significant. To what ends are they put? Although the realities of nature constrain ethical judgments about the ways in which one ought to treat the natural world, these realities must be subject to interpretive framing in light of philosophical, social, biological, and other understandings.

The teleological guidance of the "unnaturalness" argument requires one to speculate endlessly about the natural purposes of virtually all living entities and their biological components. For instance, it is not clear whether it would be ethically acceptable, on a teleological view, for one human being to donate a kidney to another or to make use of *in vitro* fertilization. By their very "unnaturalness," these practices would seem to violate the natural functions of the human beings involved. Yet these same interventions would help humans achieve their broader "natural" ends of being alive and reproducing.

The basic difficulty with the "unnaturalness" argument is that it does not explain when an intervention into nature is ethically acceptable and when it is not, why certain natural features always bear a certain moral import and therefore should not be changed. That organisms normally function in certain ways in the natural world does not indicate that it is wrong to intervene into these functions or to keep them from reaching their usual biological ends. No bright line is provided by the "unnaturalness" argument to help one distinguish between appropriate and inappropriate interventions. The context in and reasons for which the interventions are carried out have considerable import for assessing whether they are right or wrong.

Moreover, we now know that the natural world is in constant flux. Organisms do not remain static but evolve and change. Although one may appreciate and value the ways in which many aspects of the natural world unfold, there is no reason to think that there are moral requirements built into nature that all things must remain in an unaltered natural state and that humans should not influence the ways that human or nonhuman organisms function. The "unnaturalness" argument makes assumptions about the interpretation of biological phenomena and the elucidation of ethical values that does not, and could not, follow from what we have learned of the evolution and development of humans and nonhumans.

Consequently, we set aside the "unnaturalness" objection to the creation of human-nonhuman chimeras on grounds that it equates, and thereby confuses, biological description with the justification of ethical norms. It therefore provides insufficient warrant for judging the creation of human-nonhuman chimeras to be wrong.

THE SPECIES INTEGRITY ARGUMENT

An objection to the creation of human-nonhuman chimeras often implicit in the moral taboo and the "unnaturalness" arguments is that experiments employing such chimeras would cross species boundaries, which would be ethically unacceptable. No one view of why crossing species boundaries would be wrong has been proposed by those who offer the "species integrity" argument. Indeed, there is no commonly accepted view of just what is meant by species and how to distinguish one species from.

According to the classical conception of species, as depicted by Aristotle, similar biological organisms are members of a "natural kind," a species with an essential and unchanging nature. Members of species share an

essence or property that is common to all of them and that is responsible for each member being the kind that it is (Griffiths 1999). For this reason, species have explanatory priority over concrete individuals in the sense that the resemblances between individuals in a species are explicable in terms of the underlying "natural state" of each individual (Boyd 1999; Griffiths 1999; Hull 1999; Wilson 1999). They are internally homogenous and discontinuous with one another; their boundaries are real and objective. Thus, the original development of species categories was based on the presumption that to define a species involves making an objective determination about what is given straightforwardly in nature.

However, as biologists subsequently attempted to draw boundaries around groups of organisms that they observed in nature, they developed diverse views of what is meant by a species. The delineation of biological species seems to have been developed by ascribing significance to the visible appearances, functions, or behaviors of organisms. Boundaries were then drawn between groupings of living beings on this basis (de Sousa 1984). Thus, Karl Linnaeus, the father of biological taxonomy, grouped species by their visible appearance. However, biologists found that this reliance on outward appearance to capture the meaning of species did little to further scientific hypotheses and predictions, so they chose other characteristics of organisms to define species. They seemed to base their alternate species categorizations largely on biologically interesting or subjectively relevant criteria that furthered their particular scientific interests and approaches (de Sousa 1984).

Ernst Mayr (1988), for instance, focused on the mode of propagation as the most important criterion for defining species, maintaining that species are groups of interbreeding natural populations that are reproductively isolated from other such groups. One of the problems raised by his view, however, is that asexually reproducing organisms, such as bacteria, or the 5 percent of interbreeding birds that are taken to be of different species do not fit into such a classification system. In order to accommodate such exceptions, Mayr stretched his characterization of species by introducing the idea of morphological similarity. An asexual species, he maintained, contains individuals that are as structurally similar to one another as members of a sexual species. This, however, seemed to revert to the unsatisfactory Linnean characterization of species. Some biologists therefore have sought alternative criteria for defining species.

Even if another criterion for distinguishing species, say, genetic similarity, were adopted, this would not wholly resolve the difficulties encountered in

defining species. Categorizing species would become a question of setting acceptable boundaries around ever-fluctuating genetic similarities. Setting genetic species boundaries around humans and chimpanzees, for instance, seems straightforward until it becomes necessary to select a relevant threshold of genetic similarity. What threshold would produce the most accurate phylogeny between these organisms? If one were to restrict the relevant genetic grouping to a subset of the eukaryotic genome, one might not only distinguish between humans and chimpanzees, but also establish separate species categories among humans. Many would reject this result, however, because it would ignore other significant criteria that biologists have adopted for viewing all humans as members of the same species.

Because biologists have chosen differing criteria to identify species based on what has seemed important and scientifically interesting in their own research, we have a tremendous variety of ways of categorizing species. Currently, there is no general agreement, and indeed there may never be, about which categorization is correct. This lack of agreement makes it doubtful that species categorization could bear the moral weight necessary to evaluate the morality of transferring human cells into nonhuman animals. The biological categorization of species is empirical and pragmatic, a constantly developing effort that has little to do with moral judgments. Thus, even if one were to identify an unchallengeable view of what is meant by species, it would remain unclear why the possibility of transferring bodily material from one species to another, as would occur in human-nonhuman chimera studies, would be wrong.

Jason Scott Robert and Françoise Baylis (2003) attempt to answer this question. They maintain that there are no objectively given species boundaries. However, the belief that there are fixed species boundaries that exist independently has become a fixed part of conventional moral thinking. Because of this, they declare, to endow a mouse or a monkey with human cells in significant numbers or kinds would introduce moral confusion into conventional thinking and diminish the high moral status that human beings are assigned. This possibility, they argue, is so threatening to our social fabric that we need to keep tightly guarded conventional species boundaries between humans and nonhumans. Their argument is, in effect, a version of the "moral taboo" argument, for it maintains that we should not go contrary to deep, long-held societal conventions.

It is a version that requires some evidential support. Critics have argued that the claims that society fears crossing conventional species boundaries and that to cross them would create social and moral chaos need

confirmation (Rollin 2003; Charland 2003; Streiffer 2003). An alternate possibility is that society would take the creation of human-nonhuman chimeras in stride, much as it has when certain animal materials have been transferred to humans, and that it would continue to move along an orderly moral path.

We conclude that the species integrity argument provides no reliable criteria for ascertaining when the lines between species have been crossed, and, were it to do so, no clear argument about when and why crossing them would be ethically unacceptable. It offers no reasons why society should not accommodate new ways of classifying living organisms. Proponents of the species integrity argument believe that the familiarity of species categories in current use is sufficient to justify valuing and retaining them. However, that one is used to thinking about things a certain way is not a strong reason to argue against the development of new ways of thinking about the human-nonhuman chimera (Karpowicz 2003).

THE HUMAN DIGNITY ARGUMENT

In the novel, *The Island of Doctor Moreau* (Wells 1896), a visitor arrives on a remote island and discovers that a mad scientist, Moreau, is conducting experiments designed to turn animals into human beings. The resulting part-animal, part-human creatures, in Wells's fantastic scenario, struggle and ultimately fail to sustain human lives. To the visitor, this experimental manipulation raises strong concerns. "I asked him why he had taken the human form as a model. There seemed to me then, and there still seems to me now, a strange wickedness in that choice." What strikes the visitor as wrongful in Dr. Moreau's experiments is that they diminish and degrade human beings.

Similarly, the core concern that arises in deciding whether it would be ethical to create human-nonhuman chimeras in stem cell research is whether humans and nonhumans would be merged in ways that would denigrate or even eliminate the distinctive value of each, with particular emphasis on the effect on humans. The moral taboo, "unnaturalness," and species integrity arguments presume that there is something about human beings that ought to be honored and protected. It is this element that the human dignity argument addresses.

The notion of human dignity has been evoked in debates about such issues as euthanasia and reproductive cloning. Unfortunately, those who have presented the notion have tended not to elaborate on what they mean by human dignity. For instance, in a recent book, Kass (2002)

expresses concern about the threat to human dignity presented by such recent bioetchnological interventions as reproductive cloning. However, after rejecting the Greek notion of heroic dignity and what he terms a contemporary version of Kantian thought that defines dignity as "choosing for yourself, what ever you choose," he presents only a hint of what he means by the notion. Human dignity, he indicates, has to do with "the worthiness of embodied human life, and the worth of our natural desires and passions, our natural origins and attachments, our sentiments and aversions, our loves and longings" (Kass 2002, p. 18). He does not explain what it is that gives embodied human life this worthiness, thereby leaving one with a vague notion of human dignity that is open to use also by those who take an opposing view of the rightness of reproductive cloning.

Some others who use the notion of human dignity are equally mysterious about its significance. John Robertson (1994), for instance, maintains that it is a violation of human dignity to deny individuals the right to have the kind of children that they want through use of the new reproductive technologies. However, he does not attempt to explain what he means by human dignity and is therefore open to rebuttal by those who claim that it is a violation of human dignity to utilize these technologies. Because of such lack of clarity about the meaning of human dignity, some commentators, such as Ruth Macklin (2003), maintain that it is a useless notion. She asserts, for example, that it means no more than respect for autonomy. Although the violation of human dignity surely includes the loss of control over one's own choices, it means more than this in most contexts in which the notion figures. The person who voluntarily sells himself into slavery denigrates human dignity, even though he has made an autonomous choice. John Harris (1998) also finds the notion unclear and reduces it to "not using individuals as a means to the purposes of others." This, however, is a stricture that follows from the recognition of human dignity, rather than an explanation of what it means.

It was Immanuel Kant (1964; 1956) who brought the concept of human dignity to the fore of Western thought (Hill 1992; Cohen 1999). His view of human dignity is independent of his metaphysical ideas and his special understanding of the moral law (Hill 1992, p. 176). He maintains that humans have an unconditioned and incomparable worth (*Würde*) or dignity because they are moral agents whose actions can be imputed to them (Kant 1964, p. 94). Their dignity is manifested in their capacities to set ends for themselves and to act to achieve them in the practical sphere. Because they have a rational nature and the ability to act on principles,

Kant holds, humans possess a distinctive dignity that cannot be assigned a market price. Alan Gewirth (1992), a contemporary thinker influenced by Kant, maintains that the primary ground on which human agents logically must be said to have dignity is that they have purposes that they can act to fulfill. This generic purposiveness underlies the ascription of dignity to all human agents, he argues.

Although Kant's and Gewirth's views capture an important aspect of human dignity, they omit other significant factors that enter into the common understanding of this concept. Human dignity is a widely shared notion that signifies that humans typically display certain sorts of functional and emergent capacities that render them uniquely valuable and worthy of respect (Karpowicz, Cohen, and Van der Kooy 2004). It is not only the capacities for reasoning, choosing freely, and acting for moral reasons, as Kant argues, or for entertaining and acting on the basis of self-chosen purposes, as Gewirth holds, that are at the core of what is meant by human dignity. The notion also encompasses such capacities as those for engaging in sophisticated forms of communication and language, participating in interweaving social relations, developing a secular or religious world-view, and displaying sympathy and empathy in emotionally complex ways. That is, human dignity is a multi-faceted notion that is characterized by a family of unique and valuable capacities generally found in human beings. No one of these capacities is definitive of human dignity, but taken together, they set out a paradigm case of what it is to have human dignity (Cohen 2003). Further, dignity, as Kant declares, is not associated solely with those who have rank and authority. It is attributable equally to all human beings, regardless of their virtues and vices, their station in life, their disabilities, or their price on the market.

Having human dignity is conceptually distinct from behaving and bearing oneself in a dignified manner. Dignified comportment is a contingent feature displayed by some humans who respond to untoward circumstances in a noble and uplifting manner. Such individuals display what has been termed "personal dignity" (Pullman 2004) or what Aristotle termed "*arete*". Having human dignity, in contrast, is, as Gewirth (1982, pp. 27–28) states, "a characteristic that belongs permanently and inherently to every human as such." A person can behave in ways that are boorish and selfish and thereby diminish her "personal dignity" and yet retain human dignity.

Human dignity is degraded and demeaned when the family of valuable capacities at its core are deliberately and wrongfully diminished or

eliminated. This occurs when human beings are subjected to such acts as murder, torture, enslavement, rape, or maiming. These sorts of acts and practices are wrong not only because they injure humans physically and psychologically, but also because they deny them the exercise of their dignity-associated capacities, treating them instrumentally as mere things with no special value. Proponents of an argument from human dignity would maintain that to create a human-nonhuman chimera would either diminish or wholly eliminate the possibility that humans could exercise the cluster of capacities and characteristics that are associated with human dignity, treating them solely as a means to others' ends. By giving nonhumans some of the physical components necessary for development of the capacities associated with human dignity, and encasing these components in a nonhuman body where they would either not be able to function at all or function only to a highly diminished degree, those who would create human-nonhuman chimeras would denigrate human dignity. The torturer or the enslaver of human beings denies them the option of exercising the capacities associated with human dignity. The creator of the human-nonhuman chimera would do even worse—he or she knowingly would diminish or eliminate the very capacities associated with human dignity.

The argument from human dignity against the development of human-nonhuman chimeras might be criticized as a form of the species integrity argument. Helga Kuhse (2000, pp. 69–70) makes the point that "it would not be enough to say that human life has dignity because it takes the form of a featherless biped or because humans have opposing thumbs." Surely she is correct. The characteristics that are taken to define humans as a biological species have no particular ethical importance in most contexts. However, humans are not considered to have dignity because they are *homo sapiens*, but because they possess a cluster of capacities that matter ethically and that members of that species generally exhibit (Savulescu 2003).

A reverse criticism of the argument from human dignity might be raised: because human dignity is not identified with humanness but with the possession of certain capacities, only those humans with such capacities can be said to have dignity (Beyleveld and Brownsword 2001, p. 23). However, those who are human and yet display a limited subset of these capacities, say, the newborn infant or the person with severe disabilities, still have human dignity its proponents declare. We tend to ascribe it to all humans, no matter how seriously impaired or ill they may be, because there is no clear agreement about just how many dignity-associated capacities a person

must possess to be said to have human dignity. To avoid the possibility of mistakenly failing to treat those with severe disabilities as ends in themselves, human dignity proponents ascribe dignity to all humans.

Is the argument from human dignity a form of the "unnaturalness" argument? Does it presume that humans share a certain essence of humanness that must not be changed? The argument from human dignity does not maintain that there is some actual essence that corresponds to what it is to be human. Instead, it presumes an ordinary notion of humans as a group of beings who generally share certain kinds of capacities among which are some of the distinctive capacities associated with human dignity.

Another possible criticism of the argument from human dignity is that it wrongly denigrates the value of nonhuman animals. Although some who present the argument from human dignity maintain that animals have less worth than humans or even no worth, such views are not essential to the argument from human dignity. Nonhuman animals can be taken to have various characteristics and capacities of their own that give them a unique sort of worth that differs from that of humans; this need not detract from human dignity. Frans de Waal (1996, p. 210), for instance, maintains that nonhuman animals exhibit attachment and empathy; internalization of prescriptive social rules; concepts of giving, trading, and revenge; and tendencies toward peacemaking and social maintenance. However, they do not exhibit the capacities to make ethical judgments about available alternatives, to reject some alternatives on ethical grounds, to act on the basis of their judgments about those that are ethical, or to engage in speech, complex communication, or certain other dignity-associated capacities to the same degree and in the same kind as humans. The family of capacities associated with human dignity seems to belong uniquely to human beings. This is not to deny that humans have certain ethical obligations to nonhuman animals but to point out that animals, including those with capacities that resemble those of humans in several respects, have a different sort of worth from that of humans.

Although the human dignity argument needs further delineation and refinement, it does not fall into many of the difficulties to which the other arguments against the creation of human-nonhuman chimeras are prone. It maintains that it would be wrong to create human-nonhuman chimeras, but it has not yet explained in terms of the specifics of stem cell research just how doing so would violate human dignity. We therefore turn to consider what would transpire if certain human stem cells that are among the biological components especially associated with the cluster of

capacities that characterize human dignity were transferred to nonhuman animal embryos.

WHETHER DEVELOPMENT OF HUMAN-NONHUMAN CHIMERAS IN NEURAL STEM CELL RESEARCH WOULD VIOLATE HUMAN DIGNITY

At the core of the concerns related to human dignity raised by the possibility of developing human-nonhuman chimeras is that certain human components closely connected to the cluster of abilities associated with human dignity would be transferred to nonhumans. Capacities such as those for carrying out discursive and moral reasoning, engaging in complex communication, and forming multifacted social relations especially are associated with the human brain, whether one views the relationship between thought and the brain as one of materialistic identity, dualistic correlativity, or in some other way. Thus, human-nonhuman chimeric research challenges human dignity and becomes ethically problematic when it involves the introduction of substantial numbers of human brain or retinal (an outgrowth of the brain) cells into a nonhuman. Just how might such research violate human dignity?

Although it is fantastical, we at least can envision that some investigators might attempt to transplant a whole adult human brain into a nonhuman animal in order to study certain important neurological questions, resulting in a human-nonhuman chimera. To create such a chimera would violate human dignity because the resulting being could not fully exercise the dignity-related capacities associated with the human brain, due to its role as a research subject specifically produced to serve as a human proxy in experiments that it would be unethical to undertake on human beings themselves. The development of such a chimera arbitrarily would limit the ways in which certain human characteristics and capacities associated with human dignity could be exercised in a nonhuman setting and therefore would contravene human dignity. Consequently, the decision to manufacture a nonhuman research subject with a human brain and, at most, diminished capacities for various forms of human-like cognition and action would violate human dignity.

It is clear that the transfer of a whole human brain into a nonhuman animal would result in a human-nonhuman chimera that could exhibit many of the capabilities associated with human dignity. Would the transfer of human brain or retinal stem cells into nonhuman research subjects similarly result in a human-nonhuman chimera of a sort that contravenes human dignity? If human retinal stem cells, when transplanted into the

prenatal mouse or monkey, were to proliferate and develop into a whole human-like brain and if human-like capacities associated with human dignity were to emerge in such animals to some degree, the creation of this research subject would contravene human dignity. However, if the human retinal stem cells, following such a transplant, did not form a functioning human brain and were not integrated with the host's basic neurological functions, but were simply present in the nonhuman brain, the resulting being would not exhibit the cluster of distinctive capacities associated with human dignity. Such a transfer, therefore, would not violate human dignity.

Prenatal chimeras involving transplants of adult and embryonic neural human stem cells into nonhumans have been carried out, and to date none has demonstrated any evidence that such transplants result in the emergence of altered human-like features or functions in the nonhuman (Goldstein et al. 2002; Ourednik et al. 2001). It is important to understand why this is the case and to consider whether the opposite might occur in the future. In order to do this, one first needs to examine what neurobiology has to say about mammalian nervous system development.

Even if human retinal stem cells were to integrate with the mouse's or the monkey's basic neurological functions and were to replace the photoreceptors of the nonhuman, they would not control the way that the nonhuman brain functioned. Although the human retinal stem cells would become functional light-sensing cellular devices connected in a chain of similar devices that communicate messages within the animal eye or brain, the overall architecture of the animal's brain would not be affected by the presence of these cells. They simply would transmit light stimulus information to the brain of the chimera itself. The neurological functions of the nonhuman brain would remain unaltered because their organization would be governed by the animal host. The human cells would change with their environment to mimic the nonhuman host's native morphology and function and their genetic dissimilarity relative to the host would make no difference in the way in which the host brain functioned. They would become the practical equivalent of mouse or monkey cells. Thus, the human retinal cell component would not cause the unaltered mouse or monkey to develop human psychological, cognitive, or other capacities associated with human dignity.

The differences between mouse and primate brain complexity can be explained partially by differences in the number of cell cycles that occur during each species's neurogenesis (Kornack et al. 1988). *In vivo* brain

development studies have shown that primate cells divide more slowly than those of mice. Discrepancies between the brain size of primates and mice arise because primate progenitors have a longer developmental window and, although they divide slowly, they nevertheless go through many more divisions than those of developing mice. Such discrepancies reveal biological phenomena that differentiate human from nonhuman brains. Human neural progenitors or stem cells transplanted into mice or primates would produce human-like neural tissues in these nonhumans only if the cells somehow anticipated how much time was left during development and, in response, sped up their cell divisions to achieve the human-like end result—and this within the limits imposed by the experimental nonhuman host skull. However, human cells have little ability to predict anything about their nonhuman environment, or to discern when they should divide more or less rapidly to produce a human eye or brain. During development much, if not most, of a cell's behavior is not intrinsic, but rather is governed by forces arising from outside the cell itself.

What is more plausible and highly likely is that human stem cell proliferation in the mouse or monkey would be modulated by the mouse or monkey because the host's cells greatly outnumber the donor's cells. An example of such host-mediated recruitment is provided by human-mouse blood stem cell transplants in which human blood stem cells have not over-proliferated and overwhelmed the nonhuman host's blood system (Kamel-Reid and Dick 1988). Human blood stem cells do not continue to divide until human blood levels are achieved because the cells are recruited by the host, according to the host's needs. In short, the nonhuman host governs the way that these human blood stem cells function after their transfer.

Brain size is similarly regulated during development. Xenografted human stem cells would not be able to achieve human brain size and the human brain organization needed to give rise to human neural functions and behaviors when transplanted to nonhuman hosts. Both the mouse and the monkey chimeras would have to possess heads swollen many times their ordinary size to be able to accommodate a human brain. This scenario is unlikely. It is far more likely that human tissue would develop into the host's native form and would have no effect on the mouse or monkey's neural capacities. Even a monkey chimera whose thalamus and cortex were largely human-derived would not possess human capacities if the human neurons were to lie in different, nonhuman, functional networks. The same is true of even the closest relatives of the human, such as the chimpanzee, whose brain does not possess the same architecture

and organization as the human brain. The reasons why human networks differ from those of nonhuman primates are not known. It appears to have little to do with brain size itself, but instead with the time span of overall neuronal development and increases in the frequency of cell division of the neuronal progenitors that contribute to specific regions of the cortex during development (Finlay et al. 1995). It is very doubtful that a human brain could be developed outside a human body.

Given this evidence, the transplantation of human retinal stem cells into nonhuman mouse or monkey eyes or brains, as in our example, should result in the development of the same tissues and the same tissue organization that are endogenous to the mouse or monkey. This reveals that the human stem cell chimeras are not so much a test of characteristic human neural development, as a proof that human cells can contribute to a comparable, nonhuman animal's development.

One important caveat remains about the human retinal or brain stem cell chimera. This has to do with the state of the human stem cell transplant. *For the reorganization and host-driven control of transplanted human stem cells to occur, these cells must be separated or dissociated from one another when they are transplanted*. It is likely that this dissociation weakens the organization already present in a mass of cells prior to their transplantation and forces the human cells to reorganize themselves in response to the host environment. Whole organs or masses of undissociated cells should not be transferred from humans to nonhumans because doing so would risk the development of characteristic human pattern development and formation in the nonhuman animal host.

Two studies speak to this issue. First, the study noted previously that involved transplantation of undissociated goat cell masses into sheep blastocysts has shown that, during early development, the targeted replacement of the whole inner cell mass—a small clump of cells that will become the entire embryo—can bias the chimera to assume donor-only characteristics (Polzin et al. 1987). This means that the host animal develops characteristics of the animal from whom the inner cell mass was derived. In this instance, the replacement of the entire sheep inner cell mass with that of the goat resulted in the loss of any host sheep cells that could direct transplanted cell organization. The sheep was effectively replaced by the goat because only the goat cells remained to instruct the embryo's development.

Quail-chick chimeras, also mentioned previously, provide a second, striking example of interspecies-derived behavioral alterations (Balaban et

al. 1988). These chimeras were developed by transplanting whole regions of the quail brain into chicks. The resulting tissue into which those quail brain regions developed was quail-like, yet it was present in the chick body. The methodology used in this second experiment, like that of the sheep-goat experiment described above, did not use dissociated cell transplants. Instead, it involved the excision of a whole third of the chick brain region and its subsequent replacement with the corresponding region of the quail. In the resulting transplanted region, the host cells outnumbered those of the donor and thus directed its development.

Both of these studies bear little similarity to the stem cell experiments under discussion here because these studies involve the transfer of undissociated cells. The first involved the transfer of cell masses and the second of whole regions of the brain. In addition, both of these experiments used animals whose developmental stages were more closely related to each other than are either humans and monkeys or humans and mice, animals that would be used in some proposed retinal stem cell studies. It is unlikely, but theoretically conceivable, that an embryonic human cortex xenograft into chimpanzees, which are closely related to humans, could develop into a human-type neocortex and that the host chimpanzees would exhibit to some degree some human capacities relevant to human dignity. Similarly, if an experiment were conducted in which an entire chimpanzee inner cell mass were replaced with a human embryonic stem cell mass, one could theorize that this might result in a human embryo developing within a pregnant chimpanzee and would raise human dignity issues. In contrast, all available evidence indicates that the use of dissociated stem cells does not produce functional alterations in the host recipient. This is why the use of dissociated cells has been accepted in human brain cell grafts into human patients (Boer 1993).

The retinal stem cell chimeras presented in the example above would involve the dissociation of cultured human cells prior to transplantation. These cells would then interact with the developing monkey cells as they were reorganized by the host. The chimeras produced in these retinal stem cell experiments would remain functionally ordinary mice and monkeys with some human cells. The human cells would contribute to basic functions of these animals but would not cause them to exhibit the sorts of distinctive human functions and capacities that are pertinent to human dignity.

However, if we were to accept the moral taboo, "unnaturalness," or species integrity arguments discussed above, we likely would be obliged to

conclude that we should put an end to all interspecies combinations. To be consistent, both prenatal and postnatal stem cell transplant experiments, all transgenic modifications, and all xenotransplantation would have to be condemned as unethical and brought to a halt. Yet, as we have seen, the moral taboo, "unnaturalness," and species integrity arguments do not clearly demonstrate that all human-nonhuman combinations are wrong in principle. Consequently, they leave us with few compelling reasons to argue against a systematic and careful foray into the study of stem cell chimeras involving the transplantation of human brain or retinal stem cells into nonhumans. We suggest that it is appropriate to pursue such studies given certain restrictions on their design related to the argument from human dignity.

SETTING LIMITS ON STEM CELL RESEARCH INVOLVING THE TRANSFER OF HUMAN NEURAL STEM CELLS TO NONHUMANS

Scientific understanding of the development of human stem cells would be furthered through studies such as the retinal stem cell investigations discussed above. The hope of offering therapeutic benefit to those who suffer from conditions leading to blindness and other serious eye conditions drives most of such research. Yet the pursuit of scientific understanding and medical benefit should be tempered by ethical considerations.

The ethical boundaries that we provide here for research in this field are based on human dignity concerns. We have argued that psychological and cognitive capacities associated with human dignity would not develop in nonhuman hosts in the aforementioned retinal stem cell chimera experiments if methods of proceeding with this research were limited in certain ways (Karpowicz, Cohen, and Van der Kooy 2004). We offer the following guidelines for setting boundaries for experiments involving the transfer of human brain and retinal stem cells into nonhuman prenatal nonhuman animals.[1]

(1) In chimeric experiments involving transfers of human brain or retinal stem cells into early nonhuman embryos, the number of cells transferred should be limited to the smallest number necessary to reach reliable scientific conclusions in order to overcome any possibility that the resulting chimera would be considered able to develop capacities and characteristics associated with human dignity;

(2) The host animal chosen for the development of early blastocyst chimeras should not be overly morphologically or functionally related to humans in order to avoid any risk that the host's unique neurological

networks, which would just be developing, might be susceptible to human incursion; and

(3) Dissociated human stem cells, rather than postanatomical tissue transplants, should be used in the development of both early and later prenatal chimeras in order to guard against any possibility that characteristic human pattern development and formation associated with human dignity might take place in the nonhuman host. These limitations are directed toward avoiding the development in nonhumans of human-like capacities that fall into the cluster having to do with human dignity.

The juxtaposition of bodily materials of different sorts of living beings has become more acceptable since the ancients first developed the notion of the monstrous chimera. However, what is at issue today is not the mixing of materials from members of different species, which is accepted as ethical in several different research contexts, but whether the transfer of certain sorts of human materials, such as brain and retinal stem cells, to nonhuman animals would put human dignity at risk.

Some fear that in a posthuman future, society will use biotechnological manipulations of human materials to create new kinds of beings that resemble but do not fully approximate human beings. They believe that the assembly of such creatures would sacrifice that which is distinctive and significant about human beings and therefore would denigrate human dignity. We maintain that is possible to pursue the sort of neural stem cell research addressed here without violating human dignity if certain precautions are taken along the lines indicated in the above guidelines. The goal of these stem cell chimera studies is to support, rather than to denigrate, both human dignity and human well-being. Their pursuit does not threaten the belief at the core of our social ethic that human beings have a certain distinctive dignity, but instead upholds that central conviction.

We thank Ronald de Sousa, John Dick, Janet Rossant, Peter J. Cohen, Elizabeth Fisher, and Bruce Jennings for their helpful comments.

NOTE

1. We recognize that there is a remote possibility that transfers of human neural stem cells to embryonic nonhuman animals might have an effect on the germ cells of those animals. One of us has addressed this topic elsewhere (Cohen 2003), but it deserves fuller discussion in the future.

REFERENCES

Angioi, Karine; Hatier, Renée; Merle, Michel; and Duprez, Adrien. 2002. Xenografted Human Whole Embryonic and Fetal Entoblastic Organs Develop and Become Functional Adult-Like Micro-Organs. *Journal of Surgical Research* 102: 85–94.

Aquinas, Thomas. 1968. *Summa Theologica*, trans. Blackfriars. New York: McGraw Hill.

Balaban, Evan; Teillet, Marie-Aimee; and Le Douarin, Nicole. 1988. Application of the Quail-Chick Chimera System to the Study of Brain Development and Behavior. *Science* 241: 1339–42.

Bazopoulou-Kyrkanidou, Euterpe. 2001. Chimeric Creatures in Greek Mythology and Reflections in Science. *American Journal of Medical Genetics* 100: 66–80.

Beyleveld, Deryck, and Brownsword, Roger. 2001. *Human Dignity in Bioethics and Biolaw*. Oxford: Oxford University Press.

Boer, Gerard J. 1993. Ethical Guidelines for the Use of Human Embryonic or Fetal Tissue for Experimental and Clinical Neurotransplantation and Research. Network of European CNS Transplantation and Restoration (NECTAR). *Journal of Neurology* 242: 1–13.

Boyd, Richard. 1999. Homeostasis, Species and Higher Taxa. In *Species: New Interdisciplinary Essays*, ed. R. A. Wilson, pp. 141–85. Cambridge, MA: MIT Press.

Cao, Yilin; Vacanti, Joseph P.; Paige, Keith T.; et al. 1997. Transplantation of Chondrocytes Utilizing a Polymer-Cell Construct to Produce a Tissue-Engineered Cartilage in the Shape of a Human Ear. *Plastic Reconstructive Surgery* 100: 297–302.

Charland, Louis C. 2003. Are There Answers? *American Journal of Bioethics* 3 (3): W1–2.

Cohen, Cynthia B. 1999. Selling Bits and Pieces of Humans to Make Babies: "The Gift of the Magi" Revisited. *Journal of Medicine and Philosophy* 24: 288–306.

———. 2003. Creating Human-Animal Chimeras: Of Mice and Men. *American Journal of Bioethics* 3 (3): W3–5.

Crowe, Michael. 1977. *The Changing Profile of the Natural Law*. The Hague: Martinus Nijhoff.

d'Entreves, Alexander P. 1970. *Natural Law: An Introduction to Legal Philosophy*, second revised edition. London: Hutchinson.

de Sousa, Ronald. 1984. The Natural Shiftiness of Natural Kinds. *Canadian Journal of Philosophy* XIV: 561–81.

de Waal, Frans. 1996. *Good Natured: The Origins of Right and Wrong in Humans and Other Animals*. Cambridge, MA: Harvard University Press.

DeWitt, Natalie. 2002. Biologists Divided over Proposal to Create Human-Mouse Embryos. *Nature* 420: 255.

Dick, John E.; Guenechea, Guillermo; Gan, Olga I.; and Dorrell, Craig. 2001. Distinct Classes of Human Stem Cells that Differ in Proliferative and Self-Renewal Potential. *Nature Immunology* 1: 75–82.

Douglas, Mary. 1966. *Purity and Danger*. London: Routledge and Kegan Paul.

Finlay, Barbara L., and Darlington, Richard B. 1995. Linked Regularities in the Development and Evolution of Mammalian Brains. *Science* 268: 1578–84.

Flanagan, Owen J, Jr. 1991. *Varieties of Moral Personality: Ethics and Psychological Realism*. Cambridge, MA: Harvard University Press.

Franklin, Sarah. 2003. Drawing the Line at Not-Fully-Human: What We Already Know. *American Journal of Bioethics* 3 (3): W25–27.

Gewirth, Alan. 1982. *Human Rights*. Chicago: University of Chicago Press.

———. 1992. Human Dignity as the Basis of Rights. In *The Constitution of Rights: Human Dignity and American Values*, ed. Michael J. Meyer and William A. Parent, pp. 1–9. Ithaca: Cornell University Press.

Goldstein, Ronald S.; Drukker, Micha; Reubinoff, Benjamin E.; and Benvenisty, Nissim. 2002. Integration and Differentiation of Human Embryonic Stem Cells Transplanted to the Chick Embryo. *Developmental Dynamics* 225: 80–86.

Graves, Robert. 1960. *The Greek Myths*. Vol. 1. New York: Penguin.

Griffiths, Paul E. 1999. Squaring the Circle: Natural Kinds with Historical Essences. In *Species: New Interdisciplinary Essays*, ed. R. A. Wilson, pp. 210–28. Cambridge, MA: MIT Press.

Harris, John. 1998. Cloning and Human Dignity. *Cambridge Quarterly of Healthcare Ethics* 7: 163–67.

Hill, Thomas E., Jr. 1992. *Dignity and Practical Reason in Kant's Ethical Theory*. Ithaca and London: Cornell University Press .

Hull, David L. 1999. On the Plurality of Species: Questioning the Party Line. In *Species: New Interdisciplinary Essays*, ed. R. A. Wilson, pp. 23–48. Cambridge, MA: MIT Press.

Kamel-Reid, Suzanne, and Dick, John E. 1988. Engraftment of Immune-Deficient Mice with Human Hematopoietic Stem Cells. *Science* 242: 1706–9.

Kant, Immanuel. 1956. *Critique of Practical Reason*, trans. Lewis White Beck. Indianapolis: Bobbs-Merrill.

———. 1964. *Groundwork of the Metaphysics of Morals*, trans. H. J. Paton. New York: Harper and Row.

Karpowicz, Phillip. 2003. In Defense of Stem Cell Chimeras: A Response to "Crossing Species Boundaries." *American Journal of Bioethics* 3 (3): W17–19.

———; Cohen, Cynthia; and Van der Kooy, Derek. 2004. It is Ethical to Transplant Human Stem Cells into Nonhuman Embryos. *Nature Medicine* 10: 331–35.

Kass, Leon F. 1997. The Wisdom of Repugnance. *New Republic* 2: 17–26.

———. 1985. Teleology, Darwinism, and the Place of Man: Beyond Chance and Necessity? In his *Toward a More Natural Science: Biology and Human Affairs*, pp. 249–75. New York: Free Press.

———. 2001. Why We Should Ban Human Cloning Now: Preventing a Brave New World. Available at *http://www.thenewrepublic.com/052101/kass052101_print.html*. Accessed 25 May 2005.

———. 2002. *Life, Liberty, and the Defense of Dignity: The Challenge for Bioethics*. San Francisco: Encounter Books.

Kaufman, R.; Mielke, V.; Reimann, J.; et al. 1993. Cellular and Molecular Composition of Human Skin in Long-Term Xenografts on SCID Mice. *Experimental Dermatology* 2: 209–16.

Kekes, John. 1986. Moral Intuition. *American Philosophical Quarterly* 23: 83–93.

Kornack, David R., and Rakic, Pasko. 1988. Changes in Cell-Cycle Kinetics During the Development and Evolution of Primate Neocortex. *Proceedings of the National Academy of Sciences*. 95: 1242–46.

Krieger, Lisa M. 2002. Treading New Ethical Ground, Scientists Put a Bit of Man into a Mouse. *Mercury News* (8 December). Available at *http://www.sililconvalley.com/mid/silicon valley/business/columnists/gmsv/47001*. Accessed 25 May 2005.

Kuhse, Helga. 2000. Is There a Tension Between Autonomy and Dignity? In *Bioethics and Biolaw, vol. II: Four Ethical Principles*, ed. Peter Kemp, Jacob Rendtorff, and Niels Mattson Johansen, pp. 61–74. Copenhagen: Rhodos International Science and Art Publishers and Centre for Ethics and Law.

Levi-Strauss, Claude. 1978. *Myth and Meaning*. London: Routledge and Kegan Paul.

Macklin, Ruth. 2003. Dignity Is a Useless Concept. *British Medical Journal* 327: 1419–20.

Mayr, Ernst. 1988. *Toward a New Philosophy of Biology: Observations of an Evolutionist*. Cambridge, MA: Harvard University Press.

Midgely, Mary. 1978. *Beast and Man: The Roots of Human Nature*. New York: Meridian.

———. 2000. Biotechnology and Monstrosity: Why We Should Pay Attention to the "Yuk Factor." *Hastings Center Report* 30 (5): 7–15.

Ourednik, Vaclav; Ourednik Jitka; Flax Jonathan D.; et al. 2001. Segregation of Human Neural Stem Cells in the Developing Primate Forebrain. *Science* 293: 1820–24.

Polzin, V. J.; Anderson, D. L.; Anderson, G. B.; et al. 1987. Production of Sheep-Goat Chimeras by Inner Cell Mass Transplantation. *Journal of Animal Science* 65: 325–30.

Porter, Jean. 1999. *Natural and Divine Law: Reclaiming the Tradition for Christian Ethics*. Grand Rapids: William B. Eerdmans.

President's Council on Bioethics. 2003. President's Council on Bioethics Meeting of 16 October. Available at *http://bioethisprint.bioethics.gov/transcripts/oct03/session2.html*. Accessed 25 May 2005.

Pullman, Daryl. 2004. Death, Dignity and Moral Nonsense. *Journal of Palliative Care* 20: 171–78.

Raychaudhuri, S.; Sanyal, M.; Raychaudhuri, S. K.; et al. 2001. Severe Combined Immunodeficiency Mouse-Human Skin Chimeras: A Unique Animal Model for the Study of Psoriasis and Cutaneous Inflammation. *British Journal of Dermatology* 144: 931–39.

Robert, Jason Scott, and Baylis, Francoise. 2003. Crossing Species Boundaries. *American Journal of Bioethics* 3 (3): 1–13.

Rollin, Bernard E. 2003. Ethics and Species Integrity. *American Journal of Bioethics* 3 (3): 15–16.

Robertson, John A. 1994. *Children of Choice: Freedom and the New Reproductive Technologies*. Princeton, NJ: Princeton University Press.

Ross, W. D. 1930. *The Right and the Good*. Oxford: Oxford University Press.

Savulescu, Julian. 2003. Human-Animal Transgenesis and Chimeras Might Be an Expression of Our Humanity. *American Journal of Bioethics* 3 (3): 3–5.

Stout, Jeffrey. 1988. *Ethics After Babel: The Languages of Morals and Their Discontents*. Boston: Beacon Press.

Streiffer, Robert. 2003. In Defense of the Moral Relevance of Species Boundaries. *American Journal of Bioethics* 3 (3): 37–38.

Vince, Gaia. 2004. Pig-Human Chimeras Contain Cell Surprise. *New Scientist* 13: 42.

Wade, Nicholas. 2002. Is the World Ready for a Man-Mouse? *New York Times* (28 November): 15.

Wells, H. G. 1896. *The Island of Dr. Moreau*. Garden City, NY: Garden City Publishing.

Wilson, Robert A. 1999. Realism, Essence, and Kind: Resuscitating Species Essentialism? In his *Species: New Interdisciplinary Essays*, pp. 188–207. Cambridge, MA: MIT Press.

Zanjani, Esmail D.; Mackintosh, F. Roy; and Harrison, Michael R. 1991. Hematopoietic Chimerism in Sheep and Nonhuman Primates by In Utero Transplantation of Fetal Hematopoeitic Stem Cells. *Blood Cells* 17: 349–63; discussion pp. 364–66.

BEFORE THE UNITED STATES DEPARTMENT OF HEALTH AND HUMAN SERVICES

APPENDIX VOLUME 2 TO THE CITIZEN PETITION FOR RULEMAKING TO PROTECT HUMANIZED CHIMERAS UNDER THE PUBLIC HEALTH SERVICES ACT

ANIMAL LEGAL DEFENSE FUND,

Citizen petitioner,

170 E. Cotati Ave.

Cotati, CA 94931

Filing with:

KATHLEEN SEBELIUS,

In her official capacity as Secretary of the

United States Department of Health and Human Services,

200 Independence Avenue S.W.

Washington, D.C. 20201

APPENDIX VOLUME 2 TAB

Guidelines for Human Embryonic Stem Cell Research, NAT'L ACADEMY OF SCIENCES (2005) n.61-1

2007 Amendments to the National Academies' Guidelines for Human Embryonic Stem Cell Research, NAT'L ACADEMY OF SCIENCES (2007) n.61-2

2008 Amendments to the National Academies' Guidelines for Human Embryonic Stem Cell Research, NAT'L ACADEMY OF SCIENCES (2008) n.61-3

Final Report of the National Academies' Human Embryonic Stem Cell Research Advisory Committee and 2010 Amendments to The National Academies' Guidelines for Human Embryonic Stem Cell Research, NAT'L ACADEMY OF SCIENCES (2010) n.61-4

Belmont Report, The Nat'l Comm'n for the Protection of Human Subjects of Biomedical and Behavioral Research, Ethical Principles and Guidelines for the Protection of Human Subjects of Research (Apr. 18, 1979) n.82

How Tuskegee Changed Research Practices, Centers for Disease Control, available at http://www.cdc.gov/tuskegee/after.htm (last updated June 15, 2011) n.83

The Tuskegee Timeline, Centers for Disease Control, available at http://www.cdc.gov/tuskegee/timeline.htm (last updated June 15, 2011) n.84

Dolgin, *Time to ditch stand-alone stem cell oversight panels, experts say*, NATURE MEDICINE, vol. 19, p. 250 (Mar. 2013) n.137

James et al., *Contribution of Human Embryonic Stem Cells to Mouse Blastocysts*, DEVELOPMENTAL BIOLOGY vol. 295, pp. 90-102 (2006) n.143

Guidelines for Human Embryonic Stem Cell Research,

NAT'L ACADEMY OF SCIENCES (2005)

GUIDELINES FOR HUMAN EMBRYONIC STEM CELL RESEARCH

Committee on Guidelines for Human Embryonic Stem Cell Research

Board on Life Sciences
Division on Earth and Life studies

Board on Health Sciences Policy
Institute of Medicine

NATIONAL RESEARCH COUNCIL *AND*
INSTITUTE OF MEDICINE
OF THE NATIONAL ACADEMIES

THE NATIONAL ACADEMIES PRESS
Washington, D.C.
www.nap.edu

THE NATIONAL ACADEMIES PRESS 500 Fifth Street, NW Washington, DC 20001

NOTICE: The project that is the subject of this report was approved by the Governing Board of the National Research Council, whose members are drawn from the councils of the National Academy of Sciences, the National Academy of Engineering, and the Institute of Medicine. The members of the committee responsible for the report were chosen for their special competences and with regard for appropriate balance.

This material is based on work supported by the National Academies, the Ellison Medical Foundation, and the Greenwall Foundation.

Library of Congress Cataloging-in-Publication Data

Guidelines for human embryonic stem cell research / Board on Life Sciences, National Research Council, Board on Health Sciences Policy, Institute of Medicine.
p. cm.
Includes bibliographical references and index.
ISBN 0-309-09653-7 (pbk.) — ISBN 0-309-55024-6 (pdf) 1. Embryonic stem cells—Research. 2. Human embryo—Research. I. National Research Council (U.S.). Board on Life Sciences. II. National Research Council (U.S.). Board on Health Sciences Policy.
QH588.S83G85 2005
616′.02774—dc22
2005016338

Additional copies of this report are available from the National Academies Press, 500 Fifth Street, NW, Lockbox 285, Washington, DC 20055; (800) 624-6242 or (202) 334-3313 (in the Washington metropolitan area); Internet, http://www.nap.edu.

Cover: A cluster of motor neurons and neural fibers derived from human embryonic stem cells in the lab of University of Wisconsin-Madison stem cell researcher and neurodevelopmental biologist Su-Chun Zhang. The motor neurons are shown in red; neural fibers appear green and the blue specks indicate DNA in cell nuclei. These motor neurons were developed from one of James Thomson's original human embryonic stem cell lines. Copyright for the photograph is held by the University of Wisconsin's Board of Regents.

Printed in the United States of America

THE NATIONAL ACADEMIES

Advisers to the Nation on Science, Engineering, and Medicine

The **National Academy of Sciences** is a private, nonprofit, self-perpetuating society of distinguished scholars engaged in scientific and engineering research, dedicated to the furtherance of science and technology and to their use for the general welfare. Upon the authority of the charter granted to it by the Congress in 1863, the Academy has a mandate that requires it to advise the federal government on scientific and technical matters. Dr. Ralph J. Cicerone is president of the National Academy of Sciences.

The **National Academy of Engineering** was established in 1964, under the charter of the National Academy of Sciences, as a parallel organization of outstanding engineers. It is autonomous in its administration and in the selection of its members, sharing with the National Academy of Sciences the responsibility for advising the federal government. The National Academy of Engineering also sponsors engineering programs aimed at meeting national needs, encourages education and research, and recognizes the superior achievements of engineers. Dr. Wm. A. Wulf is president of the National Academy of Engineering.

The **Institute of Medicine** was established in 1970 by the National Academy of Sciences to secure the services of eminent members of appropriate professions in the examination of policy matters pertaining to the health of the public. The Institute acts under the responsibility given to the National Academy of Sciences by its congressional charter to be an adviser to the federal government and, upon its own initiative, to identify issues of medical care, research, and education. Dr. Harvey V. Fineberg is president of the Institute of Medicine.

The **National Research Council** was organized by the National Academy of Sciences in 1916 to associate the broad community of science and technology with the Academy's purposes of furthering knowledge and advising the federal government. Functioning in accordance with general policies determined by the Academy, the Council has become the principal operating agency of both the National Academy of Sciences and the National Academy of Engineering in providing services to the government, the public, and the scientific and engineering communities. The Council is administered jointly by both Academies and the Institute of Medicine. Dr. Ralph J. Cicerone and Dr. Wm. A. Wulf are chair and vice chair, respectively, of the National Research Council.

www.national-academies.org

COMMITTEE ON GUIDELINES FOR HUMAN EMBRYONIC STEM CELL RESEARCH

RICHARD O. HYNES (*Co-Chair)*, Massachusetts Institute of Technology, Cambridge, Massachusetts
JONATHAN D. MORENO (*Co-Chair)*, University of Virginia, Charlottesville, Virginia
ELIZABETH PRICE FOLEY, Florida International University, Miami, Florida
NORMAN FOST, University of Wisconsin, Madison, Wisconsin
H. ROBERT HORVITZ, Massachusetts Institute of Technology, Cambridge, Massachusetts
MARCIA IMBRESCIA, Arthritis Foundation, Lynnfield, Massachusetts
TERRY MAGNUSON, University of North Carolina, Chapel Hill, North Carolina
CHERYL MWARIA, Hofstra University, Hempstead, New York
JANET ROSSANT, Mount Sinai Hospital, Toronto, Ontario, Canada
JANET D. ROWLEY, University of Chicago, Chicago, Illinois

Board on Life Sciences Liaison to the Committee

R. ALTA CHARO, University of Wisconsin, Madison, Wisconsin

Staff

FRANCES SHARPLES, Study Director
ROBIN SCHOEN, Senior Program Officer
MATTHEW D. MCDONOUGH, Program Assistant
KATHI E. HANNA, Science Writer
NORMAN GROSSBLATT, Senior Editor

BOARD ON LIFE SCIENCES

Staff

Preface

We are pleased to offer our committee's report on guidelines for human embryonic stem cell research. This report and its recommendations are the result of many hours of committee meetings as well as a public workshop. During those sessions we heard from many dedicated and talented people who represent a wide range of views. We have tried to take these diverse perspectives into account in a report that mirrors the seriousness with which we have reflected upon them. Our task was made more difficult and also more significant by events in the worlds of science and public affairs, which altered the terrain even as we explored it. All of us on the committee have appreciated the opportunity to be part of this important and timely effort.

Great possibilities for improvements in human health are offered by research using human stem cells, both adult and embryonic. Like many scientific advances, these technologies raise questions about balancing the evident promise against the potential for inappropriate application. In the case of embryonic stem cell research, there are differing opinions within our society about the relative merits and risks of various approaches and there are philosophical differences about what is or is not appropriate. Some believe strongly that we should not turn away from the promise that embryonic stem cells will provide new therapeutic advances. Others believe that the derivation and application of human embryonic stem cells will undermine the dignity of human life. These disparate views are deeply and sincerely held and must be considered as we move forward in advancing this research. Some of the qualms arise from unfamiliarity and the "shock of the new," but others arise from concerns about the nature of human life, about ethical treatment of reproductive

materials and about exploitation of donors of such materials. Those ethical concerns need to be balanced against the duty to provide the best medical care possible, enhancing the quality of life and alleviating suffering for many people. The challenge to our society is to achieve that balance.

Scientific inquiry should not proceed unfettered, without consideration for the ethical and public policy imperatives of the society in which it operates. On the other hand, concerns about potential ethical complexities should be cause for judicious oversight and regulation, not necessarily for prohibition. Our democratic society should be capable of entertaining challenges to familiar beliefs and adapting to new conditions without yielding on its fundamental values. We believe that it is possible to do so, that human dignity will be enhanced, rather than diminished, by the great project of addressing the suffering that attends illness. Freedom of inquiry and a confident attitude toward the future are at the heart of America's civic philosophy, in which the freedom to explore controversial ideas is celebrated rather than suppressed. That is one reason that our country's scientific establishment is the envy of the world, a source of our inventive energy that was celebrated by Thomas Jefferson who wrote, "Liberty is the great parent of science and of virtue; and a nation will be great in both in proportion as it is free."

In that spirit we offer this report.

Richard O. Hynes
Jonathan D. Moreno
Co-chairs, Committee on Guidelines for
Human Embryonic Stem Cell Research

Acknowledgments

Like all National Academies reports, this one is the result of the contributions of many people. First, we sincerely thank all the speakers who participated in our workshop, "Guidelines for Human Embryonic Stem Cell Research," on October 12-13, 2004. A workshop agenda and a list of the workshop speakers with their biographies are included in Appendix C. Without their input, this report would not have been possible.

Second, we would like to thank the Ellison Medical Foundation and the Greenwall Foundation for their financial support of this activity.

This report has been reviewed in draft form by persons chosen for their diverse perspectives and technical expertise in accordance with procedures approved by the National Research Council's Report Review Committee. The purpose of this independent review is to provide candid and critical comments that will assist the institution in making its published report as sound as possible and to ensure that the report meets institutional standards of objectivity, evidence, and responsiveness to the study charge. The review comments and draft manuscript remain confidential to protect the integrity of the deliberative process. We wish to thank the following for their review of this report:

Alexander Capron, World Health Organization
Mark Fishman, Novartis
Linda Giudice, Stanford School of Medicine
Virginia Hinshaw, University of California, Davis
Brigid Hogan, Duke University
Bernard Lo, University of California, San Francisco

Michael Manganiello, Christopher Reeve Foundation
Doug Melton, Harvard University
Catherine Racowsky, Brigham and Women's Hospital
Laura Robbins, Weill Cornell Medical College
John Robertson, University of Texas Law School
Harold Shapiro, Princeton University
Harold Varmus, Memorial Sloan-Kettering Cancer Center
LeRoy Walters, Georgetown University

Although the reviewers listed above have provided many constructive comments and suggestions, they were not asked to endorse the conclusions or recommendations nor did they see the final draft of the report before its release. The review of this report was overseen by Floyd E. Bloom, Scripps Research Institute, and William H. Danforth, Washington University. Appointed by the National Research Council, they were responsible for making certain that an independent examination of this report was carried out in accordance with institutional procedures and that all review comments were carefully considered. Responsibility for the final content of this report rests entirely with the authoring committee and the institution.

Finally, we wish to acknowledge Dr. Kathi Hanna, our superb science writer, and the National Research Council staff (Fran Sharples, Robin Schoen, Matt McDonough, and Norman Grossblatt) for their thorough, thoughtful, and efficient assistance with all aspects of the preparation of this report.

Contents

Summary 1

1 Introduction 15

2 Scientific Background of Human Embryonic Stem Cell Research 29

3 Addressing Ethical and Scientific Concerns Through Oversight 47

4 Current Regulation of Human Embryonic Stem Cell Research 63

5 Recruiting Donors and Banking hES Cells 81

6 National Academies Guidelines for Research on Human Embryonic Stem Cells 97

References 109

Glossary 115

Abbreviations 121

Appendixes

A Compilation of Recommendations 123

B Committee Biographies 131

C Workshop Agenda and Speaker Biographies 137

Index 155

Summary

This report provides guidelines for the responsible practice of human embryonic stem (hES) cell research. Since 1998, the volume of research being conducted using hES cells has expanded primarily using private funds because of restrictions on the use of federal funds for such research. Although privately funded hES cell research is currently subject to many of the same oversight requirements as other biomedical research, given restricted federal involvement and the absence of federal regulations specifically designed for hES cell research, there is a perception that the field is unregulated. More accurately, there is a patchwork of existing regulations that are applicable to hES cell research, many of which were not designed with this research specifically in mind, and there are gaps in how well they cover hES cell research. In addition, hES cell research touches on many ethical, legal, scientific, and policy issues that are of concern to the public. The guidelines, which are set forth in the final chapter of the report, are intended to enhance the integrity of privately funded hES cell research both in the public's perception and in actuality by encouraging responsible practices in the conduct of that research. The body of the report provides the background and rationale for the choices involved in formulating the guidelines.

In 1998, James Thomson and co-workers became the first scientists to derive and successfully culture human embryonic stem cells (hES cells) from a human blastocyst, an early human embryo of approximately 200 cells, donated by a couple who had completed infertility treatments. Although ES cells had been derived from mouse blastocysts since 1981, this achievement with human cells was significant because of its implications for improved health. The dual capacity of hES cells for self-renewal and for differentiation into repair cells offers great potential for under-

standing disease development and progression, for regenerative medicine, and for targeted drug development.

In addition to that research accomplishment, the cloning of Dolly the sheep in 1997 using a technique called somatic cell nuclear transfer (SCNT) or, more simply, nuclear transfer (NT) provided a means of generating ES cells with defined genetic makeup. hES cell preparations could potentially be produced by using NT to replace the nucleus of a human oocyte, trigger development, and then isolate hES cells at the blastocyst stage. The advantage of using NT to derive hES cells is that the nuclear genomes of the resulting hES cells would be identical with those of the donors of the somatic cells. One obvious benefit is that this would avoid the problem of rejection if cells generated from the hES cells were to be transplanted into the donor. A more immediate benefit would be facilitation of a wide array of experiments to explore the underpinnings of genetic disease and possible forms of amelioration and cure. Some such experiments will not be possible using hES cells derived from blastocysts generated by *in vitro* fertilization (IVF), in which the nuclear genomes are not defined. Although the promise of using NT for such research is as yet unrealized, most researchers believe that it will be a critical source of both important knowledge and clinical resources. Use of NT for biomedical research, as distinct from its use to create a human being, has been considered by several advisory groups to be ethically acceptable provided that such research is conducted according to established safeguards against misuse and has undergone proper prior review. However, there is nearly universal agreement that use of NT to attempt to produce a child should not be allowed at present. The medical risks are unacceptable, and many people have additional objections to using this procedure for attempts at human procreation.

hES cells currently can be derived from three sources: blastocysts remaining after infertility treatments and donated for research, blastocysts produced from donated gametes (oocytes and sperm), and the products of NT. Ethical concerns about those sources of hES cells—combined with fears that the use of NT for research could lead to its use to produce a child—have fostered much public discussion and debate. In addition, concern has been expressed about whether and how to restrict the production of human/nonhuman chimeras in hES cell research. Research using chimeras will be valuable in understanding the etiology and progression of human disease and in testing new drugs, and will be necessary in preclinical testing of hES cells and their derivatives.

Because there is widespread agreement in the international scientific community about the potential value of hES cell research, the volume of this research has expanded since 1998, despite restrictions in the United States. First, federal legislation forbids the use of federal monies for any research that destroys an embryo; this effectively prevents any use of federal funds to derive hES cells from blastocysts. Second, research with established hES cell lines is limited by a policy announced by President George W. Bush in 2001 that restricts federal funding to research con-

ducted with specific federally approved hES cell lines already in existence before August 9, 2001. Despite the restricted use of federal funds for research of this kind, the derivation of new cell lines is proceeding legally in the private sector and in academic settings with private funds except in those states where such research has been partially or totally banned.

Privately funded hES cell research is subject to some regulation or other constraints primarily through human subjects protections regulations, limits placed on licensees by the holders of NT and hES cell patents, animal care and use regulations, state laws, and self-imposed institutional guidelines at companies and universities that are now doing or contemplating this research. Those aiming to produce biological therapies are also subject to Food and Drug Administration (FDA) regulation. However, because of the absence of federal funding for most current hES cell research, some standard protections may be lacking, and the implementation of protections is not uniform across the country. Moreover, the techniques for deriving the cells do not yet amount to fully developed standard research tools, and the development of any therapeutic application remains some years away. The best way to move forward with hES cell research in pursuit of scientific goals and new therapies is with a set of guidelines to which the U.S. scientific community will adhere. Heightened oversight also is essential to assure the public that such research is being conducted in an ethical manner.

Established criteria for deriving hES cell lines and reviewing research will help to ensure that the derivation, storage, and maintenance of cells meet a standard set of requirements for provenance and ethical review. Because not all scientists want or have the resources to derive new hES cell lines, the ability to share cell lines will create greater access for qualified scientists to participate in stem cell research. The tradition of sharing materials and results with colleagues speeds scientific progress and symbolizes to the nonscientific world that the goals of science are to expand knowledge and to improve the human condition. One key reason for the remarkable success of science since its emergence in modern form—besides the application of the scientific method itself—is the communal nature of scientific activity.

STATEMENT OF COMMITTEE TASK

The National Academies initiated this project to develop guidelines for hES cell research to advance the science in a responsible manner. The Committee on Guidelines for Human Embryonic Stem Cell Research was asked to develop guidelines to encourage responsible practices in hES cell research—regardless of source of funding—including the use and derivation of new stem cell lines derived from surplus blastocysts, from blastocysts produced with donated gametes, or from blastocysts produced using NT. The guidelines take ethical and legal concerns into account and encompass the basic science and health science policy issues related to the development and use of hES cells for research and eventual therapeutic purposes, such as

1. Recruitment of donors of blastocysts, gametes, or somatic cells including medical exclusion criteria, informed consent, the use of financial incentives, risks associated with oocyte retrieval, confidentiality, and the interpretation of genetic information that is developed from studies with these materials and that might have importance to the donors.
2. The characterization of stem cells for purposes of standardization and for validation of results.
3. The safe handling and storage of blastocysts and stem cell material and conditions for transfer of such material among laboratories.
4. Prerequisites to hES cell research (such as examination of alternative approaches), appropriate uses of hES cells in research or therapy and limitations on the use of hES cells.
5. Safeguards against misuse.

To conduct its work, the committee surveyed the current state of science in this field and probable pending developments, reviewed the policy and ethical issues posed by the research, examined professional and international regulations and guidelines that relate to hES cell research, and conducted a 2-day workshop to hear representatives of many scientific, ethical, and public policy perspectives. The committee did not revisit the debate about whether hES cell research should be pursued; it assumed that both hES cell and adult stem cell research would continue in parallel with federal and nonfederal funding.

WHAT THE GUIDELINES COVER

The guidelines are intended for the use of the scientific community, including researchers in university, industry, or other private-sector organizations. They cover all derivations of hES cell lines and all research using hES cells derived from

1. Blastocysts made for reproductive purposes and later obtained for research from IVF clinics.
2. Blastocysts made specifically for research using IVF.
3. Somatic cell nuclear transfer (NT) into oocytes.

The guidelines do not cover research with nonhuman stem cells. In addition, many but not all of the guidelines and concerns addressed in this report are common to other areas of human stem cell research, such as research with adult stem cells, fetal stem cells, or embryonic germ cells derived from fetal tissue. Institutions and investigators conducting research with such materials should consider which individual provisions of the guidelines set forth in this report are relevant to their research.

The guidelines do not apply to reproductive uses of NT, which are addressed in the 2002 report *Scientific and Medical Aspects of Human Reproductive Cloning*, in

which the National Academies stated that "Human reproductive cloning should not now be practiced. It is dangerous and likely to fail." Although these guidelines do not specifically address attempts to use NT for reproductive purposes, it continues to be the view of the National Academies that such attempts should not be conducted at this time.

MAJOR RECOMMENDATIONS

This summary provides the major recommendations made by the committee, each of which supports an operational aspect of the guidelines presented in Chapter 6. Central to the recommendations is a dual system of oversight at the institutional and national levels. This system of oversight will ensure that the highest ethical, legal, and scientific standards are met in the derivation, storage, and use of hES cells in research.

Institutional Oversight of hES Cell Research

The ethical and legal concerns involved in hES cell research make increased local oversight by research institutions appropriate. Because of the complexity and novelty of many of the issues involved in hES cell research, the committee believes that all research institutions conducting hES cell research should create special review bodies to oversee this emerging field of research. Such committees will be responsible for ensuring that all applicable regulatory requirements are met and that hES cell research is conducted in accordance with the guidelines set forth in this report.

To provide local oversight of all issues related to derivation and research use of hES cell lines and to facilitate education of investigators involved in hES cell research, all institutions conducting hES cell research should establish an Embryonic Stem Cell Research Oversight (ESCRO) committee. The committee should include representatives of the public and persons with expertise in developmental biology, stem cell research, molecular biology, assisted reproduction, and ethical and legal issues in hES cell research. The committee will not substitute for an Institutional Review Board but rather will provide an additional level of review and scrutiny warranted by the complex issues raised by hES cell research. The committee will also review basic hES cell research using preexisting anonymous cell lines that does not require consideration by an Institutional Review Board.

The ESCRO committee will assist investigators in assessing which regulations might apply to proposed research activities. The committee could serve as a clearinghouse for hES cell research proposals and could assist investigators in identifying the types and levels of review required for a given protocol. For example, the

creation of a chimera might involve both an Institutional Review Board (IRB), if cells are to be obtained from human donors for research, and an Institutional Animal Care and Use Committee (IACUC), if animals are to be used in the research. In some instances, Institutional Biosafety Committees (IBCs) and radiation safety committees might also have roles to play in research review. If hES cell research involves potential clinical applications (such as development of products to be tested in humans), FDA regulations will apply. However, care should be taken that the ESCRO committee does not duplicate or interfere with the proper functions of an IRB or other existing institutional committee. The functions of IRBs and ESCRO committees are distinct and should not be confused.

One particularly important aspect of regulatory compliance for hES cell research deals with protection of donors of blastocysts and gametes. Laboratory research that uses hES cells is generally not subject to federal regulations governing research with human subjects unless it involves personally identifiable information about the cell line's progenitors. In general, research institutions are likely already to have rules in place for research involving other biological tissues, and hES cell research, like any other form of biological or biomedical research, would be covered by these rules and in many cases will not require further review. In the case of hES cell research, however, it will be critically important for investigators and institutions to know the provenance of hES cell lines, particularly if the cell lines are imported from another institution. That would include obtaining an assurance that the process by which the cells were obtained was approved by an IRB to ensure that donors provided voluntary informed consent and that risks were minimized.

Through its Embryonic Stem Cell Research Oversight committee, each research institution should ensure that the provenance of hES cells is documented. Documentation should include evidence that the procurement process was approved by an Institutional Review Board to ensure adherence to the basic ethical and legal principles of informed consent and protection of confidentiality.

The second role of ESCRO committees is to review research proposals that involve particularly sensitive kinds of research, including all proposals to generate additional hES cell lines by any means. The vast majority of *in vitro* experiments using already derived hES cell lines are unlikely to raise serious ethical issues, and will require minimal review. Some research with hES cells, such as the creation of human/nonhuman chimeras, will need more extensive review.

Other types of studies should not be permitted at this time (such as implantation of embryos or cells into a human uterus or breeding of any interspecies chimera). Still others warrant careful consideration, including research in which identifying information about the donors is available or becomes known to the investigator and experiments involving implantation of hES cells or human neural progenitor cells into nonhuman animals. Because of the sensitive nature of some aspects of hES cell research, it is critical that the scientific community propose and

implement limits on what is to be allowed and provide clear guidance on which research activities require greater scrutiny (as discussed in the full report). Thus, a primary activity of ESCRO committees will be to ensure that inappropriate research is not conducted and that sensitive research is well justified (as explained in the full report) and subject to appropriate additional oversight. Oversight will in many instances conform to a higher standard than required by existing laws or regulations. ESCRO committees should have suitable scientific and ethical expertise to conduct their own reviews and should have the resources to coordinate the various other reviews that may be required for a particular protocol. A pre-existing committee could serve the functions of the ESCRO committee provided that it has the recommended expertise to perform the various roles described in this report.

Embryonic Stem Cell Research Oversight (ESCRO) committees or their equivalents should divide research proposals into three categories in setting limits on research and determining the requisite level of oversight:

(a) Research that is permissible after notification of the research institution's ESCRO committee and completion of the reviews mandated by current requirements. Purely *in vitro* hES cell research with pre-existing coded or anonymous hES cell lines in general is permissible provided that notice of the research, documentation of the provenance of the cell lines, and evidence of compliance with any required Institutional Review Board, Institutional Animal Care and Use Committee, Institutional Biosafety Committee, or other mandated reviews, is provided to the ESCRO committee or other body designated by the investigator's institution.

(b) Research that is permissible only after additional review and approval by an ESCRO committee or other equivalent body designated by the investigator's institution.

(i) The ESCRO committee should evaluate all requests for permission to attempt derivation of new hES cell lines from donated blastocysts, from *in vitro* fertilized oocytes, or by nuclear transfer. The scientific rationale for the need to generate new hES cell lines, by whatever means, should be clearly presented, and the basis for the numbers of blastocysts or oocytes needed should be justified. Such requests should be accompanied by evidence of Institutional Review Board approval of the procurement process.

(ii) All research involving the introduction of hES cells into nonhuman animals at any stage of embryonic, fetal, or postnatal development should be reviewed by the ESCRO committee. Particular attention should be paid to the probable pattern and effects of differentiation and integration of the human cells into the nonhuman animal tissues.

(iii) Research in which personally identifiable information about the donors of the blastocysts, gametes, or somatic cells from which the hES cells were

derived is readily ascertainable by the investigator also requires ESCRO committee review and approval.

(c) Research that should not be permitted at this time.

(i) Research involving *in vitro* culture of any intact human embryo, regardless of derivation method, for longer than 14 days or until formation of the primitive streak begins, whichever occurs first.

(ii) Research in which hES cells are introduced into nonhuman primate blastocysts or in which any embryonic stem cells are introduced into human blastocysts.

(iii) No animal into which hES cells have been introduced at any stage of development should be allowed to breed.

Because stem cell research is subject to a greater degree of public interest and scrutiny than most other kinds of laboratory research, the committee recommends that each institution should maintain through its ESCRO committee a registry of hES cell lines in use and of investigators working in this field and descriptive information on the types of hES cell research in which they are engaged. The purposes of such a registry include facilitating distribution of educational information in light of evolving ethical, legal, or regulatory issues and enabling the institution to respond to public inquiry about the extent of its involvement in hES cell research.

ADDITIONAL RECOMMENDATIONS

The committee makes several additional recommendations pertaining to the need for IRB review of procurement procedures, the need for voluntary informed consent free of inducements, adherence to standards of clinical care, and compliance with all applicable federal regulations. Those recommendations are summarized here.

Review of the Procurement Process

Research involving hES cells will require access to human oocytes and embryos, necessitating some interaction between oocyte and blastocyst donors and people or institutions seeking to procure these materials for use in hES cell research. Individuals and couples who voluntarily and with full information donate somatic cells, gametes, or blastocysts for hES cell research should be assured that their donation is made for meritorious research and that all efforts will be made by those responsible for handling, storing, and using cell lines to protect donor confidentiality. IRB review of the procurement process, combined with a full informed consent process before donation, will facilitate the ethical conduct of this research.

Regardless of the source of funding and the applicability of federal regulations, an Institutional Review Board or its equivalent should review the procurement of gametes, blastocysts, or somatic cells for the purpose of generating new hES cell lines, including the procurement of blastocysts in excess of clinical need from infertility clinics, blastocysts made through *in vitro* fertilization specifically for research purposes, and oocytes, sperm, and somatic cells donated for development of hES cell lines through nuclear transfer.

Informed Consent of Donors

The donors of sperm, oocytes, or somatic cells used to make blastocysts for research are themselves rarely the subject of the research. Nevertheless, the physical interaction needed to obtain the materials brings them under the purview of the human subjects protections system, and IRB review is required. Thus, their fully informed and voluntary consent is required before such research use.

Institutional Review Boards may not waive the requirement for obtaining informed consent from any person whose somatic cells, gametes, or blastocysts are used in hES cell research.

When donor gametes have been used in the *in vitro* fertilization process, resulting blastocysts may not be used for research without consent of all gamete donors.

In addition to ensuring voluntary informed consent of all donors, there should be no financial incentives in the solicitation or donation of blastocysts, gametes, or somatic cells for research purposes. Nonfinancial incentives also should be avoided. For example, a donor's decision should not be influenced by anticipated personal medical benefits or by concerns about the quality of later care. Thus, a potential donor should be informed that there is no obligation to make such a donation, that no personal benefit will accrue as a result of the decision to donate (except in cases of autologous transplantation), and that no penalty will result from a decision to refuse to donate.

To facilitate autonomous choice, decisions related to the production of embryos for infertility treatment should be free of the influence of investigators who propose to derive or use hES cells in research. Whenever it is practicable, the attending physician responsible for the infertility treatment and the investigator deriving or proposing to use hES cells should not be the same person.

No cash or in kind payments may be provided for donating blastocysts in excess of clinical need for research purposes.

Women who undergo hormonal induction to generate oocytes specifically for research purposes (such as for nuclear transfer) should be reimbursed only for direct expenses incurred as a result of the procedure, as determined by an Institutional Review Board. No cash or in kind payments should be provided for donating oocytes for research purposes. Similarly, no payments should be made for donations of sperm for research purposes or of somatic cells for use in nuclear transfer.

This recommendation should not be interpreted as a commentary on commercial IVF practices, but as a narrow policy position specifically with respect to hES cell research. Furthermore, as with all the policies recommended by the committee, this policy should be regularly reviewed and reconsidered as the field matures and the experiences under other policies can be evaluated.

It is widely accepted that, whenever possible, donors' decisions to dispose of their blastocysts should be made separately from their decisions to donate them for research. Potential donors should be allowed to provide blastocysts for research only if they have decided to have those blastocysts discarded instead of donating them to another couple or storing them.

Consent for blastocyst donation should be obtained from each donor at the time of donation. Even people who have given prior indication of their intent to donate to research any blastocysts that remain after clinical care should nonetheless give informed consent at the time of donation. Donors should be informed that they retain the right to withdraw consent until the blastocysts are actually used in cell line derivation.

The current regulatory system specifies basic elements of information that must be provided to prospective participants during the informed consent process. In the context of donation for research, disclosure should ensure that potential donors understand the risks involved, if any. Potential donors should be told of all options concerning the handling and disposition of their blastocysts, including freezing for later use, donation to others for reproductive use, research use, or disposing of them in accordance with the facility's policies and practices. To the extent possible, potential donors should be informed of the array of future research uses before giving consent to donate blastocysts for research. Comprehensive information should be provided to all donors that is readily accessible and at a level that will facilitate an informed decision. Written informed consent should be obtained from all those who elect to donate blastocysts or gametes.

Adherence to Standards of Clinical Care

Clinical facilities that provide assisted reproductive technology services are obligated to protect the rights and safety of their patients and to behave in an ethical

manner. Researchers should not pressure members of the fertility treatment team to generate more oocytes than necessary for the optimal chance of reproductive success. An IVF clinic or other third party responsible for obtaining consent or collecting materials should not be able to pay for or be paid for the material it obtains, except for specifically defined cost-based reimbursements. Such restrictions on payment to those who obtain the embryos discourage the production during routine infertility procedures of excess oocytes that might later be used for research purposes.

No member of the clinical staff should be required to participate in providing donor information or securing donor consent for research use of gametes or blastocysts if he or she has a conscientious objection to hES cell research. However, that privilege should not extend to the appropriate clinical care of a donor or recipient.

Consenting or refusing to donate gametes or blastocysts for research should not affect or alter in any way the quality of care provided to prospective donors. That is, clinical staff must provide appropriate care to patients without prejudice regarding their decisions about disposition of their embryos.

Researchers may not ask members of the infertility treatment team to generate more oocytes than necessary for the optimal chance of reproductive success. An infertility clinic or other third party responsible for obtaining consent or collecting materials should not be able to pay for or be paid for the material obtained (except for specifically defined cost-based reimbursements and payments for professional services).

Compliance with All Relevant Regulations

If hES cell research involves transmission of personal health information about the donors, which will increasingly be the case as cell lines approach clinical application, it will be important for investigators, institutions, and IRBs to be aware of any privacy requirements that apply through the Health Insurance Portability and Accountability Act (HIPAA). Authorization should be obtained from donors for the transmission of specific health information, which should be secured to protect donor confidentiality.

Investigators, institutions, Institutional Review Boards, and privacy boards should ensure that authorizations are received from donors, as appropriate and required by federal human subjects protections and the Health Insurance Portability and Accountability Act, for the confidential transmission of personal health information to repositories or to investigators who are using hES cell lines derived from donated materials.

As the level of hES cell research in the United States increases, it is essential that

institutions and investigators adhere to applicable regulatory requirements and, given the increasing frequency of international collaboration in hES cell research, it will be important to monitor regulatory developments in other countries. The ESCRO committees will be charged with ensuring that U.S. investigators follow standards and procedures consistent with current regulations and with the guidelines recommended in this report.

FDA's Good Laboratory Practice regulations pertain to the management of laboratories that are developing products that might eventually be introduced into humans (for example, in a clinical trial). Those regulations do not cover basic exploratory studies conducted to determine whether a test article has any potential utility or to determine its physical or chemical characteristics, but they do encompass *in vivo* or *in vitro* experiments to determine their safety—an activity that would be characteristic of the preclinical phase of hES cell research. Failure to conform to FDA regulations, although not itself a violation of law, would render any hES cell lines less useful if they are considered for tissue transplantation or other cell-based therapies.

Investigators and institutions involved in hES cell research should conduct the research in accordance with all applicable laws and guidelines pertaining to recombinant DNA research and animal care.

hES cell research leading to potential clinical application must be in compliance with all applicable Food and Drug Administration (FDA) regulations. When FDA requires that a link be maintained to the donor source, investigators and institutions must ensure that the confidentiality of the donor is protected, that the donor understands that a link will be maintained and that, where applicable, federal human subjects protections and the Health Insurance Portability and Accountability Act or other privacy protections are followed.

Banking of hES Cell Lines

As hES cell research advances, it will be increasingly important for institutions that obtain, store, and use cell lines to have confidence in the value of stored cells, that is, confidence that they were obtained ethically and with informed consent of donors, that they are well characterized and screened for safety, and that their maintenance and storage meet the highest scientific standards.

Institutions that are banking or plan to bank hES cell lines should establish uniform guidelines to ensure that donors of material give informed consent through a process approved by an Institutional Review Board, and that meticulous records are maintained about all aspects of cell culture. Uniform tracking systems and common guidelines for distribution of cells should be established.

The full report lays out recommended standards for any facility engaged in obtaining and storing hES cell lines (see Chapter 5).

National Policy Review

As individual states and private entities move into hES cell research, it is important to initiate a national effort to provide a formal context in which the complex moral and oversight questions associated with this work can be addressed. The state of hES cell research and clinical practice and public policy surrounding these topics are in a state of flux and are likely to be so for several years. Therefore, the committee believes that some body should be established to review the policies and guidelines covering appropriate practices in this field, but not to review and approve specific research protocols, an activity that will best occur at the local institutional level. Such a body should periodically review the adequacy of the guidelines proposed in this report in light of changes in the science and emergence of new issues of public interest. New policies and standards may be appropriate for issues that cannot now be foreseen. The organization that sponsors this body should be politically independent and without conflicts of interest, should be respected in the lay and scientific communities, and able to call on suitable expertise to support this effort.

A national body should be established to assess periodically the adequacy of the guidelines proposed in this document and to provide a forum for a continuing discussion of issues involved in hES cell research.

CONCLUSION

Research using hES cells offers great promise for future improvements in health care. To realize those benefits, further research will be required, including derivation of additional hES cell lines and testing of their potential. Such research is already in progress in many institutions and there is a need for a common set of standards. The guidelines provided in this report focus on the derivation, banking, and use of hES cell lines. They provide an oversight process that will help to ensure that hES cell research is conducted in a responsible and ethically sensitive manner and in compliance with all regulatory requirements pertaining to biomedical research in general. Although the committee hesitates to recommend another bureaucratic entity to oversee biomedical research, in this case it believes the burden to be justified because of the special issues involved in hES cell research and because of the diverse entities that might have a role in the review process in a research institution.

The success of hES cell research rests with those conducting and supporting it. All scientific investigators and their institutions, regardless of their fields, bear the

ultimate responsibility for ensuring that they conduct themselves in accordance with professional standards and with integrity. In particular, people whose research involves hES cells should work closely with oversight bodies, demonstrate respect for the autonomy and privacy of those who may donate gametes and embryos, and be sensitive to public concerns about research involving human embryos.

To help ensure that these guidelines are taken seriously, stakeholders in hES cell research—sponsors, funding sources, research institutions, relevant oversight committees, professional societies, and scientific journals, as well as investigators—should develop policies and practices that are consistent with the principles inherent in these guidelines. Funding agencies, professional societies, journals, and institutional review panels can provide valuable community pressure and impose appropriate sanctions to ensure compliance. For example, ESCRO committees and IRBs should require evidence of compliance when protocols are reviewed for renewal, funding agencies should assess compliance when reviewing applications for support, and journals should require that evidence of compliance accompanies publication of results.

1

Introduction

Stem cells are capable of self-renewal and also of differentiation into specialized cells. Some stem cells are more committed to a particular developmental fate than others; for example, they divide and mature into cells of a specific type or limited spectrum of types (such as heart, muscle, blood, or brain cells). Other stem cells are less committed and retain the potential to differentiate into many types of cells. It is believed that stem cells also form reservoirs of repair cells to replace cells and tissues that degenerate over the life span of the organism. The dual capacity of stem cells for self-renewal and for differentiation into particular types of cells and tissues offers great potential for regenerative medicine. The various types of stem cells differ substantially in these properties.

In 1998, scientists reported three separate sets of research findings related to the isolation and potential use of human embryonic stem cells. Two of the 1998 reports were published by independent teams of scientists that had accomplished the isolation and culture of human embryonic stem cells (hereafter referred to as hES cells) and human embryonic germ cells (hereafter referred to as hEG cells). One report described the work of James Thomson and his co-workers at the University of Wisconsin, who derived hES cells from a human blastocyst, comprising about 200 cells, donated by a couple that had received infertility treatments (Thomson et al., 1998). Their accomplishment was significant, because hES cells are considered by many to be the most fundamental and extraordinary of the stem cells; unlike the more differentiated adult stem cells or other cell types, they are pluripotent. (See the glossary for terminology used in this report.)

The second report described the successful isolation of hEG cells in the laboratory of John Gearhart and his colleagues at the Johns Hopkins University. That

team derived stem cells from primordial gonadal tissue obtained from cadaveric fetal tissue (Shamblott et al., 1998). hEG cells, which originate from the primordial reproductive cells of the developing fetus, have properties similar to those of hES cells, although there has been less research into their potential.

The third report, an article in the November 12, 1998, edition of the *New York Times*, described work funded by Advanced Cell Technology of Worcester, Massachusetts. The report was not published in a scientific journal and therefore did not meet the higher standard of peer review, but the company claimed that its scientists had caused human somatic cells to revert to the primordial state by fusing them with cow eggs. From this fusion product, a small clump of cells resembling ES cells appears to have been isolated (Wade, 1998).

In addition to those research accomplishments, the cloning of Dolly the sheep in 1997 using a technique called somatic cell nuclear transfer or, more simply, nuclear transfer (NT), illustrated another means by which to generate and isolate hES cells. hES cell preparations could potentially be produced by using NT to replace the nucleus of a human oocyte, triggering development, and then isolating hES cells at the blastocyst stage. Such a procedure was recently described by a group of Korean scientists (Hwang et al., 2004). The advantage of using NT to derive hES cells is that the nuclear genomes of the resulting hES cells would be identical with those of the donors of the somatic cells. One obvious benefit is that this would avoid the problem of rejection if cells generated from the hES cells were transplanted into the donor. Whether this approach will be technically or economically feasible is unclear. A more likely benefit of the technology is that it would further facilitate a wide range of experiments to explore the underpinnings of genetic disease and possible forms of amelioration and cure, many of which would not be possible using hES cells derived from blastocysts generated by *in vitro* fertilization (IVF), whose nuclear genomes are not defined. Although the promise of such research is as yet unrealized, most researchers believe that it will be a critical source of both important knowledge and clinical resources.

It is important to note that stem cells made via NT result from an asexual process that does not involve the generation of a novel combination of genes from two "parents." In this sense, it may be more acceptable to some than the creation of blastocysts for research purposes by IVF (National Institutes of Health, Human Embryo Research Panel, 1994). Use of NT for biomedical research, as distinct from its use to create a human being, has been considered by several advisory groups to be ethically acceptable under appropriate conditions involving the proper review and conduct of the research (NBAC, 1997, 1999a; NRC, 2002). However, there is near universal agreement that the use of NT to produce a child should not now be permitted. The medical risks are unacceptable, and many people have additional objections concerning the nature of this form of human procreation. In some countries there are statutory bans on the use of NT for reproductive purposes (see Chapter 4).

Finally, promising research has been conducted with adult stem cells (Lanza et al., 2004; Wagers and Weissman, 2004). Adult stem cells can be obtained from various tissues of adults or in some cases from neonatal tissues. A well-known example of the use of adult stem cells is bone marrow transplantation. Hematopoietic (blood-forming) adult stem cells from bone marrow or from umbilical cord blood give rise to all the cells of the blood. Skin cell transplants similarly rely on the transfer of skin stem cells. In both examples, the tissue involved naturally renews itself from its pool of stem cells—a property that can be exploited for medical use. It is possible that similar approaches can be developed for other tissues (such as muscle). However, in many other tissues, natural self-renewal appears to be a slow process, and stem cells for such tissues are correspondingly harder to characterize and isolate. There is also the possibility that some tissues may not contain a distinct subpopulation of undifferentiated stem cells at all. Furthermore, the anatomic source of the cells (such as brain or heart muscle) might preclude easy or safe access.

There are important biological differences between embryonic and adult stem cells. Embryonic stem cells show a much greater capacity for self-renewal, can be cultured to generate large numbers of cells, and are pluripotent—they have the potential for differentiation into a very wide variety of cell types. In contrast, adult stem cells appear to be capable of much less proliferation and, in general, have a restricted range of developmental capacities; that is, they can differentiate into only a limited array of cells (Wagers and Weissman, 2004). Thus most experts consider "adult stem cell research" not to be an alternative to hES and hEG cell research, but rather a complementary and important line of investigation.

hES cells currently can be derived from three sources: blastocysts remaining after infertility treatments and donated for research, blastocysts generated from donated gametes (oocytes and sperm), and the products of NT. Cadaveric fetal tissue is the only source of hEG cells. hES and hEG cells offer remarkable scientific and therapeutic possibilities, involving the potential for generating more specialized cells or tissue. This could allow the generation of new cells to be used to treat injuries or diseases involving cell death or impairment, such as Parkinson's disease, diabetes, heart disease, spinal cord injury, and hematologic and many other disorders. In addition, understanding the biology of hES and hEG cells is critical for understanding the earliest stages of human development. Ethical concerns about the sources of hES and hEG cells, however, and fears that use of NT for research could lead to the use of NT to produce a child have fostered a great deal of public discussion and debate. Concern has also been expressed about whether and how to restrict the production of human/nonhuman chimeras when conducting research with hES cells. Such research could be tremendously useful in understanding the etiology and progression of human disease and in testing new drugs, and will be necessary in preclinical testing of both adult and embryonic stem cells and their derivatives. However, some are concerned that creating chimeras would violate social conventions built around the notion of species (Robert and Baylis, 2003).

THE NEED FOR GUIDELINES

Since 1998, the volume of research being conducted with hES cells has expanded, primarily with private funds because of restrictions on the use of federal funds for such research. Those restrictions are both legislative and by executive order. Federal legislation forbids the use of federal funds for any research that destroys an embryo, that is, is "nontherapeutic" for the embryo. That effectively prevents any use of federal funds to derive hES cells from blastocysts. Research with established hES cell lines is further limited by presidential policy: the policy announced by President George W. Bush in 2001 restricts federal funding of research with hES cells to use of specific federally approved cell lines already in existence before August 9, 2001. The policy states further that funding is available only for research with hES cell lines that were derived before August 9, 2001 from frozen human blastocysts that remained at infertility clinics and that were (1) generated for reproductive purposes, (2) donated with informed consent, and (3) donated with no financial inducements.[1] Laboratories or companies that provide cells that meet those conditions (originally thought to be roughly 60 cell lines, now thought to be about 22) could list the lines in the National Institutes of Health (NIH) Human Embryonic Stem Cell Registry. To do so they were required to submit a signed assurance that their hES cells met the criteria. Once the assurance was verified, the cell lines became available for use in federally funded hES cell research. The date of August 9, 2001, was set as the cutoff point to distance the federal government from any privately funded future use of embryos for hES cell research.

Not all the original hES cell lines thought to be available for federally funded research have been viable, nor do they exhibit sufficient genetic diversity for all research endeavors and possible future clinical use. Furthermore, the roughly 22 lines now available were grown on mouse-feeder cell layers. That does not necessarily render them inadequate for research pursuing human applications, but it does raise concerns about contamination. The presence of animal feeder cells increases the risk of transfer of animal viruses and other infectious agents to humans that receive the hES cells and in turn to many others. There is also the risk that hES cells grown with nonhuman animal products will have incorporated antigenic glycolipids into their cell surface. If hES cell research and therapy are to be thoroughly investigated, cell lines that are more genetically diverse and free of animal contaminants must be available. A first step in that direction was taken in February 2005 with the publication of a paper documenting the first successful growth of hES cell lines without mouse feeder cells, although contact with a growth supplement derived

[1] "Notice of Criteria for Federal Funding of Research on Existing Human Embryonic Stem Cells and Establishment of NIH Human Embryonic Stem Cell Registry (Nov. 7, 2001)", at http://grants.nih.gov/grants/guide/notice-files/NOT-OD-02-005.html.

from mouse cells and bovine serum means that the lines are not yet completely free of contact with nonhuman materials (Xu et al., 2005).

Despite the restricted use of federal funds for research, the derivation of new cell lines is proceeding legally in the private sector and in academic settings with private funds. Some states have banned some or all forms of this research (see Chapter 4), but other states are actively promoting hES cell research. Although general regulation of laboratory research exists, there are no established regulations that specifically address procedures for hES cell research.

Several academic research centers are conducting hES cell research in this uncertain funding and regulatory climate and would benefit greatly from a set of uniform standards for conduct. Privately funded hES cell research is subject to some regulation or other constraints, primarily through human subjects protection regulations, the limits placed on licensees by the holders of NT and hES cell patents, state laws, and self-imposed institutional guidelines at companies and universities now doing or contemplating this research. Those aiming to produce biological therapies are also subject to Food and Drug Administration (FDA) regulation (see Chapter 4).

Because of the absence of federal funding for most hES cell research being conducted today, some standard protections may be lacking, and the implementation of protections is almost certainly not uniform throughout the country. The techniques for deriving the cells have not been fully developed as standardized and readily available research tools and the development of any therapeutic applications remain some years away. Because there is substantial public support for this area of research (Nisbet, 2004), and because several states are moving toward supporting this research in the absence of federal funds, heightened oversight is essential to assure the public that such research can and will be conducted ethically.

Because of the void left by restriction of federal funding and its attendant oversight of research and because of the importance that the scientific and biomedical community attaches to pursuing potential new therapies with hES cell lines, the National Academies initiated this project to develop guidelines for hES cell research to advance the science in a responsible manner. The project follows a series of reports issued by the Academies on this and related topics.

The 2002 National Academies report *Stem Cells and the Future of Regenerative Medicine* (NRC, 2002a) called for human adult stem cell and hES cell research to move forward. It also concluded that so-called therapeutic cloning, or NT for research purposes, has a separate and important potential both for scientific research and for future medical therapies. The report argued for federal funding of research deriving and using hES cells from multiple sources, including NT, asserting that, without government funding of basic research concerning stem cells, progress toward medical therapies is likely to be hindered. It noted that public sponsorship of basic research would help to ensure that many more scientists could pursue a variety of research questions and that their results would be made widely accessible in scientific journals—two factors that speed progress substantially. Public funding also offers greater opportunities for regulatory oversight and scrutiny of research.

The committee recommended that, given the ethical dilemmas and scientific uncertainties raised by hES cell research, a national advisory body made up of leading scientists, ethicists, and other stakeholders should be established at NIH. It argued that the group could ensure that proposals for federal funding to work on hES cells were justified on scientific grounds and met federally mandated ethical guidelines. The committee noted that NIH had set up similar watchdog panels, such as the Recombinant DNA Advisory Committee (RAC), which oversees genetic engineering research on the basis of an extensive set of guidelines.

In the report, *Scientific and Medical Aspects of Human Reproductive Cloning* (NRC, 2002b), the National Academies called for a "legally enforceable ban" on human reproductive cloning owing to scientific and medical concerns. The report recommended that such a ban be revisited in 5 years. Despite several legislative attempts to ban the use of NT for reproductive purposes, no such prohibition exists in federal statute, although FDA has stated that it has the authority to prohibit the use of NT for reproductive purposes on the basis of safety concerns.[2] Moreover, although a voluntary moratorium has worked in the past to delay scientific research (such as recombinant DNA research), the committee judged that a voluntary moratorium was unlikely to work for human reproductive cloning, because reproductive technology is widely accessible in numerous private fertility clinics that are not subject to federal research regulations. In addition, when the RAC (a model of successful self-regulation leading to public policy) was established and its guidelines were put into place, the vast majority of research biologists in the United States were funded by NIH or the National Science Foundation, so the potential sanction—loss of federal grants—was a strong disincentive. That would not be the case for human reproductive cloning.

Other national panels have expressed views about the regulation of reproductive cloning and the use of NT for research into new therapies. President William J. Clinton's National Bioethics Advisory Commission (NBAC) also issued two reports on the issues. In its 1997 report *Cloning Human Beings*, issued before the isolation of hES cells, NBAC wrote that hES cells could provide critical strategies for cell-based therapies and that NT could be important in averting graft rejection in recipients of such therapy (NBAC, 1997). In its 1999 report *Ethical Issues in Human Stem Cell Research* (NBAC, 1999a), NBAC recommended that federal funds be available for the derivation *and* use of hES cells and that, for the moment, federal funding be restricted to research in which the cells were derived from blastocysts that remained after IVF or were derived from fetal tissue while research with cells derived in other ways remained legal and privately funded. The commission suggested that following this recommendation would make sufficient hES cells available for research. It also noted that the issue should be revisited if studies on those

[2]See FDA letter to investigators/sponsors at http://www.fda.gov/cber/ltr/aaclone.pdf.

cell lines demonstrate the need for federal funding of research with NT-derived cell lines or cell lines from blastocysts generated for research purposes.

In its 1999 report, NBAC outlined a system of national oversight to review protocols, monitor research, and ensure strict adherence to guidelines. Although intended for research with hES cells derived from IVF blastocysts, many of the recommendations could apply equally well to blastocysts derived using NT. NBAC's regulatory paradigm was based in part on the regulatory system already in place governing fetal tissue transplantation research: strict oversight and separation of the decision to terminate a pregnancy from the decision to donate material.

In its 2002 report, *Human Cloning and Human Dignity: An Ethical Inquiry*, 10 of 17 members of President Bush's Council on Bioethics recommended a 4-year moratorium on "cloning-for-biomedical-research." They also called for "a federal review of current and projected practices of human embryo research, pre-implantation genetic diagnosis, genetic modification of human embryos and gametes, and related matters, with a view to recommending and shaping ethically sound policies for the entire field." The advocates of the moratorium argued that it "would provide the time and incentive required to develop a system of national regulation that might come into use if, at the end of the four-year period, the moratorium were not reinstated or made permanent." Furthermore, they argued that "in the absence of a moratorium, few proponents of the research would have much incentive to institute an effective regulatory system."

Seven members of the 17-member council voted for "permitting cloning-for-biomedical-research now, while governing it through a prudent and sensible regulatory regime." They argued that research should be allowed to go forward only when the necessary regulatory protections to avoid abuses and misuses of cloned embryos are in place. "These regulations might touch on the secure handling of embryos, licensing and prior review of research projects, the protection of egg donors, and the provision of equal access to benefits."

Finally, in September 2003, a worldwide movement of science academies led to a major meeting in Mexico City in which 66 academies—including the U.S. National Academy of Sciences—from all parts of the world and all cultural traditions and religions called for a global ban on the use of NT for human reproduction as a matter of urgency. The group of academies specified that no ban on NT for human reproduction should preclude hES cell research with NT blastocysts. A growing number of countries have far more permissive policies regarding such research than the United States has (Walters, 2004; see also Chapter 4).

Because there is widespread agreement in the international scientific community about the potential value of hES cell research—including the use of NT to derive hES cell lines—and because there is, at present, general agreement that NT should not be used to produce a child, the best possible way to move forward with hES cell research in pursuit of new therapies is to have a set of guidelines to which the U.S. scientific community can adhere.

A key reason for the remarkable success of science since its emergence in mod-

ern form—besides the application of the scientific method itself—is the communal nature of scientific activity. The tradition of sharing materials and results with colleagues speeds scientific progress and symbolizes to the nonscientific world that in the final analysis the goal of science is to expand knowledge and improve the human condition. Not all scientists want to or have the resources to derive new stem cell lines, so the ability to share cell lines will create greater access for qualified scientists to participate in human stem cell research. A uniform set of criteria for deriving hES cell lines and reviewing research will help to assure that research institutions that derive, store, and maintain hES cells meet a standard set of requirements for provenance and ethical review.

Another positive aspect of a set of established and generally agreed upon guidelines would be greater public confidence in the conduct of hES cell research. The integrity of privately funded hES cell research would be enhanced in the public's perception as well as in actuality by the existence of a standardized set of guidelines. Public confidence would also be increased by enhanced understanding of the research. Some of the concerns about hES cell research arise from lack of familiarity with the scientific issues. It is especially crucial that the public have access to accurate information and the scientific community needs to make greater efforts to explain what research is being proposed and why. Patient advocacy groups and those with a stake in the potential therapeutic benefits of such research have begun to provide some of the education that has been lacking. As part of the larger society, the scientific community and the lay public need to engage in constructive discussion about this and other promising new fields of biomedical research to ensure that public confidence is maintained.

A BRIEF HISTORY OF U.S. DISCUSSIONS AND POLICIES REGARDING RESEARCH INVOLVING HUMAN EMBRYOS

Public debates and deliberations about embryo research have extended over the last 30 years. In 1975, the Secretary of the Department of Health, Education, and Welfare (DHEW) announced that the department would fund no proposal for research on human embryos or on IVF unless it was reviewed and approved by a federal ethics advisory board. IVF was still an experimental technique: Louise Brown, the first IVF baby, was born in 1978 in the United Kingdom. The human subjects regulations that resulted from the work of the National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research (National Commission) required review of such work by an Ethics Advisory Board (EAB) to be appointed by the DHEW Secretary (National Commission, 1975). In 1977, NIH received an application from an academic researcher for support of a study involving IVF. After the application had undergone scientific review by NIH, it was forwarded to the EAB. At its May 1978 meeting, the EAB agreed to review the research proposal and later approved it for initiation.

With the increased public interest that followed the birth of Louise Brown that

summer, the Secretary of DHEW asked the EAB to study the broader social, legal, and ethical issues raised by human IVF. On May 4, 1979, in its report to the Secretary, the EAB concluded that federal support for IVF research was "acceptable from an ethical standpoint," provided that some conditions were met, such as informed consent for the use of gametes, an important scientific goal that was "not reasonably attainable by other means" and not maintaining an embryo "*in vitro* beyond the stage normally associated with the completion of implantation (14 days after fertilization)" (DHEW EAB 1979, 106, 107). No action was ever taken by the Secretary with respect to the board's report; for other reasons, the department dissolved the EAB in 1980. Considerable opposition to the moral acceptability of IVF was expressed by some and contributed to paralysis regarding reconstitution of the EAB (Congregation, 1987).

Because it failed to appoint another EAB to consider additional research proposals, DHEW effectively forestalled any attempts to support IVF research with federal funds, and no experimentation involving human embryos was ever funded pursuant to the conditions set forth in the May 1979 report or through any further EAB review.

A 1988 report by the congressional Office of Technology Assessment about infertility forced a re-examination of the EAB (U.S. Congress, OTA, 1988), and a later House hearing focused on its absence. The DHEW Assistant Secretary promised to re-establish an EAB, and a new charter was published, but it was never signed after the election of President George H. W. Bush (Windom, 1988). The George H. W. Bush administration did not support re-establishing an EAB. The absence of a federal mechanism for the review of controversial research protocols continued until 1993, when the NIH Revitalization Act effectively ended the *de facto* moratorium on support of IVF and other types of research involving human embryos by nullifying the regulatory provision that mandated EAB review. In response, NIH Director Harold Varmus convened a Human Embryo Research Panel (HERP) to develop standards for determining which projects could be funded ethically and which should be considered "unacceptable for federal funding."

The HERP submitted its report to the Advisory Committee to the Director in September 1994.[3] In addition to describing areas of research that were acceptable and unacceptable for federal funding, the panel recommended that under certain conditions federal funding should be made available to make embryos specifically for research purposes. Acting on this submission, the Advisory Committee to the Director formally approved the HERP recommendations (including provision for the deliberate creation of research embryos) and transmitted them to the NIH Director on December 1, 1994. On December 2, pre-empting any NIH response, President Clinton intervened to clarify an earlier endorsement of embryo research,

[3]Available at http://www.bioethicsprint.bioethics.gov/reports/past_commissions/index.html.

stating that "I do not believe that Federal funds should be used to support the creation of human embryos for research purposes, and I have directed that NIH not allocate any resources for such requests" (Office of the White House Press Secretary, 1994).

The NIH Director proceeded to implement the HERP recommendations not proscribed by the President's clarification, concluding that NIH could begin to fund research activities involving "surplus" blastocysts. But before any funding decisions could be made, Congress took the opportunity afforded by the Department of Health and Human Services (DHHS) appropriations process (then under way) to stipulate that any activity involving the creation, destruction, or exposure to risk of injury or death of human embryos for research purposes may not be supported by federal funds under any circumstances. The same legislative rider has been inserted into later annual DHHS appropriating statutes, enacting identically worded provisions into law (the so-called Dickey-Wicker amendment, named after its congressional authors). Thus, to date, no federal funds have been used for research that requires the destruction of additional human embryos, whether generated originally for reproductive purposes or for research, although the current federal policy permits research on specific cell lines derived from blastocysts prior to August 2001.

When the reports of the successful isolation of hES cell lines were published in 1998, the question arose as to whether it was acceptable to provide federal funding for hES cell research that would use embryonic stem cells that were obtained from IVF blastocysts with private funding. The NIH Director sought the opinion of the DHHS General Counsel regarding the effect of the appropriations rider to the NIH Revitalization Act. The General Counsel reported that the legislation did not prevent NIH from supporting research that uses hES cells derived using private funding because the cells themselves do not meet the statutory, medical, or biological definition of a human embryo (NIH OD, 1999). Having concluded that NIH may fund both internal and external research that uses hES cells but does not create or actively destroy human embryos, NIH delayed funding until an ad hoc working group developed guidelines for the conduct of ethical research of this kind. These guidelines prescribed the documentation and assurances that had to accompany requests for NIH funding of research with human hES cells, and designated certain areas of hES cell research that were ineligible for NIH funding:

- the derivation of hES cells from human embryos,
- research in which hES cells are utilized to create or contribute to a human embryo,
- research utilizing hES cells that were derived from human embryos created for research purposes rather than for fertility treatment,
- research in which hES cells are derived using NT, that is, the transfer of a human somatic cell nucleus into a human or animal oocyte,
- research utilizing hES cells that were derived using NT,

- research in which hES cells are combined with an animal embryo, and
- research in which NT is used for the reproductive cloning of a human.

Before any grants could be funded, the 2000 election produced a new administration, and consequently the policies that exist today. As previously noted, on August 9, 2001, President Bush announced that NIH could fund research that uses hES cells but only if the cell lines had been derived prior to that date. The President maintained further that the guidelines for hES cell research developed during the Clinton presidency and the ethics advisory committee itself were no longer needed. Instead, an NIH Stem Cell Task Force composed entirely of NIH personnel was appointed to "focus solely on the science" of stem cell research. That might be explained by the fact that many of the remaining ethical guidelines that NIH had planned to put into effect were no longer needed, because they applied to issues surrounding federal funding of research on hES cell lines yet to be derived.

Meanwhile, other countries have been active in developing laws and regulations governing research in this area (see Chapter 4). In addition, in the United States a patchwork of state laws and programs ranges from a complete ban on all hES cell research to a new program recently enacted in California that funds the development of new lines derived from both IVF blastocysts and using NT.

STATEMENT OF TASK

In light of the absence of federal guidelines, the Committee on Guidelines for Human Embryonic Stem Cell Research was asked to develop voluntary guidelines to encourage responsible practices in hES cell research—regardless of source of funding—including the use and derivation of new stem cell lines derived from surplus blastocysts, from blastocysts generated with donated gametes, and through the use of NT. The guidelines should take ethical and legal concerns into account and encompass the basic science and health sciences policy issues related to the development and use of hES cells for research and eventual therapeutic purposes, such as

1. Recruitment of blastocyst, gamete, or somatic cell donors, including medical exclusion criteria, informed consent, the use of financial incentives, risks associated with egg retrieval, confidentiality, and the interpretation of genetic information developed from studies that use these materials and might have importance to the donor.
2. The characterization of stem cells for purposes of standardization and for validation of results.
3. The safe handling and storage of blastocysts and stem cell material and the conditions for transfer of such material among laboratories.

4. Prerequisites to hES cell research (such as examination of alternative approaches), appropriate uses of hES cells in research or therapy, and limitations on the use of hES cells.
5. Safeguards against misuse.

In accordance with the stated position of the National Academies that there should be a global ban on NT for human reproduction (NRC, 2002), the guidelines developed by this committee focus exclusively on research and therapeutic uses of hES cells and NT.

To conduct its work, the committee surveyed the current state of science in this field and likely pending developments, reviewed the policy and ethical issues posed by the research, examined professional and international regulations and guidelines affecting hES cell research, and conducted a 2-day workshop with speakers who represented many scientific, ethical, and public policy perspectives. It did not revisit the debate about whether hES cell research should be pursued; rather it assumed that both hES cell and adult stem cell research would continue in parallel with federal and nonfederal funding. In addition, although the committee recognizes that successful resolution of intellectual property issues will be critically important in this evolving area of research, it was beyond its charge and beyond its capabilities to address adequately all of the legal issues that will arise. Chapter 4 briefly addresses ongoing efforts to ensure that intellectual property issues do not impede new developments in biomedical research.

The guidelines presented in Chapter 6 focus on the procurement of embryos and gametes and the derivation, banking, and use of hES cell lines. They provide an oversight process that will help to ensure that research is conducted in a responsible and ethically sensitive manner and in compliance with all regulatory requirements pertaining to biomedical research in general. These guidelines are being issued for use by the scientific community, including researchers in university, industry, or other private sector research organizations, as well as practitioners of assisted reproduction, which will be one of the sources of donated embryos and gametes.

PRECEDENTS FOR SCIENTIFIC SELF-REGULATION

Perhaps the archetype of modern scientific self-regulation in the life sciences—although primarily focused initially on safety rather than ethical issues— was the moratorium on recombinant DNA research that emerged from a meeting of several hundred scientists at the Asilomar Conference Center in California. A controversy had erupted in 1971 about an experiment that involved inserting genes from a monkey virus, SV40, which can make rodent cells cancerous, into an *E. coli* bacterial cell. Prominent scientists called for a halt to recombinant DNA research until the matter could be resolved. The 1975 Asilomar conference concluded that safeguards should be introduced into recombinant DNA work, ultimately including the creation of the NIH RAC and guidelines for federally funded recombinant DNA

research. It is generally agreed that the Asilomar conference and the measures that followed helped to reassure Congress and the public that the scientific community took its responsibilities seriously and allowed the research to go forward.

Although the recombinant DNA debate and its results have achieved a sort of iconic status in the annals of science's self-regulation, less spectacular examples have also arisen in the absence of or as a complement to government regulation of science and medicine. The government often relies on the private sector to regulate itself and supports it with the threat of sanctions. An example is the Joint Commission for the Accreditation of Health Care Organizations; failure to meet its standards can result in the loss of Medicare reimbursement. In the field of assisted reproduction, the lack of government funding has resulted in professional efforts to generate standards, such as those promulgated by the American Society for Reproductive Medicine (ASRM) and the Society for Assisted Reproductive Technologies.

Because there is no current federal support of hES cell research in which new cell lines are derived, the most applicable sets of guidelines in the United States for this purpose come from the Ethics Committee of the ASRM (ASRM, 2000, 2004b). Most international guidelines also call for some special oversight body for stem cell research to review documentation of compliance with the guidelines of various government agencies, both domestic and foreign. Such evaluation is in some cases folded into the evaluation of scientific merit; in others it is performed by stand-alone ethics review bodies. In the United States, review of scientific merit is typically conducted by the funding agency, which is often a federal agency. That will not be the case, for the time being, for most hES cell research conducted in this country.

There are clear advantages to government action, especially with regard to the legal standing of industry standards. Outstanding examples relevant to this report and to cultural environments that are similar to the United States are the British Human Fertilisation and Embryology Authority and the more recent Canadian Assisted Human Reproduction Agency. But in the absence of such arrangements, our proposals for a system of local review combined with a national oversight panel would go far toward consolidating and monitoring the policies and practices of hES cell research.

CONCLUSION

In the absence of federal guidelines broadly governing the generation and research use of hES cells, the scientific community and its institutions should step forward to develop and implement its own, much in the spirit of Asilomar, which resulted in the RAC guidelines in use today. Such guidelines are needed by the scientific community as a framework for hES cell research and would reassure the public and Congress that the scientific community is attentive to ethical concerns and is capable of self-regulation while moving forward with this important research. The premise is not to advocate that the work be done—that has already been debated with some consensus reached in the scientific community and elsewhere—

but rather to start with the presumption that the work is important for human welfare, that it will be done, and that it should be conducted in a framework that addresses scientific, ethical, medical, and social concerns. The public increasingly supports this area of research and its potential to advance human health.

The next chapter describes the current status of research involving hES cells. It also addresses possible novel sources of hES cell lines not yet developed and the use of human/nonhuman chimeras in research.

Chapter 3 focuses on ethical and policy issues and how existing and proposed guidelines address them. In Chapter 3, the committee proposes a local review mechanism to oversee research involving hES cells. It also recommends establishing a national body to periodically update the guidelines recommended in this report and assess the status of the field. Chapter 4 describes the current legal and regulatory environment of hES cell research in the United States and around the world. Chapter 5 addresses recruitment of donors and the informed consent process and makes recommendations about review of the processes by which donated materials are obtained. Chapter 5 also discusses the need for some standards in the banking and maintenance of hES cell lines. The final chapter consolidates the recommendations made in previous chapters as formal guidelines.

2

Scientific Background of Human Embryonic Stem Cell Research

INTRODUCTION

Human embryonic stem cells (hES cells) are primitive (undifferentiated) cells that can self-renew or differentiate into most or all cell types found in the adult human body (Edwards, 2004; Gardner, 2004). Differentiation is the process whereby an unspecialized cell acquires specialized features, such as those of a heart, liver, or muscle cell.

Fertilization of an oocyte by a sperm results in a one-cell zygote, which begins to divide without any increase in size (Figure 2.1). By 3-4 days after fertilization, cell division results in a compact ball of 16-32 cells known as a morula. By 5-6 days, a blastocyst is formed consisting of a sphere of about 200-250 cells. The sphere is made up of an outer layer of cells (the trophectoderm), a fluid-filled cavity (the blastocoel), and a cluster of cells in the interior (the inner cell mass). Up to this point, there has been no net growth (Figure 2.1). The cells of the inner cell mass will give rise to the embryonic disk and ultimately the fetus, but not the placenta, which arises from the trophectoderm. Neither the trophectoderm nor the inner cell mass alone can give rise to a developing fetus. After the blastocyst implants into the uterus (day 6), the cells of the inner cell mass differentiate to form the embryonic tissue layers of the developing fetus. Embryonic stem cells are usually derived from the primitive (undifferentiated) cells of the inner cell mass, which have the potential to become a wide variety of specialized cell types. Because embryonic stem cells can become all cell types of the body, they are considered to be pluripotent. Study of embryonic stem cells provides information about how an organism develops from a single cell and how healthy cells can potentially replace damaged cells in adult

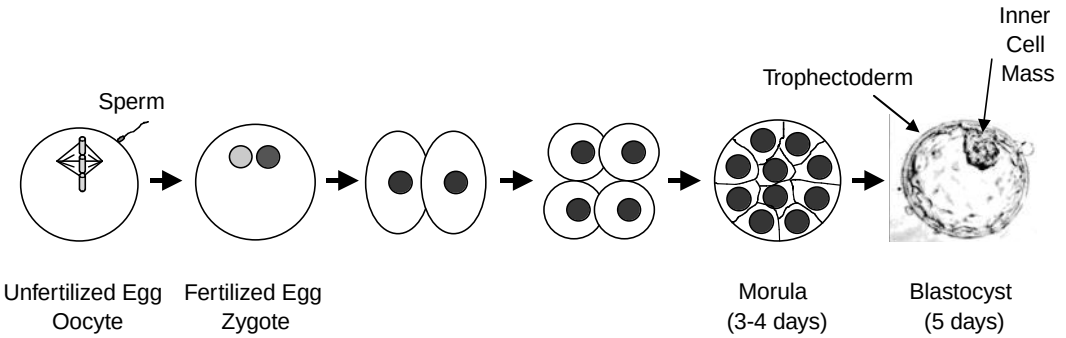


FIGURE 2.1 Preimplantation development. The oocyte (unfertilized egg) combines with sperm to form a zygote (fertilized egg). Each gamete (oocyte or sperm) is haploid (has a single set of chromosomes); the zygote and all later cells are diploid (have two sets of chromosomes). The zygote then divides approximately once a day. Since there is no growth during this period of cell division (cleavage), the cells become progressively smaller. By 3-4 days, a ball of cells (morula) has formed. By 5 days, it has become hollowed out to form a blastocyst, which consists of a sphere 0.1-0.2 mm in diameter comprising two cell types—an outer shell of trophectoderm cells and an inner collection of 30-34 cells called the inner cell mass. By day 6, the blastocyst would normally implant into the uterine wall, the trophectoderm would begin to form the placenta, and the inner cell mass would begin to form the cells and tissues of the fetus. At the blastocyst stage, cells of the inner cell mass are undifferentiated and pluripotent; that is, they have the potential to differentiate into all cells of the fetus except the placenta. If separated from the blastocyst and cultured, the cells of the inner cell mass can be converted into embryonic stem cells that are also pluripotent and can be propagated extensively while maintaining that potential. Blastocyst picture from http://stemcells.nih.gov/info/scireport/chapter3.asp.

organisms. The latter subject raises possibilities of cell-based therapies to treat disease, often referred to as regenerative medicine.

Scientists discovered how to obtain or derive embryonic stem cells from mouse blastocysts in the early 1980s (Evans and Kaufman, 1981; Martin, 1981) by culturing inner cell masses on feeder layers of mouse fibroblasts. It was later discovered that feeder cells could be replaced with culture medium containing the growth factor leukemia inhibitory factor (LIF)(Smith et al., 1988; Williams et al., 1988). Mouse ES cells (mES cells) have been studied in the laboratory, and a great deal has been learned about their essential properties and what makes them different from specialized cell types.

mES cells are shown to be pluripotent using three kinds of tests. The first and most rigorous test is to inject mES cells into the blastocoel cavity of a blastocyst (Stewart, 1993). The blastocyst is then transferred to the uterus of a pseudopregnant female (a female primed to accept implanted blastocysts). If the mES cells are pluripotent, the resulting progeny will be a chimera because it consists of a mixture

of tissues and organs derived from both the donor mES cells and the recipient blastocyst. In some cases, a fetus can be derived entirely from mES cells by providing trophectoderm cells from another source (Nagy et al., 1990, 1993). However, mES cells cannot themselves form a functional placenta and therefore are not equivalent to an intact blastocyst. The ability of mES cells to generate a complete embryo tends to decline with the number of times the cells have divided (or been "passaged") in culture.

A second approach for testing pluripotency of mES cells is to inject them into the testis or under the skin or kidney capsule of an immunodeficient mouse. If pluripotent, the injected cells form benign tumors known as teratomas. The teratomas contain differentiated tissues from all three germ layers (ectoderm, mesoderm, and endoderm). Such structures as gut, muscle (smooth, skeletal, and cardiac), neural tissue, cartilage, bone, and hair are found, but they are arranged in a disorganized manner (Martin, 1981).

A third approach for testing pluripotency of mES cells is by *in vitro* differentiation (Wiles, 1993). Spontaneous differentiation can occur if the mES cells are grown in suspension without feeders or LIF. The cells will form fluid-filled clumps called embryoid bodies, which will differentiate along the ectoderm, mesoderm, and endoderm pathways. If the embryoid bodies are allowed to attach to the tissue culture dish, they will differentiate into multiple tissue types much like teratomas.

Developmentally relevant signaling factors can also be used to induce mES cells to differentiate into specific cell types *in vitro,* including hematopoietic stem cells, beating cardiac muscle cells, neuronal progenitors, endothelial cells, and bone cells. In some cases, those differentiated cell types can be transplanted into animals to form functional tissues (Lanza et al., 2004). Such work engenders excitement about regenerative medicine using hES cells. One of the milestones of mES cell research was the development of methods to modify the cells genetically (Doetschman et al., 1987; Thomas and Capecchi, 1987). The evolution of those methods has revolutionized animal models for biomedical research by allowing one to modify endogenous genes or to tag the cells so that they can be easily visualized in the animal.

Bongso et al. (1994) first described isolation and culture of cells of the inner cell mass of human blastocysts in 1994, and techniques for deriving and culturing stable hES cell lines were first reported in 1998 (Thomson et al., 1998). The trophectoderm was removed from day-5 blastocysts, and the inner cell mass, consisting of only 30-34 cells, was placed into tissue culture. Cell lines similar to mES cells were derived after fairly extensive culture and passaging of the cells. Cells with similar properties were reported at about the same time from culturing cells isolated from fetal genital ridges—so-called human embryonic germ (hEG) cells (Shamblott et al., 1998). It had previously been shown that the germ cells in fetal mouse gonads can give rise to permanent pluripotent stem cell lines in culture, mEG cells (Matsui et al., 1992; Resnick et al., 1992). Under appropriate culture conditions, hES cells were shown to be pluripotent by differentiating into multiple tissue types (Itskovitz-Eldor et al., 2000; Reubinoff et al., 2000). Since 1998, research teams have refined the

techniques for growing hES cells *in vitro* (Amit et al., 2000; Itskovitz-Eldor et al., 2000; Klimanskaya and McMahon, 2004; Reubinoff et al., 2000). Collectively, the studies indicate that it is now possible to grow karyotypically normal hES cells (that is, with correct chromosome number) for more than a year in serum-free medium on mouse fibroblast feeder layers. Both XX (female) and XY (male) hES cell lines have been established. The cells express markers characteristic of pluripotent and proliferating cells. Work with hEG cells has also shown pluripotency and extended self-renewal, but more extensive work has been done with hES than with hEG cells.

There are differences between mouse and human ES cells (Pera and Trounson, 2004). For example, mES cells grow as rounded colonies with indistinct cell borders, while hES cell colonies are flatter and display more distinct cell borders. The two cell types also demonstrate differences in growth regulation. In general, both mES and hES cells require fibroblast feeder cell support. Current attempts to substitute for that support have required different approaches for the two species. The soluble growth factor, LIF, can substitute for a feeder cell layer in maintaining mES cells, but hES cells require a solid extracellular matrix (Matrigel) in place of the fibroblasts (Xu et al., 2005). Those examples of interspecies differences indicate that if one is to identify signals that cause stem cells to differentiate into specialized cells, work needs to continue with both hES and mES cells.

Embryonic stem cells have three important characteristics that distinguish them from other types of cells. First, hES cells express factors—such as Oct4, Sox2, Tert, Utf1 and Rex—that are associated with pluripotent cells (Carpenter and Bhatia, 2004). Second, they are unspecialized cells that renew themselves through many cell divisions. A starting population of stem cells that proliferates for many months in the laboratory can yield millions of cells. An important research challenge is to understand the signals that cause a stem cell population to remain unspecialized and to continue to proliferate until they are needed for repair of a specific tissue.

A third characteristic of hES cells is that under some physiological or experimental conditions in tissue culture they can be induced to become cells with special functions, such as cardiomyocytes (the beating cells of the heart), liver cells, nerve cell precursors, endothelial cells, hematopoietic cells, and insulin-secreting cells (Assady et al., 2001; Chadwick et al., 2003; Kaufman et al., 2001; Kehat et al., 2001; Levenberg et al., 2002; Mummery et al., 2002; Reubinoff et al., 2001; Reubinoff et al., 2000; Xu et al., 2002; Zhang et al., 2001). However, because hES cells have not yet been used in blastocyst chimera studies, researchers have been able to assess *in vivo* differentiation only after injection of hES cells into immunodeficient mice. There, the cells create teratomas in which tissues of the three embryonic germ layers are found (Thomson et al., 1998). Examples are bone and cartilage tissue, striated muscle, gut-like structures, neural rosettes, and glomerulus-like structures. More organized structures—such as hair follicles, salivary glands, and tooth buds—also form. hES cells will also create embryoid bodies and differentiate *in vitro* (Itskovitz-Eldor et al., 2000). However, those types of differentiation assays do not provide conclusive evidence that the resulting cell types are functioning

normally, nor whether hES cells have the capacity to participate in normal development in the context of the three-dimensional embryo in the reproductive tract. Such conclusive evidence requires testing in blastocyst chimeras as is routinely done with mES cells.

Understanding why ES cells are able to proliferate essentially indefinitely and retain the ability to be induced to differentiate and stop proliferating will provide important information about the regulation of normal embryonic development and the uncontrolled cell division that can lead to cancer. It is known that external signals for cell differentiation include chemicals secreted by other cells, physical contact with neighboring cells, and molecules in the microenvironment. Identifying such factors would allow scientists to find methods for controlling stem cell differentiation in the laboratory and thereby allow growth of cells or tissues that can be used for specific purposes, such as cell-based therapies.

Several methods have been shown to be effective for delivering exogenous genes into hES cells, including transfection by chemical reagents, electroporation, and viral infection (Eiges et al., 2001; Gropp et al., 2003; Ma et al., 2003; Pfeifer et al., 2002; Zwaka and Thomson, 2003). Those are all critical methodological objectives that must be met if hES cells are to be used as the basis of therapeutic transplantation.

NUCLEAR TRANSFER TO GENERATE STEM CELLS

Most work on hES cells has taken place with a relatively small number of cell lines obtained from excess blastocysts donated from *in vitro* fertilization (IVF) programs. The genetic makeup of the cells is not controlled in any way, and genetic variation among lines needs to be considered when results from different lines are compared. Experience from research with mES cells shows that ES cell lines can differ markedly in their differentiation efficiencies. Being able to control the genotype of ES cells would be valuable for various reasons, most notably the desire to generate ES cells with genotypes known to predispose to particular diseases. In the case of single-gene defects, one could achieve that goal by deriving hES cells from discarded morulae or blastocysts that were identified with preimplantation genetic diagnosis (PGD) procedures (Verlinsky et al, 2005) as carrying mutations or by generating the appropriate mutation by gene targeting of established hES cell lines. However, such approaches cannot be used if the genetic predisposition has an unknown basis or arises from multiple gene effects. Availability of hES cell lines from patients with Alzheimer's disease, type I diabetes, or many other complex diseases would provide a source of cells that could be differentiated into appropriate cell types; and the progression of the disease could then be modeled and potentially modified in culture. Given the complex interplay between genotype and environment that typifies complex chronic diseases, the availability of cell-line models would provide major new tools for diagnosis and therapy. In this context, hES cells are research tools for the study of disease, not therapeutic agents themselves.

Controlling the genotype of ES cells will also be important in the future if they are to be used directly as therapeutic tools in regenerative medicine. Transplantation of hES cells will face issues of tissue rejection common to all forms of organ or tissue transplants. As in organ or bone marrow transplantation, one solution is to develop large banks of genetically diverse hES cells to increase the chances that matches can be found for all patients who need them. That is one strong medical reason for generating additional hES cell lines from a wider spectrum of the population. Other methods to overcome tissue rejection, including genetic modification of hES cells to reduce immunogenicity and use of immunosuppressive drugs may be helpful. However, in the long run, one obvious solution would be autologous transplantation, using hES cells genetically identical with the recipient of the graft.

Generation of ES cells using nuclear transfer (NT) has the potential to produce ES cells of defined genotype to address both genetic diversity and avoidance of rejection. NT is the process by which the DNA-containing nucleus of any specialized cell (except eggs and sperm, which contain only half the DNA present in other cells) is transferred into an oocyte whose own nuclear genome has been removed (Figure 2.2). The egg can then be activated to develop and will divide to form a blastocyst, whose genetic material and genetically determined traits are identical with those of the donor of the specialized cell, not those of the donor of the oocyte. The oocyte does provide a very small amount of genetic information in the mitochondria, the "energy factories" of the cell, but the genes in the nucleus are of overriding importance, nuclear genes being responsible for the vast majority of the traits of the animal. If such a blastocyst were transferred to a uterus, the transferred blastocyst could potentially develop into a live-born offspring—a clone of the nuclear donor. NT was first developed with frog embryos and later successfully used to generate Dolly the sheep, the first mammal cloned from an adult cell (Campbell et al., 1996). Since the birth of Dolly, live cloned offspring of several other mammalian species have been reported, including mice, goats, pigs, rats, cats, and cows. The success rate of live births is very low, however, and a variety of abnormalities have been found in cloned animals (NRC, 2002b), so this is currently an unreliable technology and unsafe for application to humans. Given the safety issues associated with NT for human reproduction, there is a worldwide consensus that such efforts should be not be conducted at this time. Despite some well-publicized but undocumented claims of production of live cloned babies, the scientific community in general and this committee in particular support that moratorium.

Blastocysts derived using NT can be an important source of genetically defined ES cells. If the inner cell mass of the NT-derived blastocyst, comprising a few dozen undifferentiated cells, is removed and grown in culture, ES cells can be derived and their genotype will be identical with that of the nuclear donor. Successful derivation of pluripotent mES cells from cloned NT blastocysts has been demonstrated in mice by several groups (Kawase et al., 2000; Munsie et al., 2000; Wakayama et al., 2001). In addition, the principle of alleviating a genetic disease was demonstrated by transplantation of genetically repaired mouse NT ES cells in an immunodeficient

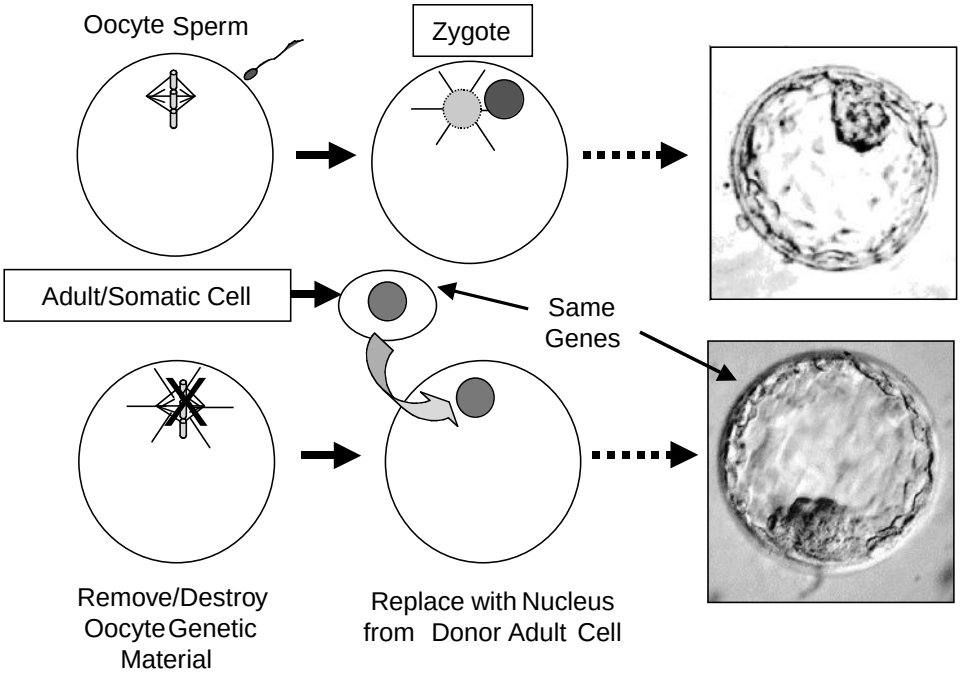


FIGURE 2.2 Comparison of Normal Preimplantation Development with Nuclear Transfer (NT). In NT, the genetic material of the oocyte is removed and replaced with a diploid nucleus from a somatic (body) cell. This divides to yield an NT blastocyst whose genes are identical with those of the donor somatic cell. NT blastocysts, like normal blastocysts, can be used to derive embryonic stem cells from their inner cell masses. The picture shown is of a normal human blastocyst (http://www.fosep.org/images/blastocyst2.gif) because pictures of human NT blastocysts are scarce and normal and NT blastocysts appear indistinguishable.

mouse (Rideout et al., 2002). Although production of normal live offspring from NT blastocysts is not very successful in any species, NT ES cells seem to be able to differentiate normally in mice and have been able to contribute extensively to adult tissues, including the germ line, in chimeras (Wakayama et al., 2001). The rate of successful production of ES cells from NT-derived blastocysts is, however, still quite low (less than 5 percent).

In 2004, the first report of an NT-derived hES cell line was made by Woo Suk Hwang and colleagues in South Korea (Hwang et al., 2004). One line was produced by transfer of a nucleus from donated ovarian cumulus cells to an enucleated host oocyte derived from the same donor. The line appeared to be pluripotent and chromosomally normal. Successful production of hES cells was again inefficient—

over 200 oocytes were used in the course of the experiments that generated a single line. However, the scientists made a number of improvements in the procedure as the experiments progressed, increasing the yield of blastocysts and suggesting that the success rate will be improved in the future. This proof of the principles behind generating NT hES cells has made plausible the derivation of more such lines from specifically defined genetic backgrounds.

It is important to note that stem cells made using NT result from an asexual process that does not involve the generation of a novel combination of genes from two "parents." In this sense, it may be more acceptable to some than the creation of blastocysts for research purposes by IVF (NIH HERP, 1994). It has also been suggested (Hurlbut, 2004) that transfer of genetically altered nuclei incapable of directing full development might make NT acceptable. However, it has been pointed out (Melton et al., 2004) that this approach faces many technical hurdles and does not avoid the need for oocyte donation. At least three methods for generating hES cells from defective embryos have been suggested. One such method involves the use of viable blastomeres extracted from a morula or blastocyst that has been declared dead due to cleavage arrest (Landry and Zucker, 2004). This proposal is untested and is technically challenging. Even if it were possible to identify unequivocally embryos with no chance of further development, the likelihood of then isolating a viable blastomere and generating an ES line is small. There has been only one published report claiming derivation of mES cell lines from isolated 8-cell blastomeres (Delhaise et al., 1996). One cell line was obtained from 52 fully viable, dissociated 8-cell stage morulae.

Two other methods of generating hES cells from defective embryos have been considered: parthenogenesis and androgenesis. In parthenogenesis, an oocyte can be activated to develop without being fertilized by a sperm. The genomic DNA of the resulting embryo is completely maternally derived, which is not compatible with survival to term. Both mouse and nonhuman primate parthenogenetic ES cell lines have been established (Kaufman et al., 1983; Cibelli et al., 2002). The results are of interest because deriving stem cells from parthenogenetic blastocysts could eliminate the requirement to produce and destroy viable blastocysts. Parthenogenetic ES cells could serve as an alternative source for autologous cell therapy. However, parthenogenetic mES cells show restricted tissue contributions in chimeras and in teratomas formed by grafting the cells under the kidney capsule (Allen et al., 1994); this is related to the lack of expression of key imprinted genes that are normally expressed from the paternal genome. In contrast with parthenogenesis, in androgenesis the entire genome comes from the male parent. Such embryos also do not survive to term. Diploid androgenetic mES cells have been derived (Mann et al., 1990), but many androgenetic ES cell chimeras died at early postnatal stages, and the ones that survived developed skeletal abnormalities. Again, the imprinting status of the cells differed from that of wild-type ES cells (Szabo et al, 1994). Thus, although the results show that androgenetic and parthenogenetic ES cells have broad developmental potential, their imprinted gene expression status is likely to

restrict their therapeutic applications. Moreover, no human parthenogenetic or androgenetic stem cell lines have been established, and more research is needed to determine whether these techniques can be applied to human oocytes for production of stem cell lines.

SOURCES OF OOCYTES FOR NT ES CELLS

At current rates of success of generation of NT blastocysts and ES cells, one major limitation of expansion of this approach will be the availability of oocytes for NT. Current and possible future sources of such oocytes include excess oocytes and unfertilized oocytes from IVF procedures, oocytes matured from ovariectomies or fetal ovaries from pregnancy terminations, oocyte donation, derivation of oocytes from nonreproductive material, and use of nonhuman oocytes.

- **Excess oocytes and unfertilized eggs from IVF procedures.** During IVF, hormonal induction is used to generate oocytes for fertilization *in vitro*. Often, more oocytes are generated than are needed for reproductive purposes, and some oocytes may be available for research donation. In addition, after IVF, not all oocytes are successfully fertilized, and unfertilized oocytes would otherwise be discarded if not donated for research. Experiments to explore use of such oocytes for NT derivation of hES cells have been approved and initiated in the United Kingdom. However, this source of oocytes is limited, and the unfertilized oocytes may be of lower quality for cell line production. It is ethically problematic to consider alteration of the IVF clinical procedure to deliberately induce more oocytes than needed for reproduction, even with the consent of the participants. Thus, this source of oocytes is likely to be limited and unreliable for any major NT ES cell program.
- **Oocytes matured from ovariectomies or fetal ovaries from pregnancy terminations.** Adult as well as fetal ovaries contain a large supply of immature oocytes, which in principle could be harvested from adult ovaries donated after removal for clinical reasons or from fetal ovaries that are obtained from legal pregnancy terminations. In the case of other mammals, it is possible to mature such oocytes in culture and achieve fertilization and normal development, although the process is not efficient (O'Brien et al., 2003). In humans, success has been limited and requires an intermediate xenograft (transplantation into an animal) of the ovarian tissue for oocyte maturation. Research on how to expand the supply and how to mature human oocytes *in vitro* could make this a reasonable source of donated material.
- **Oocyte donation.** The most reliable source of oocytes for NT ES cells today seems to be direct donation of oocytes by female donors after hormonal induction and oocyte recovery. Such third-party donation has much in common with organ donation and already occurs in some IVF programs for

reproductive purposes. However, this option raises significant issues about the risks to the donors, about a possible profit motive if excessive payment is made for donated oocytes, and about the nature of informed consent in such circumstances. Altruistic donation of oocytes by family members for generation of disease-related NT ES cells might be a good alternative source of material.

- **Derivation of oocytes from nonreproductive material.** The problems of the limited pool of oocytes for NT would be alleviated if a renewable source of oocytes can be found. The recent report that cells resembling oocytes could be formed from mouse ES cells in culture (Hubner et al., 2003) is intriguing in this regard. If confirmed and extended to human ES cells, this approach could eventually provide an extensive source of oocytes or something resembling oocytes for NT.
- **Use of nonhuman oocytes.** Obtaining large numbers of oocytes from nonhuman mammals is relatively easy, and the use of such oocytes to derive NT blastocysts and stem cells has been considered. If this were successful, the nuclear genome would be entirely human, but there could be some persistence of nonhuman mitochondria in the cells. The relevance of such interspecies mixing for the growth, potential, and safety of such cells would need to be evaluated. There has been one report of putative ES cell lines produced after transfer of human nuclei to rabbit oocytes (Chen et al., 2003), but the finding needs to be confirmed and extended before this approach can be considered feasible.

Given the strong scientific rationale for generating human NT ES cells, there is an urgent need to develop new ethically acceptable sources of cytoplasmic material for reprogramming adult nuclei. Further research into the molecular mechanisms by which the oocyte cytoplasm reprograms the adult nucleus for pluripotency should lead to methods to bypass altogether the need for oocytes to achieve NT reprogramming. In the long run, it may be possible to reprogram adult cells or nuclei directly—not by transfer into oocytes but by other means, such as fusion with pluripotent ES cells or exposure to factors from such pluripotent cells.

INTERSPECIES MIXING

Interspecies mixing happens in nature, and deliberate human-made examples, such as mules, raise no ethical concerns. However, when one of the species involved is human, there is a clear need to consider ethical issues. Hybrids, such as mules, are animals derived from interbreeding between two different species. In the case of a mule, chromosomes from a horse and a donkey are brought together through the fusion of horse and donkey gametes in fertilization to produce an animal whose every cell contains genes from both parental species. Interspecies hybrids are rarely viable and no one proposes to generate interspecies hybrids involving human ga-

metes, even if it were possible. However, there are valid scientific reasons for creating a second sort of interspecies mix in the context of hES cell research—a chimera. Chimeras, unlike genetic hybrids, consist of mixtures of cells (or, in some cases, tissues) from two different kinds of animals. Unlike the situation in hybrids, there is no commingling of genetic material in individual cells of a chimera.

Chimeras are widely used in research and medicine—xenotransplants of, for example, human skin onto mice, of human tumors into mice, and of human bone marrow into mice are already subject to regulation (for example, use of human material is regulated by Institutional Review Boards (IRBs) and animal care issues are regulated by Institutional Animal Care and Use Committees (IACUCs)). Thus, there seem to be no new ethical or regulatory issues regarding chimeras themselves. Nonetheless, because of the pluripotency of hES cells, the extent of their contributions to interspecies chimeras is uncertain, and both the need for and value of chimera experiments involving hES cells and related ethical concerns need to be considered (see Chapter 3). In stem cell research, the possible utility of interspecies mixing arises in several contexts.

Incorporation of hES Cells or Cells Derived from Them into Postnatal Animals of Another Species

Such experiments will be essential to test the potential of hES cells or their derivatives to differentiate into the desired cells and tissues and to ensure that hES cells or their derivatives do not give rise to inappropriate cell types or to tumors or have any other deleterious consequences. Such "preclinical testing" is analogous to the standard testing of drugs, transplants, and medical devices in animals before human clinical trials. It will inevitably be required by the Food and Drug Administration (FDA) en route to any application of hES cells or their derivatives or, indeed, of adult stem cells in therapeutic applications. As mentioned above, many experiments of this type have been done before and are well covered by existing regulations concerning use of human tissues and animals. The use of pig heart valves in humans is an example of routine clinical use of interspecies chimeras. The issues that are particular to hES cells concern the possibility that such cells, because of their pluripotency, could give rise to cells of the germline or the brain. That would be of less or no concern in the case of hES cell derivatives that had differentiated down particular developmental paths, for example, into cells able to make cartilage, bone, skin, or blood. But it needs consideration when pluripotent hES cells or their neural derivatives, such as neural stem cells, are used.

It seems highly unlikely that hES cells could contribute to the germline after implantation into a postnatal animal because the germline is set aside very early in fetal development. Nonetheless, the possibility could readily be addressed by ensuring that animals receiving hES cell transplants do not breed. The possibility of contribution to the brain is harder to evaluate. One purpose of introducing hES cells or human neural progenitor cells is to have them contribute to repair or regenerative

processes and to yield neurons. Production of motor neurons, sensory neurons, or neurons that secrete mediators, such as dopamine, might all contribute to combating spinal-cord injuries and neurodegenerative diseases. However, the idea that human neuronal cells might participate in "higher-order" brain functions in a nonhuman animal, however unlikely that may be, raises concerns that need to be considered. Indeed, if such cells are to be used in human therapeutic interventions, one needs to know whether they could participate in that way in the context of a treatment. Thus, there are good reasons to explore this sort of issue through animal experiments. Studies on the brain are proceeding rapidly, but there is clearly a need for more investigation, and hES cell research in this field should proceed with due care (see Chapter 3).

Incorporation of hES Cells or Cells Derived from Them into Postgastrulation Stages of Another Species

Such experiments would allow a greater opportunity for hES cells to be properly incorporated into appropriately organized tissues and would therefore offer greater opportunities to reveal the potential of such cells. Similar experiments have been invaluable in testing the capacity of neuronal progenitors derived *in vitro* from mES cells by transplantation into chicken embryos (Wichterle et al., 2002); it seems clear that there will be a need or desire to conduct similar experiments to test the potential of hES cells and their derivatives. Indeed, preliminary experiments showing that hES cells can survive and differentiate after transplantation into chicken embryos have been reported (Goldstein et al., 2002). As noted at the outset, there seems little ethical concern about many such experiments, which resemble research approaches that have been used often in the past. For example, human hematopoietic stem cell transplantation would be equivalent to current human-to-mouse bone marrow transplantation and the same could be said for many other tissues. The sensitivities, again, arise concerning neuronal and germline cells and are perhaps more of a concern than in the case of transplantation into a postnatal animal, because the hES cells might be expected to have greater opportunity to participate. As above, the issue of germline contribution could be addressed by preventing any such chimeras from breeding. The potential for incorporation into brain functions needs research and monitoring as mentioned above.

Incorporation of hES Cells into Nonhuman Blastocysts

This approach is an obvious extension of techniques widely used in research with mES cells—namely, aggregation of morulae from two mice or injection of mES cells into mouse blastocysts. In both cases, the cells can contribute extensively to any mouse that arises from implantation of such a chimeric blastocyst. Clearly, an animal (e.g., mouse) blastocyst into which human cells are transplanted raises other issues because potentially the inner cell mass, the progenitor of the fetus, would

consist of a mixture of human and mouse cells. It is not now possible to predict the extent of human contribution to such chimeras. If the recipient blastocyst were from an animal that is evolutionarily closer to a human, the potential for human contributions would appear to be greater. For these reasons, research that involves the production of such chimeras should be performed first using nonhuman primate ES cells in mouse blastocysts before proceeding to use of hES cells. The need for the use of blastocysts from larger mammals would need to be very clearly justified and nonhuman primate blastocysts should not be used at this time. Any chimeric experiments using hES cells should be subject to careful review by the institutional oversight committees described in Chapter 3. (Also see Chapter 3 for additional discussion of the ethical concerns surrounding chimeras.)

Use of Nonhuman Oocytes as Recipients of Human Somatic Nuclei in NT with the Aim of Generating hES Cell Lines Without the Need for Human Oocytes

The possibility of using nonhuman oocytes as recipients for NT was mentioned above. The procedure is not in wide use, and it is not clear how useful it will be, but it might constitute a solution to the problem of limited supplies of human oocytes. More immediately, interspecies combinations (human nucleus into nonhuman oocytes) are potentially valuable research tools that could be used to learn about reprogramming of somatic nuclei, which could be one long-term solution to the problems of tissue rejection and limited supplies of human oocytes. Such an interspecies construct would be similar to the product of human NT and would be subject to similar guidelines regarding implantation or culture beyond 14 days (the primitive streak stage) while still permitting the recovery of ES cells.

PRIORITIES FOR hES CELL RESEARCH

Although the potential for future therapeutic use of hES cells seems clear, many technical issues remain to be solved before the potential can be realized. More than a decade of research with mES cells has amply demonstrated their potential to differentiate into all cells of the body. Nonetheless, there is only limited understanding of how to direct their differentiation into well-defined paths, as would be necessary if hES cells are to be used to generate cells of specific developmental potential for therapeutic purposes. A clear example of how such research must proceed is offered by a study in which mES cells were coaxed to develop *in vitro* into precursors of motor neurons (restricted potential neuronal progenitors or neuronal stem cells), which were then transplanted into chicken embryos, where they differentiated into motor neurons (Wichterle et al, 2002). ES-cell-derived hematopoietic cells can also be used to achieve long-term hematopoietic reconstitution (Kyba et al., 2002), and cardiomyocytes from mouse ES cells have achieved reintegration into cardiac muscle (Klug et al., 1996). Much more of this type of differentiation and

transplantation research will need to be done if hES cells are to be used in regenerative medicine, and much research is needed into the various steps of such protocols.

Experimental manipulations that will need to be developed with hES cells to achieve successful applications in human medicine are described below in sequence from hES cell derivation and culture, through preclinical testing and other research uses to illustrate the spectrum of hES cell research that will be necessary in the coming years and to point out the biomedical rationales for the experiments. These are the types of essential experiments for which the guidelines proposed later in this report are designed to provide a framework for ethical and responsible conduct.

- Additional hES cell lines must be generated because experience from studies of mES cells shows that lines differ in their potential and do not always retain their potential on extended culture. Furthermore, the hES cells now available do not have adequate genetic diversity.
- hES cells of defined genetic backgrounds need to be generated. In the future, such cells could be used in autologous cellular therapy, which would avoid problems of immune rejection, but that prospect is some years away. In the immediate term, hES cells with genotypes known to predispose to particular diseases would be invaluable for research into the bases of the diseases in question and for developing tests for diagnostic and therapeutic approaches (for example, drug testing). Few such genetically defined hES cells now exist, but several sources are possible. Excess blastocysts will necessarily be produced in the course of IVF and PGD procedures designed to derive blastocysts that lack disease-promoting genotypes. Excess blastocysts that are genotypically unsuitable for reproduction would normally be discarded but instead they can be used to generate hES cells (Verlinsky et al., 2005). Such blastocysts could also be generated with IVF procedures specifically for that purpose; families with genetic predispositions might well be motivated to contribute gametes altruistically. Alternatively, hES cells of the desired genotype could be generated using NT; again, altruistic donation of oocytes and nuclei would be a suitable route.
- Genetic manipulation of hES cells is another route to the generation of hES cells with defined genetic defects where the diseases are well enough understood for the relevant genes to be known. Research with such procedures would also lay the groundwork for future manipulations, such as gene therapy, to generate autologous cells in which genetic defects have been "fixed." Such *in vitro* manipulations could eventually allow gene modifications to be controlled with precision to avoid deleterious side effects. hES cells can be genetically modified by introduction of transgenes with a variety of approaches, and homologous recombination to alter the endogenous genes of the cells is also possible (Zwaka and Thomson, 2003). Further research into genetic modification of hES cells is important.

- As discussed in the section on NT, a major current limitation of widespread use of NT is the restricted availability of human oocytes, and research into the many different possibilities for alternative sources is needed. The possibilities include the maturation of immature oocytes derived from therapeutic ovariectomies or from fetal ovaries and, perhaps, of unfertilized oocytes from IVF clinics. However, a better long-term solution of the problem would be development of methods for producing renewable sources of oocytes, such as differentiation of hES cells. Studies on the latter possibility would be invaluable.
- Nonhuman oocytes might also be used for NT, and this needs further research.
- A means of reprogramming the nuclei of somatic cells, either by culturing cells under different growth conditions or by exposing the nuclei to factors from oocyte or hES cell cytoplasm, is essential. Research on the nature of epigenetic modification and means of modifying it so that somatic cell nuclei could be reprogrammed to a state equivalent to that of ES cells would make oocytes and embryos unnecessary for generating hES cells. Success in this effort would be a major advance and, therefore, while not imminent, seems a high priority for research.
- Research is needed to understand how to maintain the self-renewing capacity of hES cells over long-term culture and expansion. In the mouse, the LIF-JAK-STAT pathway of signaling molecules is necessary and sufficient for self-renewal, but it is not sufficient to maintain hES cells in the stem cell state (Daheron et al., 2004). For therapeutic applications, it will be essential to be able to propagate and expand hES cells.
- It will also be necessary to develop culture conditions that do not include mouse feeder cells and bovine serum as in most current research. Animal products will introduce complications in any future therapeutic use of hES cells, both with respect to FDA requirements and because nonhuman materials can contribute biochemical precursors to the hES cells that render them immunogenic and therefore unsuitable for transplantation (Martin et al., 2005). Initial success has been reported in replacing mouse feeder layers (Xu et al., 2005) but additional improvements in culture conditions will need to be developed and tested.
- Detailed investigation will be needed to determine the best means of ensuring stability of genotype, epigenetic status, and phenotypic properties of ES cells grown in long-term cultures for use in human therapies.
- Research is needed to determine how to direct the development of hES cells down particular pathways to generate cells restricted to specific developmental fates. It will involve exploration of different culture conditions and investigation of growth and differentiation factors that promote specified developmental fates. Such investigations will rely on ongoing research into the developmental biology of other species but will require direct studies of

hES cells because there will be differences between ES cells of different species. Studies of nonhuman ES cell models and of hES cells must proceed in parallel.

- A related challenge will be the development of methods to separate progenitors of restricted developmental potential from hES cells (or methods to ensure complete conversion of hES cells into the desired cellular derivatives). mES cells transplanted to ectopic sites can generate benign tumors and such an outcome clearly would be undesirable in any cellular therapy. One can imagine methods for separating or removing persisting hES cells (such as sorting of undifferentiated cells or inducible suicide of inappropriate cells), but research will be required to ensure that such methods are effective.
- All the foregoing procedures will necessitate means of testing the potential of the derived cells to contribute usefully when implanted and for adverse side effects; such tests will undoubtedly be required by FDA before any therapeutic use. That requirement will necessitate development of protocols for effective and ethical testing of the potential of hES cells and their derivatives (or adult stem cells). Many tests can be conducted *in vitro* but *in vivo* tests will also be mandatory. As discussed above, some such tests present no particular ethical problems, and the technical issues can be addressed with further experimentation. However, some chimera experiments that can be easily envisaged raise issues pertaining to the possibilities of hES cell contributions to the brain or the germline. Research is needed to determine the likelihood of those potential concerns. It has been argued that their potential may be quite limited but a main purpose of developing hES cell-based therapies is to promote some participation of the implanted cells. Research will be necessary to discover the extent to which this is possible both to exploit the therapeutic potential and to avoid undesired contributions.
- One issue arising in any cell or tissue transplantation is immune rejection due to histocompatibility antigenic differences between people. This problem is confronted every day in organ transplantation and has been addressed with tissue-matching and immune suppression. Nevertheless it remains a problem and will affect any stem cell-based therapies (adult or embryonic) unless means can be found to avoid it. One such means is the use of autologous hES cells derived using a patient's own nuclei to generate genetically identical hES cells through NT. That approach is feasible and likely to be exploited, but it will face hurdles, such as oocyte availability, if it is to be widely used. The more genetically diverse hES cells there are available, the more likely that a histocompatible matching line can be found. That is a strong argument for development of stem cell banks (see Chapter 5). In parallel, research into ways of avoiding immune rejection should be encouraged both for standard organ transplantation and for future hES cell therapies. With ES cells and their derivatives, it may be possible to devise means

of suppressing histocompatibility antigens, which clearly is not feasible with organ transplants.

- In addition to therapeutic transplantation, hES cells are good candidates for testing of therapeutic drugs. If hES cells can be directed to differentiate into specific cell types, they may be more likely to mimic the *in vivo* response of cells and tissues to the drug being tested and so offer safer models for drug screening. Similarly, hES cells could be used to screen potential toxins. Toxic agents often have different effects on different animal species and cell types, and this makes it critical to have the best possible *in vitro* models for evaluating their effects on human cells. However, it remains to be determined which differentiation stages of hES-derived cells are optimal for such practical applications. For example, what differentiation stages of ES-derived cells would be best for screening drugs or toxins or for delivering potentially therapeutic drugs?

CONCLUSION

The list of hES cell research priorities underlines the need for a broadly accepted set of guidelines to assist researchers and regulators in their design of investigations, whether funded by federal, state, philanthropic, or industrial sources. The research has great promise, but much further investigation is needed to realize the potential, and the sensitivities surrounding research with hES cells require continuing attention to the ethical and public policy issues. The next chapter discusses many of the ethical concerns raised by this research and proposes a system of oversight to address ethical and public concerns.

3

Addressing Ethical and Scientific Concerns Through Oversight

The promise of human embryonic stem (hES) cell research as described in Chapter 2 raises ethical concerns that require a public policy response. This chapter addresses the primary ethical concerns, specifically public sensitivities regarding the status of the human embryo, the need to respect those who donate gametes and embryos to research, the mixing of human and nonhuman cells, and the consensus that nuclear transfer (NT) should not be used for reproductive purposes at the present time. Those concerns and the need for uniform practices and standards in the scientific and medical communities, call for an appropriate and calibrated system of oversight. Several countries have already established laws and guidance in this field and some are described in this chapter (additional discussion can be found in Chapter 4). As discussed in Chapter 1, there is a precedent for self-regulation by the scientific community and research institutions in recombinant DNA research. The initiative taken by the scientific community in the 1970s with regard to recombinant DNA research serves as a model for self-governance in hES cell research in the absence of involvement of the federal government. In this chapter the committee recommends a system of local and national oversight of hES cell research. Because in the final analysis the issues involved are scientific and moral rather than financial the proposed oversight system should apply to all hES cell research regardless of the source of funding.

ETHICAL CONCERNS

The principle ethical and religious objection to hES cell research is that the derivation of hES cells involves the destruction of the blastocyst, which is regarded

by some people as a human being. A second objection, which relates to blastocysts created for research purposes—whether through fertilization or NT—is that it is wrong to create a blastocyst with the intention of destroying it. A third objection is that some of the research depends on donor oocytes, which could result in the exploitation of women. In addition, some people are concerned about the mixing of human and nonhuman cells for research purposes. Finally, some object to the use of NT to derive hES cells because they fear that the use of NT for research purposes could lead to its use to produce a child.

The Special Status of the Human Embryo

Like all scientific work involving human embryos, hES cell research raises profound questions about the status of the human embryo, the extent to which it is justifiable to use human embryos to expand knowledge and ameliorate human suffering, and the conditions under which these goals may be pursued. Throughout its deliberations the committee was keenly aware that some view human embryos as morally equivalent to born human persons. This position takes several forms. Some argue that the identity of a future born person is present in the embryo. Others identify the moral equivalence of the human embryo to the born human person with the embryo's potentiality. Still others claim that human dignity is undermined by excessive manipulation of the human embryo regardless of the purpose and that this could lead to the abuse and exploitation of human persons more generally.

Yet even in our own society, where many hold this view in a philosophical sense, it has not been adopted as a matter of cultural practice. For example, the natural loss of an embryo in normal human reproduction is not recognized as a death that requires a funeral, and the disposal of human embryos after completion of infertility treatments is not treated as murder by the legal system. Nonetheless, in the United States in particular, hES cell research is eligible for limited federal funding because the current administration wishes to acknowledge the view of some that the destruction of embryos required to obtain new cell lines gives such lines a moral taint.

In contrast, many religious traditions—Islam, Judaism, and numerous Protestant denominations—do not recognize the human embryo before 40 days after conception as an entity that should be accorded the same moral status as a person. Among some of these traditions, there is also a strong commitment that faith must be manifest in good works and that the world itself and the persons within it should be objects of strenuous efforts to heal (National Bioethics Advisory Commission (NBAC), 1999b). To be sure, in these traditions the human embryo may have greater moral status than other collections of cells, but not so much that its cells may not be respectfully applied toward the other goals to which the faithful are committed.

There is a more general debate about the meaning of human dignity. For some, the use or creation of human embryos in research, or even the very prospect of

advances in genetics and molecular biology, represent manipulations of life that undermine human dignity. In contrast, others view the effort to heal the sick as a profound moral obligation and the restoration of health and natural functions as the promotion of human dignity. In the latter view, the undifferentiated blastocyst cells that yield hES cells are a resource that should not be squandered.

This diversity of deeply held views must be respected. However, that respect does not require that we, as a society, prohibit hES cell research, but rather that our society create institutions for the oversight of this research that, with due moral seriousness, take into account the special status of the human embryo.

Respect for Donors of Human Embryos and Gametes

Like other modern technologies associated with human reproductive capacities, hES cell research often involves donated embryos or oocytes. There is a set of minimal conditions that applies to the process of obtaining embryos and gametes for research purposes, normally from *in vitro* fertilization clinics. Those conditions are reflected in policies, guidelines, and practices in the United States and elsewhere. They include restrictions on monetary and other inducements, separation between clinical decisions and decisions to donate, and the requirement of voluntary informed consent of donors through a process that has been approved by an Institutional Review Board (IRB), as specified in federal regulations for the protection of human subjects in research (45 CFR 46.107; see Chapter 4 for further discussion). A measure of respect for donors is the assurance that research using donated materials is limited to qualified investigators and that studies have scientific merit. Those issues are discussed in greater depth below and in Chapter 5.

Transferring hES Cells into Nonhuman Animals

The transfer of hES cells into nonhuman animals has received less attention than some of the other ethical and policy issues surrounding stem cell research. The transfer of human stem cells (whether adult or embryonic) or their derivatives into nonhuman animals, creating chimeric entities, will be an important laboratory technique in research with both adult and human embryonic stem cells and may have clinical applications as well. As discussed in Chapter 2, research purposes could include understanding the mechanisms by which transplanted cells localize and differentiate in a host and using the cells in preclinical testing. Human cells also could someday be grown into functioning tissues or organs in an animal for later transfer into a patient.

A different perception of the unnaturalness of mixing tissues from different sources is the idea that there are fixed species. However, the popular notion that there are clear and distinct lines between species is a notoriously unreliable categorical scheme. Taxonomies developed since Aristotle do not necessarily countenance the idea of natural kinds, and modern scientists differ in their precise definitions of

interspecies boundaries. There is general agreement in the scientific community that these boundaries are to some extent arbitrary. As discussed in Chapter 2, some chimeras are viewed with equanimity (for example, pig heart valve transplants into humans), and one must be careful to distinguish legitimate concerns from discomfort arising from unfamiliarity. Although moral intuitions about the creation of chimeras may vary, it is a subject of deep moral concern to many thoughtful people for whom the creation of animals with certain kinds or quantities of human tissues, such as neural or germline cells, would be offensive. Accordingly, such research requires careful consideration and review.

Among the issues to be considered in the review of such proposals will be the number of hES cells to be transferred, what areas of the animal body would be involved, and whether the cells might migrate through the animal's body. The hES cells may affect some animal organs rather than others, raising questions about the number of organs affected, how the animal's functioning would be affected, and whether some valued human characteristics might be exhibited in the animal, including physical appearance.

Perhaps no organ that could be exposed to hES cells raises more sensitive questions than the animal brain, whose biochemistry or architecture might be affected by the presence of human cells. Human diseases, such as Parkinson's disease, might be amenable to stem cell therapy, and it is conceivable, although unlikely, that an animal's cognitive abilities could also be affected by such therapy. Similarly, care must be taken lest hES cells alter the animal's germline. Protocols should be reviewed to ensure that they take into account those sorts of possibilities and that they include ethically sensitive plans to manage them if they arise.

Various precautions seem reasonable in studies that involve the transfer of hES cells into nonhuman animals and should be considered in any prior review of a protocol. Questions that should be raised in this context include the following

- Are hES cells required, or can cells from other primates or animals be used?
- Has sufficient animal work preceded the proposed work involving hES cells?
- Might the cell transfer result in the animal's acquiring characteristics that are valued as distinctly human?
- If hES cells are to be transferred into an animal embryo or fetus, have studies (for example, with ES cells from other species or interspecies chimeras) suggested that the resulting creature would exhibit human characteristics that would be ethically unacceptable to find in an animal?
- If visible human-like characteristics might arise, have all those involved in these experiments, including animal care staff, been informed and educated about this?

Furthermore, donors of gametes and embryos should be informed that some of the hES cells derived from their donated cells and tissues might be transferred into nonhuman animals in the course of developing and testing their therapeutic potential (see Chapter 5).

Objections to the Use of NT for Reproductive Purposes

The ethical concerns about attempts to use NT to create children are well known. They include risks to the mother and the fetus that have been described in numerous reports by other advisory bodies and institutions (NBAC, 1997; NRC, 2002; President's Council on Bioethics, 2002). As discussed in Chapter 1, this is a matter on which the U.S. National Academies and the scientific community worldwide have spoken with virtually a single voice. Attempts to create a child by means of NT are ethically objectionable at this time because, on the basis of experience with other mammalian species, producing one child might require hundreds of pregnancies and many abnormal late-term fetuses could be produced. Furthermore, some authorities believe that there can never be a fully normal product of NT because of the differences in imprinting between the genes in a transplanted somatic nucleus and those in the oocyte nucleus that it has replaced (Jaenisch, 2004), as well as a failure of epigenetic reprogramming in general. Such concerns led to Food and Drug Administration efforts to prohibit NT for reproductive purposes.[1]

Even in the absence of moral justification for attempting NT for reproductive purposes, some groups have announced their intention to pursue that objective, even if merely to generate publicity. An oversight system for hES cell research that might include NT as a source of cell lines will reinforce the ethical and scientific consensus that NT for reproductive purposes has no place in legitimate research. The danger that the efforts will continue is far greater in the absence of systematic oversight with its attendant accountability and transparency.

THE NEED FOR AN OVERSIGHT SYSTEM

As a starting point for its deliberations, the Committee on Guidelines for Human Embryonic Stem Cell Research examined numerous other guidelines and regulations in use now or in the past to identify best practices and common features. Surveys of guidelines and regulations for embryo and/or hES cell research by this committee and others (Walters, 2004) revealed that common features of most, if not all, programs throughout the world include

- A prohibition on nuclear transfer for reproductive purposes.
- A prohibition on the culture of human embryos beyond 14 days after fertilization or when the primitive streak has appeared, whichever occurs first.

Most existing regulations and guidelines embody broad guiding principles. For example, most require that hES cell research projects aim to advance scientific and medical knowledge to benefit human health. Alternative methods (such as the use of existing hES cell lines or adult stem cells) must have been examined and shown to be

[1]See FDA letter to investigators and sponsors at http://www.fda.gov/cber/ltr/aaclone.pdf.

insufficient for projects that propose to derive new hES cell lines. And research must conform to the highest ethical and scientific standards and be conducted sensitively and in accordance with all regulatory requirements of the nation or state. For example, even under its relatively liberal policy, the United Kingdom, in its *Code of Practice for the Use of Human Stem Cell Lines*, requires that all hES cell research be conducted under special licenses obtained from the government. The rationale is, in part, to ensure protection of the status of the human embryo:

> The special regulations which govern the creation and use of human embryonic stem cells reflect the fact that the human embryo has a special moral status. The position taken by many (perhaps most) is that the embryo, unlike an infant, does not have the full rights of a person; however, its human potential gives it an intrinsic value which implies that neither its creation nor its destruction are to be treated casually, as reflected in law. A research license will not be granted unless the HFEA [Human Fertilisation and Embryology Authority] is satisfied that any proposed use of the embryos is necessary for the research and that the research is necessary or desirable for the purposes specified in the 1990 HFE Act and the 2001 Regulations. . . . Although the use of embryos for these purposes is now permitted under the law, researchers in this field should be sensitive to the fact that some people believe this practice to be morally unacceptable [MRC, 2004].

Many other sets of guidelines also contain provisions to ensure voluntary embryo donation—with a requirement of informed consent—and requirements that the confidentiality of donors be protected. Because there is no federal support in the United States for hES cell research in which new cell lines are derived, the most applicable guidelines come from the Ethics Committee of the American Society for Reproductive Medicine (ASRM, 2000; 2004b). Canada and the United Kingdom also have substantive procedural requirements regarding the recruitment of donors and informed consent. (Those and other approaches are addressed in detail in Chapter 5.) Most guidelines also call for some special oversight body for hES cell research to review documentation of compliance with the guidelines of various government agencies, both domestic and foreign (see Chapters 4 and 5). Oversight is in some cases folded into the evaluation of scientific merit; in others, it is performed by stand-alone ethics review bodies. Finally, most forms of laboratory and clinical research in the United States are subject to substantial local regulation, including provision of protections for human subjects in research, protections for laboratory animals, and the many considerations that must be addressed for research and testing of new drugs and medical devices. (The applicability of those regulatory systems to hES cell research is addressed in Chapter 4.)

In considering the ethical and policy issues that arise in connection with hES cell research, the committee subscribes to the consensus of many bioethics bodies throughout the world that a system of oversight of hES cell research should be in place. Examples of current and former national bioethics bodies taking such a view are the 1994 National Institutes of Health Human Embryo Research Panel, the National Bioethics Advisory Commission, the U.K. Human Fertilisation and Em-

bryology Authority, and others (see Chapter 4 for elaboration). Unfortunately, the U.S. government has not established such a regulatory system, although many regulations are relevant to these activities, and seems unlikely to do so in the near future, especially in the absence of a substantial federal presence in this field because of the current limitations on the use of federal funding.

However, nonfederally funded hES cell research is going forward in the absence of federal regulation specific to such research, and it is incumbent on the scientific community to exercise the same sort of self-discipline as it has exercised in the past with regard to novel areas of research, such as recombinant DNA in the 1970s. In the absence of a federal regulatory regime designed specifically to provide comprehensive coverage of hES cell research, the committee proposes an oversight system with both local and national components that meets the important goals identified by the other advisory bodies, including the President's Council on Bioethics in its report on NT (President's Council on Bioethics, 2002):

- To support the current consensus against attempts to create children through NT;
- To create a forum for further deliberation on these questions;
- To ensure that legitimate research includes efforts to gather information from animal models and other avenues before utilizing hES cells; and
- To show respect for the deep moral concerns of those who have ethical objections to the research.

RECOMMENDATIONS

Institutional Oversight of hES Cell Research

The ethical and legal concerns involved in hES cell research make increased local oversight by research institutions appropriate. Because of the complexity and novelty of many of the issues involved in hES cell research, the committee believes that all research institutions engaged in hES cell research should create and maintain Embryonic Stem Cell Research Oversight (ESCRO) committees to

1. Provide oversight for all issues related to derivation and use of hES cell lines.
2. Review and approve the scientific merit of research protocols.
3. Review compliance of all in-house hES cell research with all relevant regulations (see Chapter 4) and the guidelines presented in this report (see Chapter 6).
4. Maintain registries of hES cell research conducted at the institution and hES cell lines derived or imported by institutional investigators.
5. Facilitate education of investigators involved in hES cell research.

An ESCRO committee will assist investigators in assessing which regulations

might apply to proposed research activities (see Chapter 4 for a fuller discussion). It could serve as a clearinghouse for hES cell research proposals and could assist investigators in identifying the types and levels of review required for a given protocol. For example, the creation of a human/nonhuman chimera may involve review by both an IRB and an Institutional Animal Care and Use Committee (IACUC). In some instances, Institutional Biosafety Committees (IBCs), radiation safety committees, and other groups may also have roles to play in research review (see Chapter 4 for further discussion of the roles of these committees). If hES cell research involves potential clinical applications (such as development of products to be tested in humans), FDA regulations will apply. However, care should be taken that the ESCRO committee does not duplicate or interfere with the proper functions of an IRB or other existing institutional committees. The functions of IRBs and ESCRO committees are distinct and should not be confused.

One particularly important aspect of regulatory compliance for some hES cell research is protection of donors of blastocysts and gametes, which is a matter for IRB review. On the other hand, laboratory research with existing hES cells is generally not covered by federal regulations governing research with human subjects (Department of Health and Human Services [DHHS] regulations at 45 CFR 46, subparts A through D[2]) unless the research involves personally identifiable information about a cell line's progenitors (see Chapter 4). Such research does not need IRB review but should be reviewed by an ESCRO committee. In general, research institutions are likely already to have rules in place for research involving other biological tissues, and, as with any other form of biological or biomedical research, hES cell research would be covered by these rules. But in the case of hES cell research, it will be critically important for investigators and institutions to know the provenance of hES cell lines, particularly if the cell lines are imported to the institution from another site. This would include obtaining an assurance that the process by which the cells were procured was approved by an IRB to ensure that donors provided voluntary informed consent and that risks were minimized (see Chapters 4 and 5). The IRB could be situated at the institution where the cells originated or at the institution where the stem cell research is to be conducted, or it could be independent (non-local). As described in Chapter 5, only one IRB need approve the procurement process, but the institution where the research is to be conducted should obtain evidence of such review. In all cases, the ESCRO committee should

[2]DHHS has codified its human subjects protections regulations at 45 CFR 46, subparts A through D. Other agencies have signed onto subpart A, which is referred to as the Common Rule. In this report, DHHS regulations are cited because they are more inclusive than the Common Rule alone, providing protections also to pregnant women, viable fetuses, children, and prisoners. FDA also has codified subpart A of the regulations at 21 CFR 50 and 56 but with slightly different interpretations. In some cases, FDA regulations *and* HHS regulations might apply to research.

ensure that the procurement process has been appropriate by requiring documentation that it was approved by an IRB and adhered to basic principles of ethically responsible procurement. (See Chapter 5 for commentary on requirements for informed consent, payment, timing of consent, and coding of samples.)

The second role of the ESCRO committee is to review research proposals that involve particularly sensitive kinds of research. It is important to note that the vast majority of *in vitro* experiments using already derived hES cell lines are unlikely to raise serious ethical issues and will require minimal review. However, proposals to generate additional hES cell lines by any means will require more extensive review. Some other experiments will also warrant careful consideration, including research in which the identity of the donors of the blastocysts or gametes from which the hES cells were derived is readily ascertainable by the investigator and experiments involving implantation of hES cells or human brain cells into nonhuman animals. Because of the sensitive nature of some aspects of hES cell research, it is critical that the scientific community propose and implement limits on what is to be allowed and provide clear guidance on which research activities require greater scrutiny. Thus, a primary activity of the ESCRO committee will be to ensure that inappropriate research is not conducted and that controversial research is well justified and subject to appropriate additional oversight. Oversight will in many instances conform to a higher standard than is currently required by laws or regulations.

Among those studies that should not be conducted at this time are any that involve *in vitro* culture of any intact human embryo, regardless of derivation method, for longer than 14 days or until formation of the primitive streak begins, whichever occurs first. This is a widely recognized international standard that avoids research on embryos after the formation of the precursors of the brain and central nervous system. Research in which hES cells are introduced into nonhuman primate blastocysts, or in which animal or human ES cells are introduced into human blastocysts, should also not be conducted at this time. These kinds of studies could produce creatures in which the lines between human and nonhuman primates are blurred, a development that could threaten to undermine human dignity. Finally, although it is unlikely, hES cells introduced into nonhuman hosts might be able to generate gametes, so any such human/nonhuman chimeras should not be allowed to breed (see Chapter 2 for further discussion). In all those cases, future scientific advances might render the concerns moot or might raise new concerns, so the category of currently nonpermissible experiments will need review in the future (see later discussion of a national review panel).

The ESCRO committee must have suitable scientific, medical, and ethical expertise to conduct its own review and should have the resources needed to coordinate the management of the various other reviews that may be required for a particular protocol. Besides scientists and ethicists, its membership should also include at least one person from the community. A pre-existing committee could serve the functions of the ESCRO committee provided that it has the recommended expertise and representation to perform the various roles described in this report.

For example, an institution might elect to constitute an ESCRO committee from among some members of an IRB. But the ESCRO committee should not be a subcommittee of the IRB, as its responsibilities extend beyond human subject protections. Furthermore, much hES cell research does not require IRB review.

Because stem cell research is subject to a greater degree of public interest and scrutiny than most other laboratory and clinical research, the committee believes that each institution should maintain through its ESCRO committee a registry of hES cell lines in use and of investigators working with them and descriptive information on the types of hES cell research in which they are engaged. The purposes of such a registry include facilitating distribution of educational information in light of evolving ethical, legal, or regulatory issues and enabling an institution to respond to public inquiry about the extent of its involvement in hES cell research. The ESCRO committee should also play a central role in educating investigators—including research staff, fellows, and students—on ethical, legal, and policy issues in stem cell research. That might include developing and maintaining a web-based primer, such as those commonly used at research institutions that support human subjects research.

The foregoing concerns give rise to the following recommendations.

Recommendation 1:
To provide local oversight of all issues related to derivation and research use of hES cell lines and to facilitate education of investigators involved in hES cell research, all institutions conducting hES cell research should establish an Embryonic Stem Cell Research Oversight (ESCRO) committee. The committee should include representatives of the public and persons with expertise in developmental biology, stem cell research, molecular biology, assisted reproduction, and ethical and legal issues in hES cell research. The ESCRO committee would not substitute for an Institutional Review Board but rather would provide an additional level of review and scrutiny warranted by the complex issues raised by hES cell research. The committee would also serve to review basic hES cell research using preexisting anonymous cell lines that does not require consideration by an Institutional Review Board.

Recommendation 2:
Through its Embryonic Stem Cell Research Oversight (ESCRO) committee, each research institution should ensure that the provenance of hES cells is documented. Documentation should include evidence that the procurement process was approved by an Institutional Review Board to ensure adherence to the basic ethical and legal principles of informed consent and protection of confidentiality.

Recommendation 3:
Embryonic Stem Cell Research Oversight (ESCRO) committees or their equivalents should divide research proposals into three categories in setting limits on research and determining the requisite level of oversight:

(a) Research that is permissible after notification of the research institution's ESCRO committee and completion of the reviews mandated by current requirements. Purely *in vitro* hES cell research with pre-existing coded or anonymous hES cell lines in general is permissible provided that notice of the research, documentation of the provenance of the cell lines, and evidence of compliance with any required Institutional Review Board, Institutional Animal Care and Use Committee, Institutional Biosafety Committee, or other mandated reviews is provided to the ESCRO committee or other body designated by the investigator's institution.

(b) Research that is permissible only after additional review and approval by an ESCRO committee or other equivalent body designated by the investigator's institution.

(i) The ESCRO committee should evaluate all requests for permission to attempt derivation of new hES cell lines from donated blastocysts, from *in vitro* fertilized oocytes, or by nuclear transfer. The scientific rationale for the need to generate new hES cell lines, by whatever means, must be clearly presented, and the basis for the numbers of blastocysts and oocytes needed should be justified. Such requests should be accompanied by evidence of Institutional Review Board approval of the procurement process.

(ii) All research involving the introduction of hES cells into nonhuman animals at any stage of embryonic, fetal, or postnatal development should be reviewed by the ESCRO committee. Particular attention should be paid to the probable pattern and effects of differentiation and integration of the human cells into the nonhuman animal tissues.

(iii) Research in which personally identifiable information about the donors of the blastocysts, gametes, or somatic cells from which the hES cells were derived is readily ascertainable by the investigator also requires ESCRO committee review and approval.

(c) Research that should not be permitted at this time:

(i) Research involving *in vitro* culture of any intact human embryo, regardless of derivation method, for longer than 14 days or until formation of the primitive streak begins, whichever occurs first.

(ii) Research in which hES cells are introduced into nonhuman primate blastocysts or in which any ES cells are introduced into human blastocysts.

In addition:

(iii) No animal into which hES cells have been introduced at any stage of development should be allowed to breed.

***Recommendation 4*:**

Through its Embryonic Stem Cell Research Oversight (ESCRO) committee, each research institution should establish and maintain a registry of investigators conducting hES cell research and record descriptive information about the types of research being performed and the hES cells in use.

Investigators who collaborate across national boundaries should respect the ethical standards and procedural protections applicable in all the relevant jurisdictions.

***Recommendation 5*:**

If a U.S.-based investigator collaborates with an investigator in another country, the Embryonic Stem Cell Research Oversight (ESCRO) committee may determine that the procedures prescribed by the foreign institution afford protections equivalent with these guidelines and may approve the substitution of some or all of the foreign procedures for its own.

The committee hesitates to recommend another bureaucratic entity to oversee the biomedical research system, but in this case it believes the burden to be justified because of the special issues involved in hES cell research and the diverse entities that might have a role in the review process in a research institution. A coordination function is crucial. In some cases, smaller institutions may wish to avail themselves of the services of larger facilities that have ESCRO committees.

The creation of an ESCRO committee to perform functions unique to hES cell oversight does not relieve institutions or scientific investigators, regardless of their field, of the ultimate responsibility to ensure that they conduct themselves in accordance with professional standards and integrity. In particular, people whose research involves hES cells should work closely with oversight bodies, demonstrate respect for the autonomy and privacy of those who donate gametes and embryos, and be sensitive to public concerns about research involving human embryos.

Need for a National Perspective

As individual states and private entities move into the field of hES cell research, it is important to initiate a national effort to provide a formal context in which the complex moral and oversight questions associated with this work can be addressed. The state of the science of hES cell research and the clinical practice and public policy surrounding these topics are in a state of flux and are likely to be so for several years. Therefore, the committee believes that some entity needs to be estab-

lished to review the policies and guidelines covering appropriate practices in this field but not to review and approve specific research protocols, an activity that will best occur at the local institutional level. Such national bodies have been established in most other countries where hES cell research has been debated and approved—such as Australia, Canada, Israel, Singapore, and the United Kingdom (see Chapter 4)—usually under government auspices. Some of those bodies also have responsibility for reviewing individual research proposals, and such centralized review entities may serve well in smaller jurisdictions where public funds are being used in the research. However, in line with the longstanding practice in the United States of using local review boards for human subjects research, animal research, and biohazards, the committee believes that local review of individual research proposals by ESCRO committees (with involvement of IRBs, IACUCs, IBCs, and other panels as described above) will be the best mechanism of oversight of hES cell research. Nonetheless, there will be a need for continuing consideration of new issues that arise from scientific advances, clinical applications, or public policy concerns that will need to be discussed in a central forum. Such a forum should from time to time review the adequacy of the guidelines proposed in this report (Chapter 6) in light of changes in science and the emergence of new issues of public interest. New policies and standards may be appropriate for issues that cannot currently be foreseen.

The organization that sponsors the public forum should be one that is respected in the lay and scientific communities, is politically independent without conflicts of interest, and is able to call on suitable expertise to support the effort. Its membership should include nationally and internationally recognized authorities in the scientific, medical, ethical, and legal issues associated with hES cell research, and representatives of the public. The proposed national body must pay careful attention to evidence and argumentation in its deliberations, as well as taking into account the diverse views of the public on these sensitive and evolving issues.

To help ensure that these guidelines are taken seriously, the various stakeholders in hES cell research—sponsors, funding sources, research institutions, relevant oversight committees, professional societies, and scientific journals, as well as investigators—should develop policies and practices that are consistent with these guidelines and adhere to the recommendations of the national panel. Funding agencies, professional societies, journals, and institutional review panels can provide valuable community pressure and sanctions to ensure compliance. For example, ESCRO committees and IRBs should require evidence of compliance when protocols are reviewed for renewal, funding agencies should assess compliance when reviewing applications for support, and journals should require that evidence of compliance accompanies publication of results.

***Recommendation 6*:**
A national body should be established to assess periodically the adequacy of the guidelines proposed in this document and to provide a forum for a continuing discussion of issues involved in hES cell research.

The Just Distribution of the Benefits of hES Cell Research

Billions of dollars will be committed to hES cell research from public and private sources in the coming years. It is not yet clear exactly what specific therapeutic benefits will emerge from this investment, but there is reason for concern that they will not be equitably distributed in our current health-care system. Skeptics may argue that the social investment in science that still requires much research before any health benefits will be realized is not merited when so many basic, often technology-intensive, health services are not adequately provided.

The therapeutic possibilities inherent in hES cells can mean vastly improved lives for millions of disease sufferers, and the successful practice of regenerative medicine could yield substantial reductions in health-care expenditures. It is critical that hES cell research, especially as it approaches clinical application, serve the needs of all populations. There must be a concerted effort to ensure diversity not just in the genetic makeup of cell lines but in the approaches to clinical care. Our current health-care system is not well designed for the just distribution of the benefits of research. Besides the excellent scientific work that will surely be accomplished, institutions involved in hES cell research should concern themselves with ensuring genetic diversity in the development of cell lines and in devising health-care systems that can make the long-term benefits of this work widely available.

Recommendation 7:
The hES cell research community should ensure that there is sufficient genetic diversity among cell lines to allow for potential translation into health-care services for all groups in our society.

CONCLUSION

The proposed local ESCRO committees and national forum should help to ensure that conventional and well founded research practices and protections apply to hES cell research. Among those practices is the use of *in vitro* and animal models before interventions that involve human subjects. Protections include minimizing the use of human gametes or embryos and ensuring that recruitment, disclosure, informed consent, and risk assessment procedures are in accord with the highest ethical standards. The consensus on prohibition of NT for reproductive purposes can also be reinforced with a rigorous system of oversight of hES cell research. With this system in place, the scientific community will signal its respect for the views of those who have ethical reservations about the research and provide an opportunity for those views to be expressed. As some have observed, when many people find a practice morally troubling—particularly one that is novel—that is an indication that further consideration is required. An initial reaction of moral alarm need not be

decisive. Many practices that are now regarded as morally noncontroversial, such as blood transfusions, organ transplants and *in vitro* fertilization, were once seen by many as shocking and unacceptable; others that are now regarded as unacceptable, such as blood-letting, were conventional. Moral perceptions are sharpened with experience, through the growth of knowledge, and the consideration of various viewpoints. Even if the underlying principles do not change, the interpretation and application of the principles often do.

The next chapter addresses the specific regulatory issues that might apply to hES cell research.

4

Current Regulation of Human Embryonic Stem Cell Research

It would be a mistake to assume that the restrictions on federal funding for human embryonic stem (hES) cell research result in an absence of oversight of such work. At present, many federal regulations already govern various aspects of hES cell research, including

- Human subjects protection for donors of somatic cells and oocytes and for some donors of embryos.
- Medical privacy protections.
- Laboratory standards for investigators whose work will result in products that require Food and Drug Administration (FDA) approval.
- Safety reviews of laboratory work that involves genetic alteration of hES cell lines.
- Animal care committee reviews of hES cell research that uses nonhuman animals.
- Various rules governing the importation of biological materials or the transfer of medical data from other countries.

However, there is a perception that the field is unregulated. In fact, the field is subject to a patchwork of regulations, many not designed with this research specifically in mind, and the patchwork has some gaps in its coverage.

This chapter reviews current state and federal regulation of hES cell research in the United States, noting where gaps in regulatory coverage are addressed by the guidelines proposed later in this report (Chapter 6). It also offers some examples of how the proposed guidelines would operate in conjunction with current regulations

and presents comparisons with regulations in other nations that have substantial hES cell research programs. Recommendations about the application of existing regulatory conventions to hES cell research are offered.

Finally, although the committee recognizes that successful resolution of intellectual property issues will be critically important in this evolving area of research, it was beyond its charge and beyond its capabilities to address adequately all of the legal issues that will arise. In the context of privately funded research it is particularly difficult to explore mechanisms by which discoveries made using hES cells can be made widely accessible for the benefit of human health. However, the committee believes that best practices can be developed and followed. Several policy statements developed regarding patenting and licensing issues more generally applied in biomedical science can serve as aspirational goals for the hES cell research community. In particular, in 2004 NIH issued *Best Practices for the Licensing of Genomic Inventions*.[1] This document aims to maximize the public benefit whenever Public Health Service-owned or -funded technologies are transferred to the commercial sector. In this document NIH recommends that "whenever possible, non-exclusive licensing should be pursued as a best practice. A non-exclusive licensing approach favors and facilitates making broad enabling technologies and research uses of inventions widely available and accessible to the scientific community." In addition, the National Academies is developing recommendations for NIH on intellectual property rights in genomic- and protein-related innovation (forthcoming, 2005). The reader is encouraged to review these documents, which aim to facilitate responsible patenting and licensing practices by the scientific community.

REGULATION OF PROCUREMENT OF GAMETES, SOMATIC CELLS, AND BLASTOCYSTS

Whether it involves receiving donated blastocysts that would otherwise be discarded after infertility treatment or procuring gametes and somatic cells to make blastocysts specifically for research purposes, the procurement process often requires oversight by an Institutional Review Board (IRB), whose membership and functions are described in Department of Health and Human Service (DHHS) regulations at 45 CFR 46.107-115 and in FDA regulations at 21 CFR 56.107-115.[2] IRB

[1] http://a257.g.akamaitech.net/7/257/2422/06jun20041800/edocket.access.gpo.gov/2004/pdf/04-25671.pdf.

[2] DHHS has codified its human subjects protection regulations at 45 CFR 46, Subparts A through D. Other federal research agencies have signed onto Subpart A, which is referred to as the Common Rule. In this report, the DHHS regulations are cited in discussing the protection of human subjects of research because they are more inclusive than the Common Rule alone. The DHHS regulations extend additional protections to vulnerable populations, such as pregnant women, viable fetuses, prisoners, and children. FDA also has codified Subpart A of the regulations at 21 CFR 50 and 56, although with slightly different interpretations. In some cases, FDA regulations and HHS regulations might apply to research.

review is the primary means of implementing the research protections found in the federal regulations, which generally require that human research be undertaken with the informed and voluntary consent of the subjects, that the risks to subjects be minimized, and that the research be approved and monitored by an IRB. The federal regulations generally are triggered when research is funded by the federal government, when privately funded research is aimed at developing data for a product to be approved by FDA, or when privately funded research takes place at institutions that have agreed to adopt the protections more broadly than required by law. In addition, some states, such as California and New Jersey, have adopted legislation requiring IRB review and many of the substantive protections of the federal regulations with regard to hES cell research conducted in those states.[3]

Research involving hES cells will require access to human oocytes and blastocysts, which in turn will necessitate some interaction between donors of oocytes and blastocysts and the people or institutions seeking to procure these materials for use in hES cell research. The federal regulations governing human subjects research define human subjects research as involving either

(1) obtaining data from a living individual through intervention or interaction with the individual; or
(2) obtaining private (i.e., individually identifiable) information about a living individual (45 CFR 46.102(f)).

The DHHS Office for Human Research Protections (OHRP) has made it clear that hES cell research "that involves neither interactions nor interventions with living individuals or obtaining identifiable private information is not considered human subjects research [and therefore] IRB review is not required for such research."[4] According to OHRP, merely asking couples whether they wish to donate their surplus blastocysts for research does not render them "human subjects of research" if no data on them are being gathered and there is no substantive interaction with them other than gaining their consent.[5]

On the other hand, where physical interaction is needed to obtain biological materials, such as in the case of donors whose sperm, oocytes, or somatic cells are used to make blastocysts for research, the interaction brings them under the purview of the human subjects protections system and IRB review is required, even though the donors are not themselves the subjects of scientific study. Thus, their fully informed and voluntary consent is required before such research use.

[3]See http://www.ncsl.org/programs/health/genetics/rt-shcl.htm.

[4]*Guidance for Investigators and Institutional Review Boards Regarding Human Embryonic Stem Cells, Germ Cells and Stem Cell-Derived Test Articles,* OHRP/DHHS, Mar. 19, 2002, at 3.

[5]OHRP staff briefing to the committee, January 8, 2005, interpreting 45 CFR 46.102(f).

Whether it is blastocyst donation or the donation of gametes and somatic cells, even where the federal regulations require informed consent, IRBs are permitted to waive the requirement if certain conditions are met (45 CFR 46. 116(8)(d)), that is, if the research is of minimal risk, waiver of consent would not adversely affect rights and welfare of subjects, and obtaining consent is impracticable. In the case of gamete or somatic cell donation, in which the donors must be present at the time of donation, not all those conditions apply, and waiver of consent cannot be granted. In the case of blastocyst donation, the committee finds that informed consent should be required in all cases (see Chapter 5): a waiver should not be granted even when the specified conditions can be met.

Although OHRP requires IRB review of the procurement process for blastocyst donors only under certain conditions, this committee finds that the best way to ensure that protections are in place for all potential donors is to require IRB review at all times for the process by which somatic cells, gametes, and blastocysts are obtained to ensure that risks are minimized and voluntary and informed consent is provided. (Consent issues are addressed in greater detail in Chapter 5.) In contrast, as noted below in the discussion of privacy protections, when research is to be conducted on hES cell lines that have already been derived through a procurement process approved by an IRB, the committee does not find that there is need for additional IRB review of work with coded or anonymous cell lines.

Recommendation 8:
Regardless of the source of funding and the applicability of federal regulations, an Institutional Review Board or its equivalent should review the procurement of gametes, blastocysts, or somatic cells for the purpose of generating new hES cell lines, including the procurement of blastocysts in excess of clinical need from *in vitro* fertilization clinics, blastocysts made through *in vitro* fertilization specifically for research purposes, and oocytes, sperm, and somatic cells donated for development of hES cell lines derived through nuclear transfer.

Recommendation 9:
Institutional Review Boards may not waive the requirement for obtaining informed consent from any person whose somatic cells, gametes, or blastocysts are used in hES research.

Requiring informed consent before donation of gametes, somatic cells, or blastocysts and requiring oversight by such a body as an IRB would bring U.S. practices into conformity with the practices in Australia, Canada, Israel, Singapore, the United Kingdom, and other major centers of hES cell research. That, in turn, will not only ensure the ethical conduct of procurement practices in the United States but also facilitate collaboration with investigators subject to regulations in the other countries.

THE PRIVACY RULE AND HUMAN SUBJECTS PROTECTIONS FOR RESEARCH WITH BIOLOGICAL MATERIALS: IMPLICATIONS FOR hES CELL RESEARCH

In many cases, medical information about donors will be collected at the time of gamete or blastocyst donation. The primary purpose of collecting such information is to permit a coded link to be maintained between the resulting hES cell lines and information about the genetic or infectious disease status of the donors. The information could facilitate some types of research (such as genetics research) or might be needed to enhance suitability screening for downstream tissue transplantation uses (see later discussion of FDA donor suitability rules).

How such donor information is collected and managed can affect whether the human subjects protections described above apply and whether federal privacy protections apply. Thus, a key determinant is whether the resulting cell lines will be managed in a way that makes the donors' identities readily ascertainable to investigators. If so, both sets of protections apply.

When investigators wish to work with existing lines rather than obtain materials to derive new lines, those lines may be accompanied by medical or other information about the donors. Work with hES cell lines whose identifiers render identity of the original donors readily ascertainable to the investigators would be a form of human subjects research that requires IRB review because the work might well reveal information about the donors. But properly obscuring donor identities can exempt work with cell lines from the requirement of IRB review. In that situation, OHRP has declared that *in vitro* research or research in animals that involves the use of an hES cell line that "retains a link to identifying [donor] information" (such as the use of a code) will not be considered human subjects research subject to the federal regulations if

(1) the investigator and research institution do not have access to identifiable private information related to the cell line; and
(2) a written agreement is obtained from the holder of the identifiable private information related to the cell line providing that such information will not be released to the investigator under any circumstances.[6]

OHRP has stated that, when those two conditions are satisfied, the research is not considered to involve human subjects, because the donors' identities cannot be readily ascertained by the investigator or associated with the cell line. By necessary implication, the OHRP *Guidance* dictates that any hES cell researcher who has access to personally identifiable information regarding the donors, including medi-

[6]See also *Guidance on Research Involving Coded Private Information or Biological Specimens,* OHRP/DHHS, Aug. 10, 2004.

cal information, will fall within the regulatory purview, and IRB review will be required. Thus, when medical information required for FDA donor suitability rules is collected (see below), human subjects protections are triggered unless the information is carefully coded and managed.

In addition to human subjects protections, if donor health information is attached to hES cell lines, federal privacy protections under the Health Insurance Portability and Accountability Act of 1996 (HIPAA; PL 104-191) might apply. The Privacy Rule of HIPAA might be applicable to hES cell research if the investigator obtains personal health information (PHI) on donors and the investigator is a "covered entity" (most likely a provider that transmits information in electronic format, such as a physician or hospital).[7] The Privacy Rule would permit PHI obtained by the researcher to be "deidentified," for example, statistical data would be aggregated or stripped of individual identifiers (45 CFR 164.514(b)) so that it could be used or disclosed without restriction.

If an hES cell investigator is employed by a covered entity and does not wish to "deidentify" PHI related to donors of somatic cells, gametes, or blastocysts (presumably because the identifying information may be expected to contribute relevant scientific information or assist in FDA review), HIPAA requires either of these

- A valid "authorization" from the donor before the PHI is used or disclosed (45 CFR 164.508).
- Appropriate documentation that an IRB or a privacy board has granted a waiver or alteration of the authorization requirement that satisfies 45 CFR 164.512(i).[8]

The criteria for approving an authorization waiver or alteration must be consistent with the criteria for IRB waiver of the informed consent:

(1) PHI is protected by a plan to guard against unauthorized disclosure, so there is no more than "minimal risk" to privacy;
2) The research could not practicably be conducted without the requested waiver or alteration; and
3) The research could not practicably be conducted without access to and use of the PHI (45 CFR164.512(i)(2)(ii)(A)-(C)).

[7]See 65 Fed. Reg. 82,799 (Dec. 28, 2000) (defining *covered entities*).

[8]An example of a situation in which a waiver of authorization requirements may be deemed appropriate by an IRB is a study that involves the use of PHI on numerous people whose contact information is unknown. The research would be impracticable to conduct if authorization were required, and an IRB could waive all the authorization requirements if the waiver criteria were satisfied. If the IRB approves such a waiver, the receipt of the requisite documentation of the approval permits a covered entity to use or disclose PHI in connection with a particular research project without authorization.

In sum, FDA's donor suitability rules (discussed below) may require collection of medical record information on donors of somatic cells, gametes, or blastocysts whose biological materials were used to derive new hES cell lines. In such cases, both federal human subjects protections and the Privacy Rule might apply to the research uses of the information, depending on how it is collected and transmitted in conjunction with the cell lines. Thus, if hES cell research involves the transmission of PHI on the donors, which will increasingly be the case as cell lines approach clinical application, it will be important for investigators, institutions, and IRBs to be aware of any Privacy Rule requirements that apply and to seek authorization from donors, as appropriate, for the transmission of health information.

***Recommendation 10*:**
Investigators, institutions, Institutional Review Boards, and privacy boards should ensure that authorizations are received from donors, as appropriate and required by federal human subjects protections and the Health Insurance Portability and Accountability Act, for the confidential transmission of personal health information to repositories or to investigators who are using hES cell lines derived from donated materials.

REGULATION OF *IN VITRO* AND ANIMAL STUDIES THAT USE hES CELL LINES

In general, state law does not affect the practice of *in vitro* or animal studies with hES cells. There are, however, sources of federal regulation for this research.

Once the cell lines are established, as noted above, federal regulations governing human research and HIPAA regulations apply only if information being used or developed might personally identify the original donors and progenitors. Thus, *in vitro* or animal studies that use hES cell lines do not require IRB review if the tracking codes that link the donors to the cell lines are properly managed. However, a host of other federal regulations apply to even purely laboratory, preclinical research with hES cell lines.

Recombinant DNA Research

Some of the research being done on hES cell lines will require some degree of genetic manipulation (see Chapter 2 for a description of these experiments). Research institutions are responsible for ensuring that all recombinant DNA research conducted at or sponsored by them is conducted in compliance with the National Institutes of Health *Guidelines for Research Involving Recombinant DNA Molecules*.[9] Institutional authority and responsibility place accountability for the safe

[9]Available at http://www4.od.nih.gov/oba/rac/guidelines/guidelines.html.

conduct of such research at the local level, and oversight is managed through an Institutional Biosafety Committee (IBC), a review body registered with NIH and appointed by an institution to review and approve potentially biohazardous lines of research.[10]

The need for IBCs grew out of the Asilomar Conference, when scientists agreed to self-regulate recombinant DNA research to avoid any potential threats to human health or the environment. Much of that research was initially reviewed case-by-case, not only by IBCs but also by a federal-level committee, the Recombinant DNA Advisory Committee (RAC). Over time RAC's role has evolved, first toward a focus on human gene transfer therapy study approvals and more recently toward human gene transfer therapy policy development, with authority to approve gene transfer therapy studies lodged solely in FDA's jurisdiction. To the extent possible, review of individual recombinant DNA research proposals has been delegated to local IBCs, and they remain as the guardians of public safety with regard to all recombinant DNA research and other potentially biohazardous research. They focus their review on safety, not on compliance with human subjects protections or other aspects of state and federal law governing the ethical conduct of scientific research. Many experiments are reviewed and approved by IBCs without any input from RAC.

At present, RAC is an advisory committee whose goal is to consider the current state of knowledge and technology regarding recombinant DNA. This includes review but not approval of human gene transfer trials, and assessment of the ability of DNA recombinants to survive in nature and the potential for transfer of genetic material to other organisms. A major role for RAC is to examine clinical trials that involve the transfer of recombinant DNA to humans. Currently, all human gene transfer trials in which NIH funding is involved (either directly or indirectly) are registered with the RAC. Protocols that contain unique and/or novel issues are discussed in a public forum. In addition, RAC advises the NIH director and his/her staff in a number of activities, including the preparation of materials required in legal actions, international coordination of biotechnology regulations, and the review of regulations proposed by other federal agencies.

In contrast to RAC's role, FDA's role is to determine whether a sponsor may begin studying a gene transfer product and, ultimately, whether it is safe and effective for human use. FDA regulates the products evaluated in human gene transfer clinical trials that are intended for eventual sale in the United States and is responsible for reviewing serious adverse events that occur in a gene transfer study.

Animal Care and Use

Increasingly, hES cell research might also involve the manipulation of hES cells in a nonhuman animal, such as a mouse. Laboratory work with nonhuman animals

[10]See http://www4.od.nih.gov/oba/IBC/IBCrole.htm.

is governed by its own set of federal laws and regulations, and any hES cell research that involves insertion of hES cells or their derivatives into animals is already subject to animal welfare protections. The Animal Welfare Act constitutes congressional policy to ensure the most humane use of animals in research. Some animals that might be used by hES cell investigators are not covered by the act, but most are covered.[11] In addition, the Public Health Service (PHS) Policy on Humane Care and Use of Laboratory Animals requires that each institution receiving PHS support for an activity involving any live vertebrate animals establish an appropriate institutional animal care and use program, including an Institutional Animal Care and Use Committee (IACUC) with specific responsibilities as described in the PHS policy.[12]

Laboratory Practice

In addition to special regulations governing recombinant DNA research and research that uses animals, the federal government has regulations pertaining to the management of laboratories where products that might ultimately be introduced into humans (as in a clinical trial) are being developed. FDA's Good Laboratory Practice (GLP) regulations establish standards for nonclinical laboratory studies. These do not include basic exploratory studies performed to determine whether a test article has any potential utility or to determine its physical or chemical characteristics but they do encompass *in vivo* or *in vitro* experiments in which test articles are studied to determine their safety—an activity that would be characteristic of the preclinical phase of hES cell research. Failure to conform to GLP regulations, although not itself a violation of law, would render any hES cells less useful in the future if they were considered for clinical trials of tissue transplantation or other cell-based therapies.[13]

Recommendation 11:
Investigators and institutions involved in hES cell research should conduct the research in accordance with all applicable laws and guidelines pertaining to recombinant DNA research and animal care. Institutions should consider adopting Good Laboratory Practice standards for some or all of their basic hES cell research.

REGULATION OF CLINICAL RESEARCH WITH CELL LINES AND DIFFERENTIATED TISSUE

Clinical research aimed at obtaining FDA approval or new labeling of drugs, devices, or biologics is subject to regulation by FDA. It must be conducted in

[11]Animal Welfare Act (as amended), 7 USC §§ 2131-56.
[12]http://grants.nih.gov/grants/olaw/references/phspol.htm.
[13]http://www.fda.gov/ora/compliance_ref/bimo/7348_808/part_I.html.

compliance with FDA's regulations governing investigational new drugs (INDs) or investigational device exemptions (IDEs), regardless of source of funding. Thus, all human studies conducted under INDs and IDEs are subject to FDA's own regulations concerning IRB review and informed consent (21 CFR 50 and 56), which are roughly parallel to the DHHS regulations at 45 CFR 46.

Transplantation of hES cells or tissues developed from hES cell lines is a form of "cell-based therapy" and is generally regulated by FDA as a biologic, drug, or device. The regulations entail a variety of premarket notifications and approvals based on safety and efficacy data; the precise requirements depend on the primary mode of action (drug or device), in accordance with the Food, Drug and Cosmetic Act and its amendments (21 USC Section 301 *et seq*). Biologics are subject to additional precautions based on the Public Health Service Act, aimed primarily at control of transmission of infectious disease (42 USC, Chapter 6A, Part F).

Because hES cell research is likely to lead to clinical applications that involve the transfer of cells or tissue into humans they will also be subject to FDA's comprehensive tissue transplantation regulations.[14] Of course, many investigators will be engaged in basic research with no intent to pursue an immediate clinical application, and much of what follows does not necessarily apply to such investigators. But failure to follow FDA's tissue transplantation regulations may result in FDA's refusal to use materials from the laboratories in question in later clinical trials. If so, investigators might have to derive new cell lines in accordance with the regulations if their materials are to be acceptable for development into transplantable tissue.

FDA's new, more comprehensive approach to regulating tissue transplantation was announced in February 1997.[15] Although only partially implemented as of 2005, FDA already requires registration by all establishments that recover, process, store, label, package, or distribute "human cells, tissues, and cellular and tissue-based products" (HCT/Ps) or that screen or test donors of them. The registration requirement is applicable to establishments involved in the derivation and management of hES cell lines and resulting tissues that will be used for transplantation into humans.

In addition, as of May 2005, FDA's "current good tissue practices" (CGTP) will include rules governing the process for procuring human blastocysts, oocytes, sperm, and somatic cells for use in research leading to clinical applications. The rules will include donor screening to prevent the spread of communicable diseases and a tracking system that will permit tracking from each human cell line or tissue back to the original donor. For work with existing cell lines, CGTP rules already govern the methods and facilities used for the manufacture of HCT/Ps to prevent the introduction or transmission of communicable diseases by these cells, tissues, and products. As with the registration requirements, the rules apply to HCT/Ps that are destined for transplantation into humans.

[14]http://www.fda.gov/cber/tissue/tissue.htm.
[15]http://www.fda.gov/cber/tissue/tissue.htm.

Once a donation has been made, the resulting tissue must be coded in a fashion that permits tracking back to the original donor if that is needed, and a summary of relevant information about the donor must accompany the cell line or tissue whenever it is passed to a new facility.[16]

Because those rules require some kind of tracking system that will maintain a connection between the donor and the endproduct, such as transplantable tissue, the FDA tissue rules have an effect on the operation of human subjects protections, as well as the HIPAA Privacy Rule. The net effects are that

- Work on completely anonymous hES cell lines will not be human subjects research, but this tissue may well be disfavored by FDA if investigators wish to use it for clinical trials. FDA will prefer that trials use tissue for which there is a traceable history back to the donors and their medical histories.
- Work on hES cell lines with identifiers linking them to the donors will be subject to federal regulations governing human subjects research and, in the case of covered entities, HIPAA privacy protections unless the identifiers are coded and managed in a fashion that renders the donors effectively unidentifiable to the investigators.

Finally, work with hES cell lines that were grown on mouse feeder cells may face a special obstacle if an investigator wishes to use them to develop transplantable tissue for human clinical trials. FDA's regulations define xenotransplantation to include any procedure that involves the transplantation of human body fluids, cells, tissues or organs that have had *ex vivo* contact with live nonhuman animal cells, tissues, or organs. Tissue transplantation from cell lines grown on nonhuman feeder cells would be considered xenotransplantation and would require additional FDA review.[17]

For hES cell investigators who plan to obtain cell lines from outside the United States, it is worth noting that FDA's new tissue regulations also govern the importation of cell lines and derived tissues for use in clinical transplantation, and importation must be approved by FDA, whose regulations pursuant to Section 361 of the Public Health Service Act are designed to prevent the transmission of communicable diseases.

Also of relevance to researchers working with cell lines from other countries, there are medical privacy requirements in other countries that must be considered whenever transnational collaborations are contemplated.[18] For collaborations with

[16]See § 1271.55 of the new regulations, as presented in "Eligibility Determination for Donors of Human Cells, Tissues, and Cellular and Tissue-Based Products", *Federal Register* Vol. 69, No. 101, amending 21 CFR Parts 210, 211, 820, and 1271, 69 FR 29786 (May 25, 2004).

[17]http://www.fda.gov/cber/xap/xap.htm.

[18]"New International Guidelines on the Transfer of Personal Health Data," William R. M. Long and Julia Barnes, *Medical Research & Policy News*, Volume 4 Number 4, February 16, 2005 Page 157, ISSN 1539-4530.

members of the European Union, Iceland, Liechtenstein, and Norway, medical information about donors that accompanies cell lines must comply with the guidelines issued by the International Organisation for Standardisation (ISO).[19] Those rules generally preclude the transfer of medical data about identifiable persons unless consent has been obtained and the country receiving the data has an adequate system for medical data protection.[20] Despite the passage of HIPAA, the United States has not been deemed to have such a system, although individual institutions may devise systems that meet the European requirements.

Many forms of hES cell research, however, can be exempted from the rules, provided that the data are rendered anonymous. Under the ISO guidelines, anonymization means rendering data "nonpersonal," that is, the codes do not directly or indirectly reveal the identity of the donors.[21] Given the varied ways in which anonymous is interpreted under HIPAA, ISO guidelines, and federal human subjects research rules, investigators and institutions need to be attentive to the concerns of all appropriate bodies before working with cell lines that are understood to be anonymized.

***Recommendation 12*:**
hES cell research leading to potential clinical application must be in compliance with all applicable Food and Drug Administration (FDA) regulations. If FDA requires that a link to the donor source be maintained, investigators and institutions must ensure that the confidentiality of the donor is protected, that the donor understands that a link will be maintained, and that, where applicable, federal human subjects protections and Health Insurance Portability and Accountability Act or other privacy protections are followed.

U.S. STATE LAW ON hES CELL RESEARCH

State law rarely addresses the regulation of medical research. It does, however, often address the status of embryos. In this respect, it is relevant to hES cell research.

[19]ISO 22857: 2004(E)—"Health informatics—Guidelines on data protection to facilitate trans-border flows of personal health information."

[20]The ISO guidelines are based on four other pieces of transnational legislation: "Recommendations of the Council of the OECD concerning Guidelines on the Protection of Privacy and Trans-border flows of Personal Data" [OECD, Sept. 23, 1980, and "Guidelines for the Security of Information Systems," OECD, 1996.]; the "Council of Europe Recommendation R(97)5 on the Protection of Medical Data" (Council of Europe Publishing, Strasbourg, Feb. 12, 1997); actions of the U.N. General Assembly, Dec. 14, 1990; and the EU Data Protection Directive (Directive 95/46/EC of the European Parliament and of the Council of Oct. 24, 1995, on the protection of individuals with regard to the processing of personal data and on the free movement of such data. OJL 281, Nov. 23, 1995, p. 31). The latter directive was last amended by Regulation (EC) No. 1882/2003 of the European Parliament and of the Council of Sept. 29, 2003, OJL 289, Oct. 31, 2003, p. 1.

[21]Recital 26 of the EU Data Protection Directive provides that the principles of protection shall not apply to data rendered anonymous in such a way that the data subject is no longer identifiable.

Courts have held that dispositional authority over an embryo in general belongs to the progenitors.[22] Moreover, case law suggests that destruction of an embryo does not require the consent of anonymous gamete donors, although in the context of couples who disagreed over the disposition of embryos, the consent of both partners has been required before release of an embryo for reproductive purposes, particularly in the absence of a prior agreement between the partners.[23] In the absence of a joint decision regarding disposition, however, current law will result in leaving the embryo in a frozen state. Fertility clinics have sought to avoid such conflicts by asking couples to agree in advance on the terms on which embryos can be released for reproductive use, kept frozen, discarded, or released for research.

A number of states, such as Louisiana, Maine, Massachusetts, Minnesota, New Hampshire, North Dakota, Pennsylvania, and Rhode Island, have enacted legislation to prohibit or limit research with human embryos,[24] with the definition of embryo occasionally merged with the definition of fetus.[25] In some cases, these state laws restricting embryo research have been challenged successfully in court, on grounds such as unconstitutional vagueness.[26] But most U.S. states have no laws or regulations specifically addressing hES cell research. Of the laws that do exist, many focus exclusively on nuclear transfer (NT) research. For example, as of March 2005, Arkansas, Iowa, Michigan, North Dakota, and South Dakota had laws that clearly forbid the use of NT for research purposes.[27] Missouri forbids the use of state funds for NT research.[28] Other states, such as Rhode Island and Virginia (less clear from the text of the law), have banned NT for reproductive purposes but have not addressed its use for research purposes.[29] In states that do not forbid NT research, it remains legal and subject to the federal regulations described above. New Jersey and California, however, have adopted laws that add extra state regulation to the field of hES cell and NT research, most notably by expanding the jurisdiction of IRBs to review the research and by prohibiting the sale of embryos.[30] In California, however, research funded pursuant to the Proposition 71 initiative will be exempt

[22]See York v. Jones, 717 F. Supp. 421 (E.D. Va. 1989); Del Zio v. Presbyterian Hosp., No. 74 Civ. 3558 (CES), 1978 U.S. Dist. LEXIS 14450 (S.D.N.Y. Nov. 9, 1978).

[23]See, e.g., In re Marriage of Litowitz, 48 P.3d 261 (Wash. 2002); J.B. v. M.B., 783 A.2d 707 (N.J. 2001); A.Z. v. B.Z., 725 N.E.2d 1051 (Mass. 2000); Kass v. Kass, 696 N.E.2d 174 (N.Y. 1998); Davis v. Davis, 842 S.W.2d 588 (Tenn. 1992).

[24]See http://www.kentlaw.edu/islt/TABLEIII.htm (last visited March 24, 2005).

[25]See, e.g., Massachusetts, where a statute prohibits the use of embryos for experimental purposes. See Mass. Gen. Laws Ann. ch. 112, 12J (prohibiting experimentation on live fetus either before or after it is implanted in uterus).

[26]Forbes v. Woods, 71 F. Supp. 2d 1015 (1999); Lifchez v. Hartigan, 735 F. Supp. 1361 (1990).

[27]http://www.ncsl.org/programs/health/genetics/rt-shcl.htm.

[28]http://www.ncsl.org/programs/health/genetics/rt-shcl.htm.

[29]http://www.ncsl.org/programs/health/genetics/rt-shcl.htm.

[30]http://www.ncsl.org/programs/health/genetics/rt-shcl.htm.

from many aspects of this law and subject instead to new guidelines to be adopted by the newly created California Institute for Regenerative Medicine.

State laws on dispositional authority over embryos and on hES cell research are in flux and are largely untested in the courts. Investigators working with NT or hES cell lines are well advised to seek advice on the latest rules applicable in their states.

REGULATION OF hES CELL AND NT RESEARCH IN OTHER COUNTRIES

There is no international consensus yet on whether and how to pursue hES cell research. For example, in February 2005, a committee of the U.N. General Assembly abandoned attempts to craft a global treaty on NT research and satisfied itself with a plurality vote in favor of a nonbinding resolution calling for a ban on all forms of human cloning or genetics research that are contrary to "human dignity," a phrase left to the interpretation of member countries.[31] Thus, the regulation of hES cell research varies from country to country. In many cases, there is no law explicitly addressing such research. In some countries, such as Poland and Italy, the research is forbidden or substantially curtailed. In others, however, there seems to be a trend toward liberalization of the laws. France and Germany, for example, have taken steps to permit research on cell lines derived from surplus *in vitro* fertilization (IVF) blastocysts,[32] and Japan[33] and Sweden[34] have lifted restrictions on making blastocysts for research with NT.

Given the increasing frequency of international collaboration in hES cell research, it is important to monitor regulatory developments in other countries. As the guidelines recommended by this committee in Chapter 6 require that the provenance of hES cell lines be consistent with the ethical standards and procedures adopted here, understanding the points of similarity and difference between the guidelines and the rules in other countries will help investigators and the ESCRO committees proposed in Chapter 3 to manage collaboration.

Some countries place limitations on the importation of cell lines whose origins are inconsistent with their laws. Australia, for example, adopted the Research Involving Human Embryos Act in 2002 and the Human Cloning Act, which prohibits NT for reproductive or therapeutic purposes.[35] Of possible importance to U.S.

[31]Associated Press, U.N. Group Calls for Cloning Ban, Feb. 18, 2005.

[32]"Europe Sends Mixed Signals on Stem-Cell Work," Victoria Knight, *Wall Street Journal* Jan. 26 2005. Note that that German liberalization applies only to cell lines produced prior to 2002. See http://www.germany-info.org/relaunch/education/new/edu_stemcells.html.

[33]http://web2.innovationworld.net/biotechconnect/000312.html.

[34]http://www.geocities.com/giantfideli/art/CellNEWS_Sw_thera_cloning.html.

[35]Research Involving Human Embryos Act, 2002, No. 145, 2002, An Act to regulate certain activities involving the use of human embryos, and for related purposes (http://scaleplus.law.gov.au/html/comact/browse/TOCN.htm); Prohibition of Human Cloning Act 2002, No. 144, 2002, An Act to prohibit human cloning and other unacceptable practices associated with reproductive technology, and for related purposes (http://scaleplus.law.gov.au/html/comact/browse/TOCN.htm).

investigators seeking to collaborate with Australian centers, Australia forbids the importation of cloned, parthenogenetic, androgenetic, or chimeric embryos (a chimeric embryo is defined as one in which nonhuman cells have been introduced into a human embryo). It is also an offense to create a human embryo by any method other than fertilization and for any purpose other than for the treatment of infertility. So-called hybrid embryos are specifically forbidden and such entities are defined to include an animal egg into which the nucleus of a human cell has been introduced. Commercial trading in human eggs, sperm, or embryos is not allowed. Those bans are backed by criminal sanctions with prison terms of up to 15 years, depending on the offense.

Australia's law allows research to be performed on embryos remaining in excess of clinical need, and the consent requirements for donors are consistent with those outlined in this committee's recommendations (see Chapter 5). Research is subject to oversight by a new committee, the National Health and Medical Research Council Licensing Committee, which has the authority to review research programs, grant licenses, and maintain a database regarding the licenses granted. That committee also has the authority to inspect licensee facilities to ensure compliance with its licensing conditions.

The United Kingdom has adopted an approach that depends on a central licensing authority, called the Human Fertilisation and Embryology Authority (HFEA). The role of HFEA is to monitor and license clinics that carry out any of the established IVF or other assisted reproductive technology procedures and to regulate human embryo research and the storage of reproductive materials. As in the present committee's Recommendation 8 above, donors in the United Kingdom must give consent for use of their gametes or embryos in research. Egg and sperm donors are paid a nominal fee and reasonable expenses.[36]

HFEA will grant a license to make embryos for research only if the research program meets the purposes outlined in U.K. law. Allowable research purposes include increasing knowledge of genetic disorders, developing better contraceptive techniques, and advancing the treatment of infertility. As of early 2005, HFEA had granted 28 research licenses, including 10 related to hES cells and two related to parthenogenesis.[37] Two licenses were granted for work with NT blastocysts.[38]

The United Kingdom also has created a Stem Cell Bank, launched by the Medical Research Council in September 2002. The bank exists to establish fully characterized and quality-controlled cell lines (see Chapter 5 for a discussion on banking). The cell lines will be supplied to accredited scientific research teams and eventually to pharmaceutical companies to enable the development of broad-ranging cell therapies.[39]

[36]http://www.hfea.gov.uk/PressOffice/Archive/34673456).

[37]http://www.hfea.gov.uk/Research.

[38]See "British to Clone Human Embryos for Stem Cells," Rick Weiss, *Washington Post*, February 9, 2005; Page A02; see also http://www.hfea.gov.uk/PressOffice/Archive/1092233888.

[39]http://www.hfea.gov.uk/PressOffice/Backgroundpapers/Stemcellresearch.

Israel does not have a central licensing authority, but it does have well-developed guidelines emerging out of the work of the Bioethics Advisory Committee of the Israel Academy of Sciences and Humanities, and, because the Health Ministry delegates decisions regarding new genetic research involving human beings to the Helsinki Committee for Genetic Experiments on Human Subjects, it also has a centralized review process for hES cell research.[40] Consistent with the guidelines proposed in this report, the Israeli guidelines require informed consent from donors of surplus blastocysts. The guidelines state that best practices include mentioning research uses from the beginning of the IVF process and separating the medical team responsible for the IVF treatment and donation from the scientific teams involved in embryo research who receive the donation. As in the recommendations made in the next chapter, buying and selling of embryos is forbidden in Israel, but making new embryos solely for research, including blastocysts made by NT, is permissible. Research and possible applications must be justifiable in terms of the benefit that it offers humanity, and confidentiality and privacy of the donors should be respected. As in the recommendation proposed in Chapter 3 for purely *in vitro* work on hES cell lines, Israel allows such work to be conducted without further need for specific ethical authorization.

In June 2002, Singapore's Bioethics Advisory Committee released its report *Ethical, Legal and Social Issues in Human Stem Cell Research, Reproductive and Therapeutic Cloning*, in which it recommended that NT be permitted under centralized regulation. Consistent with the guidelines proposed here, the regulatory framework should require the informed voluntary consent of donors, prohibit the commerce and sale of donated materials, require strong scientific justification before making new embryos solely for research purposes, and stipulate that no one shall be under a duty to participate in any manner of research on human stem cells to which he or she has a conscientious objection. The report has been presented to the relevant ministries, and the government will decide on the recommendations later.[41]

Canada is still debating legislation to regulate assisted reproductive technologies and embryo research, but it operates under guidelines that incorporate both centralized and local review. Under the guidelines issued by the Canadian Institute for Health Research,[42] review and approval by the central Stem Cell Oversight Committee, by local research ethics boards (REBs), and, where appropriate, by animal care committees is required for all research involving the derivation, *in vitro* study, and clinical trial of hES cell lines. At any time, however, the local REB or animal care committee may refer an hES cell research proposal to the Stem Cell Oversight Committee for ethics review if it considers the research to be within the oversight committee's purview according to the above criteria. Such decisions by the

[40]http://www.academy.ac.il/bioethics/articles/embryonic_ibc_report.pdf.
[41]www.bioethics-singapore.org.
[42]http://www.cihr-irsc.gc.ca/e/15349.html.

REB or animal care committee are not subject to appeal. Like the guidelines recommended in this report, the Canadian guidelines require a medical rationale for the research, the informed consent of donors, protection of donors' privacy, and a prohibition on payment to donors (see Chapter 5). And like the current policy of the U.S. government (but unlike that of New Jersey or California), the Canadian guidelines prohibit public financial support for making embryos solely for research or of research in which hES cells are combined with a nonhuman embryo.[43]

CONCLUSION

Despite the lack of federal funding for most hES cell research underway in the United States, several sets of federal regulations govern various aspects of hES cell research—human subjects protections for oocyte and some blastocyst donors, medical privacy protections, laboratory and safety standards, animal welfare requirements, and rules governing the importation of biological materials or the transfer of medical data from other countries. In many other countries where hES cell research is permitted and publicly funded, its practice is regulated by statute or other government policy. Those regulations address matters such as whether embryos may be made solely for research purposes; whether they may be made using NT, parthenogenesis, or androgenesis; whether human hES cells may be combined with nonhuman materials; and whether facilities and researchers must be licensed before engaging in hES cell research.

As hES cell research in the United States increases, it is essential that institutions and investigators adhere to existing applicable regulatory requirements, and given the increasing frequency of international collaboration in hES cell research, it will be important to monitor regulatory developments in other countries. The ESCRO committees proposed in this report will be charged with ensuring that U.S. investigators follow standards and procedures consistent with current regulations and with the guidelines recommended in this report. Various jurisdictions differ in their mechanisms for oversight and review. As discussed in Chapter 3, the committee recommends both local review of hES cell research by an institutional ESCRO committee and the establishment of a national body to serve as a forum for considering new developments in the scientific, clinical, and public policy issues surrounding hES cell research and for periodic review of the relevant guidelines. The distinction between local review and oversight and national consideration of larger policy issues is in line with current U.S. practice in other fields. An analogy is the current use of local institutional IBCs to regulate recombinant DNA research and the RAC to consider policy issues related to gene therapy. The dual mechanism will fulfill oversight and monitoring functions equivalent to the various systems mandated by other countries.

[43]http://www.cihr-irsc.gc.ca/e/1487.html.

5

Recruiting Donors and Banking hES Cells

The emergence of assisted reproductive technology (ART) more than 20 years ago has enabled many couples to overcome fertility problems. Nationwide, 107,587 ART procedures were performed in 2001 at 385 medical centers in the United States and U.S. territories; they resulted in the birth of 40,687 infants from 29,344 pregnancies (Wright et al., 2004). Nationally, 75 percent of ART treatments used fresh, fertilized embryos from the patients' own oocytes; 14 percent used thawed embryos from the patients' oocytes; 8 percent used fresh, fertilized embryos from donor oocytes; and 3 percent used thawed embryos from donor oocytes. Thus, procedures can involve gametes from the couples themselves or from donors.

Various ART procedures result in the production of more embryos than are needed. Couples can choose to cryopreserve (freeze) and store these "extra" embryos for future attempts at establishing pregnancy. Embryos are often cryopreserved in *in vitro* fertilization (IVF) practices because transfer of more than three embryos per cycle increases risks for the mother and offspring and cryopreserved embryos offer fairly high pregnancy rates upon eventual transfer (Klock, 2004). Frozen embryos accumulate at a rate of about four per cycle. It is estimated that more than 400,000 embryos are stored in the United States (Hoffman et al., 2003), and there are nearly 16,000 embryos in storage in Canada (Baylis et al., 2003).

Once a couple decides to terminate their fertility treatment, for whatever reason, they have a number of options regarding the disposition of these embryos: they can donate them to another couple, they can make them available for quality assurance activities, they can donate them for research purposes, they can dispose of them, or they can store them indefinitely (Hoffman, et al., 2003). Many industrialized countries have developed laws or guidelines to govern the disposition of em-

bryos. National regulations vary from eternal preservation to 5-year and 10-year preservation limits (Moutel et al., 2002; Grubb, 1996).

In addition to excess blastocysts, there might be excess gametes—oocytes and sperm that have been collected for IVF procedures from the couples themselves or from donors—that are no longer needed for reproductive purposes. Women not seeking infertility treatments might elect to donate oocytes for research purposes as an adjunct to a clinical intervention (such as oophorectomy) or as a straightforward altruistic donation specifically for research.

A number of studies have shown that some couples are willing to donate unneeded blastocysts for research purposes—as many as 25 percent in some studies (Bangsboll et al., 2004; Burton and Sanders, 2004; Klock, 2004; McMahon et al., 2003). The attitudes of couples who have undergone IVF range from almost parental concern for the embryos to regarding them as medical byproducts with little relationship to a couple's having a living child. Respondents positively disposed to donation commented on their desire not to waste blastocysts, a desire to help infertile couples, or a desire to advance scientific knowledge. Those with negative views commented on the embryo as a potential child and expressed concerns about a perceived lack of control over the type of research to be performed (McMahon et al. 2003).

Ethical principles dictate that potential donors of gametes or blastocysts for human embryonic stem cell (hES cell) research be able to make voluntary and informed choices about whether and how to donate their materials for research and that there be a clear option of "informed refusal," that is, the right to preclude any research use of embryos. Because of concerns about possible coercion or exploitation of potential donors and controversy regarding the moral status of embryos, it is important that precautions be taken in recruiting donors and ensuring their informed voluntary consent. Some of the protections offered through existing federal regulations can be adapted for application to hES cell research, such as adherence to principles of informed consent and a requirement that an Institutional Review Board (IRB) review the consent process. In addition, Food and Drug Administration (FDA) regulations should be considered for some types of research, specifically if there is a need to retain identifying information about the donors. That has implications for the consent process and for plans to protect confidentiality and privacy of information. Because of privacy concerns, certain provisions of the Health Insurance Portability and Accountability Act (HIPAA) might also apply. (Those regulatory requirements were discussed in Chapter 4.)

In this chapter, the committee makes specific detailed recommendations for IRB review of procurement (as recommended in the previous chapter); for the consent processes for obtaining somatic cells, gametes, and blastocysts for use in hES cell research; and for storing and maintaining cell lines once derived. Important safeguards must be in place to ensure that materials are collected ethically and that, once obtained, they are used for scientifically meritorious research (see also Chapters 2 and 3) with the confidentiality of donors protected.

REVIEW OF THE PROCUREMENT AND INFORMED CONSENT PROCESS

As discussed in Chapter 4, although the federal regulations governing human subjects research apply directly only to federally sponsored research or research conducted to secure FDA approval, many research institutions have implemented policies that require that all human subjects research conducted at the institution—regardless of the source of funding—abide by the federal requirements, primarily IRB review and the need for voluntary informed consent of subjects.

If an institution abides by the research regulations, it must invoke IRB review whenever human subjects research is conducted unless the research is exempt under the regulations. In addition, if hES cell lines obtained from donated materials are maintained with tracking codes, which might be required for research intended for clinical application, such research could transform donors into "research subjects" because study of the tissue could reveal information about them (unless the information was coded in such a way as to be unidentifiable by the investigator). Because FDA donor-suitability rules for transplants of cells or tissues from hES cell lines (discussed in Chapter 4) will probably require such tracking back to the donors, best practices suggest treating the donors as though they might be research subjects—that is, obtaining IRB review and approval of the consent process—to avoid problems later. In addition, even in the absence of tracking information, the process of donation could benefit from IRB experience in assessing the potential for inducements and risks and in reviewing the consent processes—all of which is relevant to the recruitment of donors of somatic cells, gametes, and blastocysts. As discussed in Chapter 4, this committee recommends that an IRB review the process by which material is obtained and that in all cases donors of cells, gametes, or blastocysts provide their informed consent. That requirement should extend to donors of gametes used in the IVF process.

***Recommendation 13*:**
When donor gametes have been used in the *in vitro* fertilization process, resulting blastocysts may not be used for research without consent of all gamete donors.

The committee recognizes that this recommendation might eliminate from research some blastocysts that are in excess of clinical need, but that should not impose a major impediment to research, and the requirement for voluntary informed consent of all donors is an absolute prerequisite.

Thus, a researcher who wishes to obtain human oocytes or blastocysts for hES cell research must either request and obtain IRB review at his or her own institution (if one exists) to ensure that the informed consent provisions of the federal regulations at 45 CFR 46.116-117 and FDA regulations at 21 CFR 50.20-27 are followed or require that the fertility clinic have its own process for obtaining review from some other duly constituted IRB. The hES cell researcher should maintain a written record documenting the IRB review. IRB documentation should include an assur-

ance of compliance with the relevant requirements in this report and relevant regulations and a copy of the consent form used for procurement purposes.

Ensuring that Donation Is Voluntary

Preceding sets of guidelines have emphasized the critical requirement of voluntary donation, including the explicit prohibition of monetary inducement or promise of therapeutic benefit. The original National Institutes of Health guidelines for hES cell research developed in 2000 stated "To ensure that the donation of human embryos in excess of the clinical need is voluntary, no inducements, monetary or otherwise, should have been offered for the donation of human embryos for research purposes. Fertility clinics and/or their affiliated laboratories should have implemented specific written policies and practices to ensure that no such inducements are made available." Likewise, the Canadian guidelines state "Neither the oocyte nor the sperm from which the embryos were created, nor the embryos themselves, were obtained through commercial transactions, including exchange for service." The European Commission and the U.K. Medical Research Council have instituted similar prohibitions. And the provisions of California's Proposition 71, passed in 2004, similarly prohibit payment to donors. Thus, there is virtual unanimity that to avoid any temptation for individuals to create extra embryos for research purposes, no payments should be offered for donation of residual embryos created for reproductive purposes in IVF programs. It is also agreed that there should be no added expense or burden to patients when residual blastocysts are donated and all storage costs for frozen blastocysts should be assumed by the investigators once donation has been confirmed.

The explanation of such unanimity might lie in the view that the treatment of the developing human embryo as an entity deserving of respect may be undermined by the introduction of a commercial motive into the solicitation or donation of fetal or embryonic tissue for research purposes. But although the potential for pressure is probably greatest when financial incentives are present, some nonfinancial incentives also should be avoided. For example, a donor's decisions should not be influenced by anticipated personal medical benefits or by concerns about the quality of later care. Any suggestion of personal benefit to a donor or to a person known to the donor should be avoided. (For obvious reasons, the use of nuclear transfer [NT] to develop hES cells for autologous transplantation requires that the recipient be specified.) Thus, a potential donor should be informed that there is no obligation to donate, that no personal benefit will accrue as a result of a decision to donate (except in cases of autologous transplantation), and that no penalty will result from a decision to refuse to donate. Similarly, people who elect to donate stored blastocysts for research should not be reimbursed for the costs of storage before the decision to donate, because this may be interpreted as an incentive to donate.

Recommendation 14:
To facilitate autonomous choice, decisions related to the production of embryos for infertility treatment should be free of the influence of investigators who propose to derive or use hES cells in research. Whenever it is practicable, the attending physician responsible for the infertility treatment and the investigator deriving or proposing to use hES cells should not be the same person.

Recommendation 15:
No cash or in kind payments may be provided for donating blastocysts in excess of clinical need for research purposes.

Recruiting and Paying Donors of Gametes for Research Purposes

Although there is widespread consensus that donors should not be paid for blastocysts they donate for research, there is less consensus about inducements for women to donate oocytes or men to donate sperm for research purposes. It is probably least problematic when women opt to donate oocytes for research in conjunction with a clinical procedure already scheduled (such as IVF or oophorectomy). It is most problematic in the case of oocyte donation solely for research purposes, because the invasiveness and risks of the procedure suggest that financial remuneration is most deserved, but at the same time there is a greater likelihood of enticing potential donors to do something that poses some risk to themselves. Of course, some women might wish to donate oocytes solely for research for nonfinancial motives; such a desire might exist among women who have family or friends affected by a particular disease that might be better understood or treated in the future if hES cells were used.

If the need for oocytes in hES cell research increases, it is possible that donations from clinical procedures or for nonfinancial motives may prove insufficient to meet the demand. In such cases—for example, for research involving NT or for research requiring blastocysts that have not been frozen—investigators might want to recruit oocyte donors. In the context of human subjects research, use of advertising to recruit subjects is not considered objectionable, but it is deemed worthy of review. In the context of clinical research, FDA considers direct advertising for study subjects to be the start of the informed consent process and subject selection; therefore, advertisements should be reviewed and approved by an IRB.

No matter how donors are recruited, the issue of whether they should be paid remains. Paying research subjects is "a common and long-standing practice in the United States" (Dickert et al., 2002; Anderson and Weijer, 2002), perhaps because of the need to provide incentives as part of recruitment and because moral principles of fairness and gratitude support providing payment to those who bear the burdens of research on behalf of society. But how much money gamete donors should receive and what they should receive payment for (for example, time, inconvenience, dis-

comfort, or level of risk) are still contested because of fears that remuneration—or some level of remuneration—will undermine voluntary informed consent.

Although the consensus is that remuneration of participants in research should be just and fair, there is little agreement in theory or in practice about what constitutes just or fair payment. Moreover, federal regulations and guidance are relatively quiet on the subject, warning about "undue influence" without specifying what counts as undue. One difficulty is that "undue influence" depends on context. The level at which remuneration is set will influence the decisions of some more than others. A major ethical concern is that payments should not be so high as to create an undue influence or offer undue inducement that could compromise a prospective donor's evaluation of the risks or the voluntariness of her choices. That concern is greatest when studies involve significant risks. Other concerns are that payments should not be so low as to recruit disproportionately high numbers of economically disadvantaged persons and that they should compensate participants fairly for their contribution to research.

In its guidance on "Payment to Research Subjects," FDA notes that "financial incentives are often used when health benefits to subjects are remote or nonexistent. The amount and schedule of all payments should be presented to the IRB at the time of initial review. The IRB should review both the amount of payment and the proposed method and timing of disbursement to assure that neither are coercive or present undue influence" (21 CFR 50.20). In particular, the FDA guidance indicates that payment should be prorated for the time of participation in the study rather than extended to study completion, because the latter could compromise a participant's right to withdraw at any time.

Many argue that research subjects, or in this case gamete donors, should be paid for their time and inconvenience, as well as their direct expenses, but are concerned about providing payment for incurring risk, a practice that some ethicists would rule out altogether. However, attitudes may differ considerably when the risk is a minor and transient symptom or discomfort (such as sleepiness or dizziness) rather than a substantial harm. Some arguments for limiting payment to time and inconvenience reflect a belief that participation in research should be an altruistic act. It is almost certainly true, however, that the prospect of financial remuneration motivates many people to participate in research and that it is often a necessary and sometimes a sufficient condition for their participation.

Thus, although payments to volunteers in research studies can be characterized as compensation, honoraria, or inducements, it is widely agreed that volunteers should be reimbursed for direct expenses. Similarly, offering a small or token honorarium after participation is generally accepted. The consensus is less clear on whether volunteers should be paid for time and lost wages. Some consider that a form of compensation and there is disagreement about whether amounts should depend on income. The value placed on a person's time depends in part on the person's socioeconomic status, but there are concerns about using poverty as a justification for perpetuating differential payments.

Inducements are commonly provided for competent adult research subjects and some argue that oocyte donation should be treated in a similar fashion and that it is inappropriately paternalistic to prohibit competent women from making an informed choice. Others believe that the reproductive context makes this special and that payment should be prohibited. Underlying those principled concerns is a more pragmatic debate about whether (and how much) payment is needed to ensure a sufficient supply of oocytes for stem cell research.

Recommendation 16:
Women who undergo hormonal induction to generate oocytes specifically for research purposes (such as for nuclear transfer) should be reimbursed only for direct expenses incurred as a result of the procedure, as determined by an Institutional Review Board. No cash or in kind payments should be provided for donating oocytes for research purposes. Similarly, no payments should be made for donations of sperm for research purposes or of somatic cells for use in nuclear transfer.

This recommendation is based, in part, on the recognition that payments to oocyte donors raise concerns that might undermine public confidence in the responsible management of hES cell research. Following the recommendation will ensure consistency between procurement practices here and in other countries that have major hES cell research programs, thus facilitating international collaborations and the sharing of hES cell lines across national borders. It also ensures consistency with the limitations enacted in California in Proposition 71, facilitating collaboration between California investigators and those in the rest of the country.

The committee recognizes the strengths of all the arguments surrounding this issue. The recommendation should not be interpreted as a commentary on commercial IVF practices, but as a narrow policy position specifically with respect to hES cell research. Further, as with all the policies recommended by the committee, this policy should be regularly reviewed and reconsidered as the field matures and the experiences under other policies can be evaluated.

Finally, it is important to note that oocyte donation is not without risks. Oocyte donors undergoing ovulation induction have a small risk of severe ovarian hyperstimulation syndrome (OHSS). OHSS may affect 2-5 percent of women undergoing stimulation and can sometimes require hospital admission (Orvieto, 2005; ASRM, 2004a; Endo et al., 2002). Careful monitoring and adjustment of the medication regimen during the stimulation treatment can reduce the risk of OHSS. Risks posed by donation must be clearly articulated and understood by the prospective donor. In the United States—where insurance coverage varies and often does not cover research-related costs—the donor must be informed of whether and how much compensation is available if she is injured as a result of research. In general, compensation is not assured.

TIMING OF THE DECISION TO DONATE EXCESS BLASTOCYSTS

It is widely accepted that, whenever possible, donors' decisions to dispose of their blastocysts should be made separately from their decisions to donate them for research. Potential donors should be allowed to provide blastocysts for research only if they have decided to have those blastocysts discarded instead of donating them to another couple or storing them. If the decision to discard the blastocysts precedes the decision to donate them for research purposes, the research will determine only how their destruction occurs, not whether it occurs (NBAC, 1999a). The U.K. Medical Research Council guidelines emphasize the separation of tissue collection from the practice of research: "Those collecting embryos or adult cells/tissues, or involved in the process of fetal termination, and those responsible for the clinical care of the donor, should not knowingly be involved in research on those human tissues."

That separation may not always be possible, particularly because the couple may be informed of several options simultaneously at the outset of treatment for infertility or after its completion. Some infertility programs provide patients with multiple consent forms at the outset of treatment, forms that include options to donate to research, discard, or transfer any embryos that remain. When embryos are created for infertility treatment, couples are often asked to stipulate what should be done with frozen embryos in the event of future contingencies, such as death, divorce, or the inability of the clinic to contact them at a later date (ASRM, 2002). In addition, given growing public awareness about hES cell research, some couples might request at the outset of treatment that they be provided the opportunity to donate unneeded embryos to research. However, even if couples indicated at the outset of their clinical treatment that they chose to donate excess embryos for research, that decision must be confirmed before the embryos are thawed for research use (Lo et al., 2004).

Recommendation 17:
Consent for blastocyst donation should be obtained from each donor at the time of donation. Even people who have given prior indication of their intent to donate to research any blastocysts that remain after clinical care should nonetheless give informed consent at the time of donation. Donors should be informed that they retain the right to withdraw consent until the blastocysts are actually used in cell line derivation.

INFORMED CONSENT REQUIREMENTS

Prospective donors of blastocysts or gametes that remain after infertility treatment and donors of gametes for research should receive timely, relevant, and appropriate information to make informed and voluntary choices. Before considering the potential research use of the blastocysts, a prospective donor should have been

presented with the option of storing the embryos, donating them to another woman or couple, donating them to research, or discarding them.

The current regulatory system specifies basic elements of information that must be provided to prospective participants during the informed consent process. In the context of donation for research, disclosure should ensure that potential donors understand the risks involved, if any. Donors should be told of all options concerning the care and disposition of their embryos, including freezing for later use, donation to others for reproductive use, research use, or discard without research use (Lo et al., 2004). To the extent possible, donors should be informed of the variety of future research uses before giving consent to donate blastocysts for research. Written informed consent must be obtained from all those who elect to donate blastocysts or gametes. Comprehensive information must be provided to all donors that is readily accessible and at a level that will enable an informed decision to be made.

Potential Discovery of Clinically Significant Information

If the identity of the donor is to be retained in a way that is ascertainable to the investigator, donors should be informed of the possibility that relevant clinical information might be discovered in the course of the research (for example, a genetic mutation conferring carrier status). There is ongoing debate about whether findings from research should be communicated to research subjects (donors would be considered subjects if identifiable information about them were known to researchers), either upon completion of a study or at some later date in time. This issue is relevant to all research, not just research involving hES cell lines. The obligation to report such findings to the donors depends in large part on the reliability of the findings and the significance of the information to human health.

MacKay has written that preliminary results do not yet constitute "information" since "until an initial finding is confirmed, there is no reliable information" to communicate to subjects, and that "even . . . confirmed findings may have some unforeseen limitations" (MacKay, 1984). McKay and others have argued that subjects should not be given information about their individual research results until the findings have been confirmed through the development of a reliable, accurate, and valid confirmatory test (MacKay, 1984; Fost and Farrell, 1989). On the other hand, those who believe that persons have the right to research results cite the principle of autonomy, which dictates that persons have a right to know what has been learned about them, and that therefore, interim results should be shared with subjects (Veatch, 1981).

Confusion about the appropriateness of returning individual research findings has increased as a result of HIPAA's Standards for Privacy of Individually Identifiable Health Information (the Privacy Rule; see Chapter 4). The Privacy Rule provides an individual the right of access to information about himself or herself, including personal research results obtained in the course of clinical care, with

limited exceptions. The Privacy Rule not only gives patients a right to see their own records but also requires that patients be notified of their right to see such records. This regulatory requirement is most likely to lead to an increase in the number of persons who are aware of and exercise their right to request and receive research findings, all of which will have implications for the researcher. Investigators will have to be prepared to include, and IRBs to review, plans for how to respond to subjects' requests for disclosure of research findings. Clearly, in the clinical context it is the utility and validity of the information that should dictate a decision to recontact individuals. It is less clear whether an investigator, who has no therapeutic relationship with the person, has the same obligation.

Another important requirement must be considered in the decision to report research findings to subjects—the Clinical Laboratory Improvement Amendments of 1988 (CLIA). CLIA regulations do not permit the return of research results to patients or subjects if the tests were not conducted in a CLIA-approved laboratory. Thus, if a research laboratory is not CLIA-approved, it should not be reporting its results to subjects. In some circumstances, repeating the test in a CLIA-approved laboratory may be feasible and appropriate.

In any case, donors should be clearly informed in the consent process whether they will have the opportunity to receive individual results from the project. Whether it is appropriate to return the results will depend on several factors and should be subject to IRB review.

Recommendation 18:
In the context of donation of gametes or blastocysts for hES cell research, the informed consent process, should, at a minimum, provide the following information:

a. A statement that the blastocysts or gametes will be used to derive hES cells for research that may include research on human transplantation.
b. A statement that the donation is made without any restriction or direction regarding who may be the recipient of transplants of the cells derived, except in the case of autologous donation.
c. A statement as to whether the identities of the donors will be readily ascertainable to those who derive or work with the resulting hES cell lines.
d. If the identities of the donors are retained (even if coded), a statement as to whether donors wish to be contacted in the future to receive information obtained through studies of the cell lines.
e. An assurance that participants in research projects will follow applicable and appropriate best practices for donation, procurement, culture, and storage of cells and tissues to ensure, in particular, the traceability of stem cells. (Traceable information, however, must be secured to ensure confidentiality.)
f. A statement that derived hES cells and/or cell lines might be kept for many years.

g. A statement that the hES cells and/or cell lines might be used in research involving genetic manipulation of the cells or the mixing of human and nonhuman cells in animal models.
h. Disclosure of the possibility that the results of study of the hES cells may have commercial potential and a statement that the donor will not receive financial or any other benefits from any future commercial development;
i. A statement that the research is not intended to provide direct medical benefit to the donor(s) except in the case of autologous donation.
j. A statement that embryos will be destroyed in the process of deriving hES cells.
k. A statement that neither consenting nor refusing to donate embryos for research will affect the quality of any future care provided to potential donors.
l. A statement of the risks involved to the donor.

In addition, donors could be offered the option of agreeing to some forms of hES cell research but not others. For example, donors might agree to have their materials used for deriving new hES cell lines but might not want their materials used, for example, for NT. The consent process should fully explore whether donors have objections to any specific forms of research to ensure that their wishes are honored.

ADHERENCE TO STANDARDS OF CLINICAL CARE

Clinical facilities providing ART services have an obligation to protect the rights and safety of their patients and to behave in an ethical manner. Researchers must not pressure members of the fertility treatment team to generate more embryos than necessary for the optimum chance of reproductive success. An IVF clinic, or other third party responsible for obtaining consent and/or collecting materials should not be able to pay for or be paid for the material it obtains (apart from specifically defined, cost-based reimbursements). Placing such restrictions on paying those who obtain the embryos discourages the creation during routine infertility procedures of excess embryos that would later be used for research purposes.

Finally, no member of the medical or nursing staff should be under any duty to participate in providing donor information or securing donor consent for research use of gametes or blastocysts if he or she has a conscientious objection. However, this privilege does not extend to the appropriate clinical care of a donor or recipient.

***Recommendation 19*:**
Consenting or refusing to donate gametes or embryos for research should not affect or alter in any way the quality of care provided to prospective donors. That is, clinical staff must provide appropriate care to patients without prejudice regarding their decisions about disposition of their embryos.

Recommendation 20:
Clinical personnel who have a conscientious objection to hES cell research should not be required to participate in providing donor information or securing donor consent for research use of gametes or blastocysts. That privilege should not extend to the care of a donor or recipient.

Recommendation 21:
Researchers may not ask members of the infertility treatment team to generate more oocytes than necessary for the optimal chance of reproductive success. An infertility clinic or other third party responsible for obtaining consent or collecting materials should not be able to pay for or be paid for the material obtained (except for specifically defined cost-based reimbursements and payments for professional services).

Restricting payment of those who obtain the embryos discourages the production of excess embryos during routine infertility procedures for later use in research. Other measures can be taken to ensure that conflicts of interest are appropriately managed. For example, the embryologist in the ART program who makes the determination that an oocyte has failed to fertilize or develop sufficiently for implantation should not be a member of the hES research team.

BANKING AND DISTRIBUTION OF CELL LINES

Once donated materials are obtained from couples or individuals, several additional standards should be applied to the storage, maintenance, and distribution of cell lines for research use. People and institutions responsible for these activities must maintain the highest ethical, legal, and scientific standards (Brivanlou et al., 2003). Cell lines might be stored at several institutions as part of individual research collections or might be deposited in more central repositories or banks. Developing standardized practices for obtaining, screening, processing, validating, and storing cell lines, and distributing them to users will provide confidence to researchers and the public that the materials are of high quality and of optimal use to researchers.

Several models exist for the banking of human biological materials. The most relevant is the U.K. Stem Cell Bank, which was established to provide researchers with an independent national stem cell resource:[1]

> The Cell Bank will offer a vital resource to support the advance of research in this exciting area. At the same time it will develop important safeguards, by ensuring that cell lines which could ultimately provide the basis for clinical treatment are appropriately characterized and also handled and stored under conditions that are

[1]http://www.nibsc.ac.uk/divisions/cbi/stemcell.html.

> properly controlled. This will not only provide high quality starting materials to facilitate the development of stem cell therapy, but, in providing a centralized resource for researchers, should also reduce the use of surplus embryos for the development of stem cell lines by individual teams.

One of the conditions of the U.K. bank's establishment was the development of an extensive code of practice for its operations (Medical Research Council, 2004). In addition, it has a clear system of governance, which involves a steering committee for policy, a management committee, and a user and clinical liaison committee.

Tissue-banking policies and practices in connection with a wide array of human cells, tissues, and organs have been established by several public and private entities in the United States, including the National Cancer Institute,[2] the National Heart, Lung and Blood Institute,[3] and private entities, such as Coriell[4] and the American Type Culture Collection.[5] In addition, the U.S. Office for Human Research Protections (OHRP) has issued two guidance documents: *Issues to Consider in Research Use of Stored Data or Tissues*[6] and *Guidance on Research Involving Coded Private Information or Biological Specimens*.[7]

The guidelines developed by those groups and the U.K. Stem Cell Bank generally adhere to key ethical principles that focus on the need for consent of donors and a system for monitoring adherence to ethical, legal, and scientific requirements. For example, a common requirement is that any identifiable tissue (including coded tissue) that is collected requires IRB review at the site of collection and informed consent of the subject. In addition, most require that, when possible, the informed consent process include information about the repository and the conditions under which materials will be shared. Other policies address the need to protect the privacy of donors. Several models exist for protecting subjects whose specimens are used for research, including the honest-broker model, in which a tissue bank trustee ensures strict control of information flows associated with research that uses banked tissues (see the model developed by OHRP[8]).

Procedurally, it is common practice that there be a clear policy and system for evaluating requests for samples to see whether each request is consistent with the conditions for sharing samples and with the original informed consent.

At the repository management level, there typically are requirements for safety, security, and risk assessments; validation of submitted material; culturing and ex-

[2]www.cancerdiagnosis.nci.nih.gov/specimenes/brochure.html; www.cancerdiagnosis.nci.nih.gov/specimens/legal.html.
[3]www.nhlbi.nih.gov/funding/policies/repos-gl.htm.
[4]http://locus.umdnj.edu/.
[5]http://www.atcc.org/.
[6]http://www.hhs.gov/ohrp/humansubjects/guidance/reposit.htm.
[7]http://www.hhs.gov/ohrp/humansubjects/guidance/cdebiol.pdf.
[8]http://www.hhs.gov/ohrp/humansubjects/guidance/reposit.htm.

pansion of cell line; process control; packaging, labeling, and distribution; and documentation and data management. Those requirements, in addition to routine quality assurance and control, will be as critical in hES cell research as in any other field that uses human materials. As hES cell research advances, it will be increasingly important for institutions that are obtaining, storing, and using cell lines to have confidence in the value of stored cells—that is, that they were obtained ethically and with the informed consent of donors, that they are well characterized and screened for safety, and that the conditions under which they are maintained and stored meet the highest scientific standards.

Recommendation 22:
Institutions that are banking or plan to bank hES cell lines should establish uniform guidelines to ensure that donors of material give informed consent through a process approved by an Institutional Review Board, and that meticulous records are maintained about all aspects of cell culture. Uniform tracking systems and common guidelines for distribution of cells should be established.

Recommendation 23:
Any facility engaged in obtaining and storing hES cell lines should consider the following standards:

(a) Creation of a committee for policy and oversight purposes and creation of clear and standardized protocols for banking and withdrawals.
(b) Documentation requirements for investigators and sites that deposit cell lines, including
- **(i) A copy of the donor consent form.**
- **(ii) Proof of Institutional Review Board approval of the procurement process.**
- **(iii) Available medical information on the donors, including results of infectious-disease screening.**
- **(iv) Available clinical, observational, or diagnostic information about the donor(s).**
- **(v) Critical information about culture conditions (such as media, cell passage, and safety information).**
- **(vi) Available cell line characterization (such as karyotype and genetic markers).**

A repository has the right of refusal if prior culture conditions or other items do not meet its standards.

(c) A secure system for protecting the privacy of donors when materials retain codes or identifiable information, including but not limited to

(i) A schema for maintaining confidentiality (such as a coding system).
(ii) A system for a secure audit trail from primary cell lines to those submitted to the repository.
(iii) A policy governing whether and how to deliver clinically significant information back to donors.

(d) The following standard practices:
(i) Assignment of a unique identifier to each sample.
(ii) A process for characterizing cell lines.
(iii) A process for expanding, maintaining, and storing cell lines.
(iv) A system for quality assurance and control.
(v) A website that contains scientific descriptions and data related to the cell lines available.
(vi) A procedure for reviewing applications for cell lines.
(vii) A process for tracking disbursed cell lines and recording their status when shipped (such as number of passages).
(viii) A system for auditing compliance.
(ix) A schedule of charges.
(x) A statement of intellectual property policies.
(xi) When appropriate, creation of a clear Material Transfer Agreement or user agreement.
(xii) A liability statement.
(xiii) A system for disposal of material.

(e) Clear criteria for distribution of cell lines, including but not limited to evidence of approval of the research by an Embryonic Stem Cell Research Oversight committee or equivalent body at the recipient institution.

The committee also notes and commends recent efforts at the federal level by the National Institutes of Health[9] to encourage the sharing and dissemination of important research resources. Restricted availability of unique research resources, such as hES cell lines, upon which further studies are dependent, can impede the advancement of research. To the extent possible, the committee encourages practices that make cell lines readily available in a timely fashion to the research community for further research, development, and application.

[9]See NIH's Policy on Sharing of Model Organisms for Biomedical Research at http://grants.nih.gov/grants/guide/notice-files/NOT-OD-04-042.html.

SUMMARY

Individuals and couples who voluntarily and with full information donate somatic cells, gametes, or blastocysts for hES research must be assured that the research will be meritorious and that all possible efforts will be made by those with responsibility for handling, storing, and using resulting cell lines to protect donor confidentiality. The combination of IRB review of the procurement process and a process of fully informed consent before donation will contribute to the ethical conduct of the research. Once hES cells are derived, the proper banking and distribution of hES cell lines will maintain the covenant between donor and scientific community.

6

National Academies Guidelines for Research on Human Embryonic Stem Cells

1.0 Introduction
2.0 Establishment of an Institutional Embryonic Stem Cell Research Oversight Committee
3.0 Procurement of Gametes, Blastocysts or Cells for hES Generation
4.0 Derivation of hES Cell Lines
5.0 Banking and Distribution of hES Cell Lines
6.0 Research Use of hES Cell Lines
7.0 International Collaboration
8.0 Conclusion

1.0 INTRODUCTION

In this chapter we collect all the recommendations made throughout the report and translate them into a series of formal guidelines. These guidelines focus on the derivation, procurement, banking, and use of human embryonic stem (hES) cell lines. They provide an oversight process that will help to ensure that research with hES cells is conducted in a responsible and ethically sensitive manner and in compliance with all regulatory requirements pertaining to biomedical research in general. The National Academies are issuing these guidelines for the use of the scientific community, including researchers in university, industry, or other private-sector research organizations.

1.1(a) What These Guidelines Cover

These guidelines cover all derivation of hES cell lines and all research that uses hES cells derived from

(1) Blastocysts made for reproductive purposes and later obtained for research from *in vitro* fertilization (IVF) clinics.
(2) Blastocysts made specifically for research using IVF.
(3) Somatic cell nuclear transfer (NT) into oocytes.

The guidelines do not cover research that uses nonhuman stem cells.

Many, but not all, of the guidelines and concerns addressed in this report are common to other areas of human stem cell research, such as

(1) Research that uses human adult stem cells.
(2) Research that uses fetal stem cells or embryonic germ cells derived from fetal tissue; such research is covered by federal statutory restrictions at 42 U.S.C. 289g-2(a) and federal regulations at 45 CFR 46.210.

Institutions and investigators conducting research using such materials should consider which individual provisions of these guidelines are relevant to their research.

1.1(b) Reproductive Uses of NT

These guidelines also do not apply to reproductive uses of nuclear transfer (NT), which are addressed in the 2002 report *Scientific and Medical Aspects of Human Reproductive Cloning*, in which the National Academies recommended that "Human reproductive cloning should not now be practiced. It is dangerous and likely to fail." Although these guidelines do not specifically address human reproductive cloning, it continues to be the view of the National Academies that research aimed at the reproductive cloning of a human being should not be conducted at this time.

1.2 Categories of hES Cell Research

These guidelines specify categories of research that:

(a) Are permissible after currently mandated reviews and proper notification of the relevant research institution.
(b) Are permissible after additional review by an Embryonic Stem Cell Research Oversight (ESCRO) committee, as described in Section 2.0 of the guidelines.
(c) Should not be conducted at this time.

Because of the sensitive nature of some aspects of hES cell research, these guidelines in many instances set a higher standard than is required by laws or regulations with which institutions and individuals already must comply.

1.2(a) hES Cell Research Permissible after Currently Mandated Reviews

Purely *in vitro* hES cell research that uses previously derived hES cell lines is permissible provided that the ESCRO committee or equivalent body designated by the investigator's institution (see Section 2.0), receives documentation of: i) the provenance of the cell lines; ii) appropriate informed consent in their derivation; and iii) evidence of compliance with any required review by an Institutional Review Board (IRB), Institutional Animal Care and Use Committee (IACUC), or Institutional Biosafety Committee (IBC), or other mandated review.

1.2(b) hES Cell Research Permissible Only after Additional Review and Approval

(1) Generation of new lines of hES cells by whatever means.
(2) Research involving the introduction of hES cells into nonhuman animals at any stage of embryonic, fetal, or postnatal development; particular attention should be paid to the probable pattern and effects of differentiation and integration of the human cells into the nonhuman animal tissues.
(3) Research in which the identity of the donors of blastocysts, gametes, or somatic cells from which the hES cells were derived is readily ascertainable or might become known to the investigator.

1.2(c) hES Cell Research That Should Not Be Permitted at This Time

The following types of research should not be conducted at this time:

(1) Research involving *in vitro* culture of any intact human embryo, regardless of derivation method, for longer than 14 days or until formation of the primitive streak begins, whichever occurs first.
(2) Research in which hES cells are introduced into nonhuman primate blastocysts or in which any embryonic stem cells are introduced into human blastocysts.

In addition:

(3) No animal into which hES cells have been introduced at any stage of development should be allowed to breed.

1.3 Obligations of Investigators and Institutions

All scientific investigators and their institutions, regardless of their field, bear the ultimate responsibility for ensuring that they conduct themselves in accordance with professional standards and with integrity. In particular, people whose research involves hES cells should work closely with oversight bodies, demonstrate respect for

the autonomy and privacy of those who donate gametes, blastocysts, or somatic cells and be sensitive to public concerns about research that involves human embryos.

2.0 ESTABLISHMENT OF AN INSTITUTIONAL EMBRYONIC STEM CELL RESEARCH OVERSIGHT COMMITTEE

To provide oversight of all issues related to derivation and use of hES cell lines and to facilitate education of investigators involved in hES cell research, each institution involved in hES cell research should establish an Embryonic Stem Cell Research Oversight (ESCRO) committee. The committee should include representatives of the public and persons with expertise in developmental biology, stem cell research, molecular biology, assisted reproduction, and ethical and legal issues in hES cell research. It must have suitable scientific, medical, and ethical expertise to conduct its own review and should have the resources needed to coordinate the management of the various other reviews required for a particular protocol. A pre-existing committee could serve the functions of the ESCRO committee provided that it has the recommended expertise and representation to perform the various roles described in this report. For example, an institution might elect to constitute an ESCRO committee from among some members of an IRB. But the ESCRO committee should not be a subcommittee of the IRB, as its responsibilities extend beyond human subject protections. Furthermore, much hES cell research does not require IRB review. The ESCRO committee should:

(1) Provide oversight over all issues related to derivation and use of hES cell lines.
(2) Review and approve the scientific merit of research protocols.
(3) Review compliance of all in-house hES cell research with all relevant regulations and these guidelines.
(4) Maintain registries of hES cell research conducted at the institution and hES cell lines derived or imported by institutional investigators.
(5) Facilitate education of investigators involved in hES cell research.

3.0 PROCUREMENT OF GAMETES, BLASTOCYSTS, OR CELLS FOR hES GENERATION

3.1. An IRB, as described in federal regulations at 45 CFR 46.107, should review the procurement of all gametes, blastocysts, or somatic cells for the purpose of generating new hES cell lines, including the procurement of blastocysts in excess of clinical need from infertility clinics, blastocysts made through IVF specifically for research purposes, and oocytes, sperm, and somatic cells donated for development of hES cell lines derived through NT or by parthenogenesis or androgenesis.

3.2. Consent for donation should be obtained from each donor at the time of donation. Even people who have given prior indication of their intent to donate to research any blastocysts that remain after clinical care should nonetheless give informed consent at the time of donation. Donors should be informed that they retain the right to withdraw consent until the blastocysts are actually used in cell line derivation.

3.3. When donor gametes have been used in the IVF process, resulting blastocysts may not be used for research without consent of all gamete donors.

3.4a. No payments, cash or in kind, may be provided for donating blastocysts in excess of clinical need for research purposes. People who elect to donate stored blastocysts for research should not be reimbursed for the costs of storage prior to the decision to donate.

3.4b. Women who undergo hormonal induction to generate oocytes specifically for research purposes (such as for NT) should be reimbursed only for direct expenses incurred as a result of the procedure, as determined by an IRB. No payments, cash or in kind, should be provided for donating oocytes for research purposes. Similarly, no payments should be made for donations of sperm for research purposes or of somatic cells for use in NT.

3.5. To facilitate autonomous choice, decisions related to the creation of embryos for infertility treatment should be free of the influence of investigators who propose to derive or use hES cells in research. Whenever it is practicable, the attending physician responsible for the infertility treatment and the investigator deriving or proposing to use hES cells should not be the same person.

3.6. In the context of donation of gametes or blastocysts for hES cell research, the informed consent process, should, at a minimum, provide the following information.

(a) A statement that the blastocysts or gametes will be used to derive hES cells for research that may include research on human transplantation.
(b) A statement that the donation is made without any restriction or direction regarding who may be the recipient of transplants of the cells derived, except in the case of autologous donation.
(c) A statement as to whether the identities of the donors will be readily ascertainable to those who derive or work with the resulting hES cell lines.
(d) If the identities of the donors are retained (even if coded), a statement as to whether donors wish to be contacted in the future to receive information obtained through studies of the cell lines.
(e) An assurance that participants in research projects will follow applicable and appropriate best practices for donation, procurement, culture, and

storage of cells and tissues to ensure, in particular, the traceability of stem cells. (Traceable information, however, must be secured to ensure confidentiality.)

(f) A statement that derived hES cells and/or cell lines might be kept for many years.

(g) A statement that the hES cells and/or cell lines might be used in research involving genetic manipulation of the cells or the mixing of human and nonhuman cells in animal models.

(h) Disclosure of the possibility that the results of study of the hES cells may have commercial potential and a statement that the donor will not receive financial or any other benefits from any future commercial development.

(i) A statement that the research is not intended to provide direct medical benefit to the donor(s) except in the case of autologous donation.

(j) A statement that embryos will be destroyed in the process of deriving hES cells.

(k) A statement that neither consenting nor refusing to donate embryos for research will affect the quality of any future care provided to potential donors.

(l) A statement of the risks involved to the donor.

In addition, donors could be offered the option of agreeing to some forms of hES cell research but not others. For example, donors might agree to have their materials used for deriving new hES cell lines but might not want their materials used, for example, for NT. The consent process should fully explore whether donors have objections to any specific forms of research to ensure that their wishes are honored.

3.7. Clinical personnel who have a conscientious objection to hES cell research should not be required to participate in providing donor information or securing donor consent for research use of gametes or blastocysts. That privilege should not extend to the care of a donor or recipient.

3.8. Researchers may not ask members of the infertility treatment team to generate more oocytes than necessary for the optimal chance of reproductive success. An infertility clinic or other third party responsible for obtaining consent or collecting materials should not be able to pay for or be paid for the material obtained (except for specifically defined cost-based reimbursements and payments for professional services).

4.0 DERIVATION OF hES CELL LINES

4.1. Requests to the ESCRO committee for permission to attempt derivation of new hES cell lines from donated embryos or blastocysts must include evidence of IRB approval of the procurement process (see Section 3.0 above).

4.2. The scientific rationale for the need to generate new hES cell lines, by whatever means, must be clearly presented, and the basis for the numbers of embryos and blastocysts needed should be justified.

4.3. Research teams should demonstrate appropriate expertise or training in derivation or culture of either human or nonhuman ES cells before permission to derive new lines is given.

4.4. When NT experiments involving either human or nonhuman oocytes are proposed as a route to generation of ES cells, the protocol must have a strong scientific rationale. Proposals that include studies to find alternatives to donated oocytes in this research should be encouraged.

4.5. Neither blastocysts made using NT (whether produced with human or nonhuman oocytes) nor parthenogenetic or androgenetic human embryos may be transferred to a human or nonhuman uterus or cultured as intact embryos *in vitro* for longer than 14 days or until formation of the primitive streak, whichever occurs first.

4.6. Investigators must document how they will characterize, validate, store, and distribute any new hES cell lines and how they will maintain the confidentiality of any coded or identifiable information associated with the lines (see Section 5.0 below).

5.0 BANKING AND DISTRIBUTION OF hES CELL LINES

There are several models for the banking of human biological materials, including hES cells. The most relevant is the U.K. Stem Cell Bank. The guidelines developed by this and other groups generally adhere to key ethical principles that focus on the need for consent of donors and a system for monitoring adherence to ethical, legal, and scientific requirements. As hES cell research advances, it will be increasingly important for institutions that are obtaining, storing, and using cell lines to have confidence in the value of stored cells—that is, that they were obtained ethically and with the informed consent of donors, that they are well characterized and screened for safety, and that the conditions under which they are maintained and stored meet the highest scientific standards. Institutions engaged in hES research should seek mechanisms for establishing central repositories for hES cell lines—through partnerships or augmentation of existing quality research cell line repositories and should adhere to high ethical, legal, and scientific standards. At a minimum, an institutional registry of stem cell lines should be maintained.

5.1 Institutions that are banking or plan to bank hES cell lines should establish uniform guidelines to ensure that donors of material give informed consent through

a process approved by an IRB and that meticulous records are maintained about all aspects of cell culture. Uniform tracking systems and common guidelines for distribution of cells should be established.

5.2 Any facility engaged in obtaining and storing hES cell lines should consider the following standards:

(a) Creation of a committee for policy and oversight purposes and creation of clear and standardized protocols for banking and withdrawals.

(b) Documentation requirements for investigators and sites that deposit cell lines, including
   (i) A copy of the donor consent form.
   (ii) Proof of Institutional Review Board approval of the procurement process.
   (iii) Available medical information on the donors, including results of infectious-disease screening.
   (iv) Available clinical, observational, or diagnostic information about the donor(s).
   (v) Critical information about culture conditions (such as media, cell passage, and safety information).
   (vii) Available cell line characterization (such as karyotype and genetic markers).

A repository has the right of refusal if prior culture conditions or other items do not meet its standards.

(c) A secure system for protecting the privacy of donors when materials retain codes or identifiable information, including but not limited to
   (i) A schema for maintaining confidentiality (such as a coding system).
   (ii) A system for a secure audit trail from primary cell lines to those submitted to the repository.
   (iii) A policy governing whether and how to deliver clinically significant information back to donors.

(d) The following standard practices:
   (i) Assignment of a unique identifier to each sample.
   (ii) A process for characterizing cell lines.
   (iii) A process for expanding, maintaining, and storing cell lines.
   (iv) A system for quality assurance and control.
   (v) A website that contains scientific descriptions and data related to the cell lines available.
   (vi) A procedure for reviewing applications for cell lines.
   (vii) A process for tracking disbursed cell lines and recording their status when shipped (such as number of passages).
   (viii) A system for auditing compliance.

(ix) A schedule of charges.
(x) A statement of intellectual property policies.
(xi) When appropriate, creation of a clear Material Transfer Agreement or user agreement.
(xii) A liability statement.
(xiii) A system for disposal of material.

(e) Clear criteria for distribution of cell lines, including but not limited to evidence of approval of the research by an Embryonic Stem Cell Research Oversight committee or equivalent body at the recipient institution.

6.0 RESEARCH USE OF hES CELL LINES

Once hES cell lines have been derived, investigators and institutions, through ESCRO committees and other relevant committees (such as an IACUC, an IBC, or a radiation safety committee) should monitor their use in research.

6.1 Institutions should require documentation of the provenance of all hES cell lines, whether the cells were imported into the institution or generated locally. Notice to the institution should include evidence of IRB-approval of the procurement process and of adherence to basic ethical and legal principles of procurement. In the case of lines imported from another institution, documentation that these criteria were met at the time of derivation will suffice.

6.2. *In vitro* experiments involving the use of already derived and coded hES cell lines will not need review beyond the notification required in Section 6.1.

6.3. Each institution should maintain a registry of its investigators who are conducting hES cell research and ensure that all registered users are kept up to date with changes in guidelines and regulations regarding the use of hES cells.

6.4. All protocols involving the combination of hES cells with nonhuman embryos, fetuses, or adult animals must be submitted to the local IACUC for review of animal welfare issues and to the ESCRO committee for consideration of the consequences of the human contributions to the resulting chimeras. (See also Section 1.2(c)(3) concerning breeding of chimeras.)

6.5. Transplantation of differentiated derivatives of hES cells or even hES cells themselves into adult animals will not require extensive ESCRO committee review. If there is a possibility that the human cells could contribute in a major organized way to the brain of the recipient animal, however, the scientific justification for the experiments must be strong, and proof of principle using nonhuman (preferably primate) cells, is desirable.

6.6. Experiments in which hES cells, their derivatives, or other pluripotent cells are introduced into nonhuman fetuses and allowed to develop into adult chimeras need more careful consideration because the extent of human contribution to the resulting animal may be higher. Consideration of any major functional contributions to the brain should be a main focus of review. (See also Section 1.2(c)(3) concerning breeding of chimeras.)

6.7. Introduction of hES cells into nonhuman mammalian blastocysts should be considered only under circumstances in which no other experiment can provide the information needed. (See also Sections 1.2(c)(2) and 1.2(c)(3) concerning restrictions on breeding of chimeras and production of chimeras with nonhuman primate blastocysts.)

6.8 Research use of existing hES cells does not require IRB review unless the research involves introduction of the hES cells or their derivatives into patients or the possibility that the identity of the donors of the blastocysts, gametes, or somatic cells is readily ascertainable or might become known to the investigator.

7.0 INTERNATIONAL COLLABORATION

If a U.S.-based investigator collaborates with an investigator in another country, the ESCRO committee may determine that the procedures prescribed by the foreign institution afford protections consistent with these guidelines, and the ESCRO committee may approve the substitution of some of or all of the foreign procedures for its own.

8.0 CONCLUSION

The substantial public support for hES cell research and the growing trend by many nonfederal funding agencies and state legislatures to support this field requires a set of guidelines to provide a framework for hES cell research. In the absence of the oversight that would come with unrestricted federal funding of this research, these guidelines will offer reassurance to the public and to Congress that the scientific community is attentive to ethical concerns and is capable of self-regulation while moving forward with this important research.

To help ensure that these guidelines are taken seriously, stakeholders in hES cell research—sponsors, funding sources, research institutions, relevant oversight committees, professional societies, and scientific journals, as well as investigators—should develop policies and practices that are consistent with the principles inherent in these guidelines. Funding agencies, professional societies, journals, and institutional review panels can provide valuable community pressure and impose appropriate sanctions to ensure compliance. For example, ESCRO committees and IRBs should require evidence of compliance when protocols are reviewed for renewal,

funding agencies should assess compliance when reviewing applications for support, and journals should require that evidence of compliance accompanies publication of results.

As individual states and private entities move into hES cell research, it will be important to initiate a national effort to provide a formal context in which the complex moral and oversight questions associated with this work can be addressed on a continuing basis. Both the state of hES cell research and clinical practice and public policy surrounding these topics are in a state of flux and are likely to be so for several years. Therefore, the committee believes that a national body should be established to assess periodically the adequacy of the policies and guidelines proposed in this document and to provide a forum for a continuing discussion of issues involved in hES cell research. New policies and standards may be appropriate for issues that cannot now be foreseen. The organization that sponsors this body should be politically independent and without conflicts of interest, should be respected in the lay and scientific communities, and able to call on suitable expertise to support this effort.

References

Allen, N. D., Barton, S. C., Hilton, K., Norris, M. L., and Surani, M. A. 1994. A functional analysis of imprinting in parthenogenetic embryonic stem cells. Development. 120:1473-1482.

Amit, M., Carpenter, M. K., Inokuma, M. S., Chiu, C. P., Harris, C. P., Waknitz, M. A., Itskovitz-Eldor, J., and Thomson, J. A. 2000. Clonally derived human embryonic stem cell lines maintain pluripotency and proliferative potential for prolonged periods of culture. Develomental Biology. 227:271-278.

Anderson J. A., and Weijer C. 2002. The research subject as wage earner. Theoretical Medicine and Bioethics. 23(4-5):359-376.

American Society for Reproductive Medicine (ASRM). Ethics Committee. 2000. Financial incentives in recruitment of oocyte donors. Fertility and Sterility. 74(2):216-220.

ASRM. Ethics Committee. 2002. Donating spare embryos for embryonic stem-cell research. Fertility and Sterility. 78(5):957-960.

ASRM. Ethics Committee. 2004a. Ovarian hyperstimulation syndrome. Fertility and Sterility. 82(Supplement 2):S81-86.

ASRM. Ethics Committee. 2004b. Donating spare embryos for embryonic stem-cell research. Fertility and Sterility. 82(Supplement 2):S224-S227.

Assady, S., Maor, G., Amit, M., Itskovitz-Eldor, J., Skorecki, K. L., and Tzukerman, M. 2001. Insulin production by human embryonic stem cells. Diabetes. 50:1691-1697.

Bangsboll S., Pinborg A., Yding Andersen C., and Nyboe Andersen A. 2004. Patients' attitudes towards donation of surplus cryopreserved embryos for treatment or research. Human Reproduction. 19(10):2415-2419.

Baylis F., Beagan B., Johnston J., and Ram N. 2003. Cryopreserved human embryos in Canada and their availability for research. Journal of Obstetrics and Gynaecology Canada. 25(12):1026-1031.

Bioethics Advisory Committee of Singapore. 2002. Ethical, Legal and Social Issues in Human Stem Cell Research, Reproductive and Therapeutic Cloning. Released June 21.

Bongso, A., Fong, C. Y., Ng, S. C., and Ratnam, S. 1994. Isolation and culture of inner cell mass cells from human blastocysts. Human Reproduction. 9:2110-2117.

Brivanlou, A. H., Gage, F. H., Jaenisch, R., Jessell, T., Melton, D., and Rossant, J. 2003. Setting Standards for Human Embryonic Stem Cells. Science. 300:913-916.

Burton P. J., and Sanders K. 2004. Patient attitudes to donation of embryos for research in Western Australia. The Medical Journal of Australia. 180(11):559-561.

Campbell, K. H., McWhir, J., Ritchie, W. A., and Wilmut, I. 1996. Sheep cloned by nuclear transfer from a cultured cell line. Nature. 380:64-66.

Carpenter, M. K., and Bhatia, M. 2004. Characterization of Human Embryonic Stem Cells, Vol. 1 (Amsterdam, Elsevier).

Chadwick, K., Wang, L., Li, L., Menendez, P., Murdoch, B., Rouleau, A., and Bhatia, M. 2003. Cytokines and BMP-4 promote hematopoietic differentiation of human embryonic stem cells. Blood. 102:906-915.

Chen, Y., He, Z. X., Liu, A., Wang, K., Mao, W. W., Chu, J. X., Lu, Y., Fang, Z. F., Shi, Y. T., Yang, Q. Z., Chen, D. Y., Wang, M. K., Li, J. S., Huang, S. L., Kong, X. Y., Shi, Y. Z., Wang, Z. Q., Xia, J. H., Long, Z. G., Xue, Z. G., Ding, W. X., and Sheng, H., Z.. 2003. Embryonic stem cells generated by nuclear transfer of human somatic nuclei into rabbit oocytes. Cell Research. 13:251-263.

Cibelli, J. B., Grant, K. A., Champman, K. B., Lanza, R. P., Vrana, K. E., Cunniff, K., Worst, T., Green, H., Walker, S., Gutin, P., Vilner, L., Tabar, V., Dominko, T., Kane, J., Wettstein, P., Studer, L., and West, M. 2002. Parthenogenetic stem cells in nonhuman primates. Science. 295:819.

Congregation of the Doctrine of the Faith. 1987. Instruction on Respect for Human Life in Its Origin and on the Dignity of Procreation. Vatican City.

Daheron, L., Opitz, S. L., Zaehres, H., Lensch, W. M., Andrews, P. W., Itskovitz-Eldor, J., and Daley, G. Q. 2004. LIF/STAT3 signaling fails to maintain self-renewal of human embryonic stem cells. Stem Cells. 22:770-778.

Delhaise, F., Bralion, V., Schuurbiers, N., and Dessy, F. 1996. Establishment of an embryonic stem cell line from 8-cell stage mouse embryos. European Journal of Morphology. 34:237-243.

Department of Health, Education, and Welfare (DHEW). Ethics Advisory Board (EAB). 1979. Report and Conclusions: HEW Support of Research Involving Human In Vitro Fertilization and Embryo Transfer. Washington, D.C.: U.S. Government Printing Office.

Dickert N., Emanuel E., and Grady C. 2002. Paying research subjects: an analysis of current policies. Annals of Internal Medicine. 136(5):368-373.

Doetschman, T., Gregg, R. G., Maeda, N., Hooper, M. L., Melton, D. W., Thompson, S., and Smithies, O. 1987. Targeted correction of a mutant HPRT gene in mouse embryonic stem cells. Nature. 330:576-578.

Edwards, R. G., ed. 2004. History of Embryo Stem Cells (Amsterdam, Elsevier).

Eiges, R., Schuldiner, M., Drukker, M., Yanuka, O., Itskovitz-Eldor, J., and Benvenisty, N. 2001. Establishment of human embryonic stem cell-transfected clones carrying a marker for undifferentiated cells. Current Biology. 11:514-518.

Endo, T., Honnma, H., Hayashi, T., Chida, M., Yamazaki, K., Kitajima, Y., Azumaguchi, A., Kamiya, H., and Kudo, R. 2002. Continuation of GnRH agonist administration for 1 week, after hCG injection, prevents ovarian hyperstimulation syndrome following elective cryopreservation of all pronucleate embryos. Human Reproduction. 17(10):2548-2551.

Evans, M. J., and Kaufman, M. H. 1981. Establishment in culture of pluripotential cells from mouse embryos. Nature. 292:154-156.

Fost, N., and Farrell, P.M. 1989. A prospective randomized trial of early diagnosis and treatment of cystic fibrosis: A unique ethical dilemma. Clinical Research. 37:495–500.

Gardner, R. L. 2004. Pluripotent Stem Cells from Vertebrate Embryos: Present Perspective and Future Challenges, Vol. 1 (Amsterdam, Elsevier).

Goldstein, R. S., Drukker, M., Reubinoff, B. E., and Benvenisty, N. 2002. Integration and differentiation of human embryonic stem cells transplanted to the chick embryo. Developmental Dynamics. 225:80-86.

Gropp, M., Itsykson, P., Singer, O., Ben-Hur, T., Reinhartz, E., Galun, E., and Reubinoff, B. E. 2003. Stable genetic modification of human embryonic stem cells by lentiviral vectors. Molecular Therapy. 7:281-287.

Grubb, A. 1996. The Human Fertilisation and Embryology Act (Statutory Storage Period for Embryos) Regulations 1996 (S.I. 1996 No. 375). Medical Law Review. 4(2):211-215.

Hoffman, D. I., Zellman, G. L., Fair, C. C., Mayer, J. F., Zeitz, J. G., Gibbons, W. E., and Turner, T. G. 2003. Cryopreserved embryos in the United States and their availability for research. Fertility and Sterility. 79(5):1063-1069.

Hubner, K., Fuhrmann, G., Christenson, L. K., Kehler, J., Reinbold, R., De La Fuente, R., Wood, J., Strauss, J. F., 3rd, Boiani, M., and Scholer, H. R. 2003. Derivation of oocytes from mouse embryonic stem cells. Science. 300:1251-1256.

Hurlbut. 2004. PCB; http://www.bioethics.gov/transcripts/dec04/dec3full.html.

Hwang, W. S., Ryu, Y. J., Park, J. H., Park, E. S., Lee, E. G., Koo, J. M., Jeon, H. Y., Lee, B. C., Kang, S. K., Kim, S. J., Ahn, C., Hwang, J. H., Ky Young Park, K. Y., Cibelli, J. B., and Moon, S. Y. 2004. Evidence of a pluripotent human embryonic stem cell line derived from a cloned blastocyst. Science. 303:1669-1674.

Itskovitz-Eldor, J., Schuldiner, M., Karsenti, D., Eden, A., Yanuka, O., Amit, M., Soreq, H., and Benvenisty, N. 2000. Differentiation of human embryonic stem cells into embryoid bodies compromising the three embryonic germ layers. Molecular Medicine. 6:88-95.

Jaenisch, Rudolph. 2004. Human Cloning — The Science and Ethics of Nuclear Transplantation. The New England Journal of Medicine. 351(27):2787-2791.

Kaufman, D. S., Hanson, E. T., Lewis, R. L., Auerbach, R., and Thomson, J. A. 2001. Hematopoietic colony-forming cells derived from human embryonic stem cells. PNAS. 98:10716-10721.

Kaufman, M. H., Robertson, E. J., Handyside, A. H., and Evans, M. J. 1983. Establishment of pluripotential cell lines from haploid mouse embryos. J. Embryol. Exp. Morphol. 73:249-261.

Kawase, E., Yamazaki, Y., Yagi, T., Yanagimachi, R., and Pedersen, R. A. 2000. Mouse embryonic stem (ES) cell lines established from neuronal cell-derived cloned blastocysts. Genesis. 28:156-163.

Kehat, I., Kenyagin-Karsenti, D., Snir, M., Segev, H., Amit, M., Gepstein, A., Livne, E., Binah, O., Itskovitz-Eldor, J., and Gepstein, L. 2001. Human embryonic stem cells can differentiate into myocytes with structural and functional properties of cardiomyocytes. Journal of Clinical Investigation. 108:407-414.

Klimanskaya, I., and McMahon, J. 2004. Isolation and Maintenance of Human ES Cells: Detailed Procedures and Alternatives, Vol 1 (Amsterdam, Elsevier).

Klock, S. C. 2004. Embryo disposition: the forgotten "child" of in vitro fertilization. International Journal of Fertility and Womens Medicine. 49(1):19-23.

Klug, M. G., Soonpaa, M. H., Koh, G. Y., and Field, L. J. 1996. Genetically selected cardiomyocytes from differentiating embryonic stem cells form stable intracardiac grafts. Journal of Clinical Investigation. 98:216-224.

Kyba, M., Perlingeiro, R. C., and Daley, G. Q. 2002. HoxB4 confers definitive lymphoid-myeloid engraftment potential on embryonic stem cell and yolk sac hematopoietic progenitors. Cell. 109: 29-37.

Landry, D. W., and Zucker, H. A. 2004. Embryonic death and the creation of human embryonic stem cells. The Journal of Clinical Investigation.114:1184-1186.

Lanza, R., Gearhart, J., Hogan, B., Melton, D. W., Pedersen, R., Thomson, J., and West, M. 2004. Handbook of Stem Cells, Vol. 1 (Amsterdam, Elsevier).

Levenberg, S., Golub, J. S., Amit, M., Itskovitz-Eldor, J., and Langer, R. 2002. Endothelial cells derived from human embryonic stem cells. PNAS. 99:4391-4396.

Lo, B., Chou, V., Cedars, M. I., Gates, E., Taylor, R. N., Wagner, R. M., Wolf, L., Yamamoto, K. R. 2004. Informed consent in human oocyte, embryo, and embryonic stem cell research. Fertility and Sterility. 83(3):559-563.

Ma, Y., Ramezani, A., Lewis, R., Hawley, R. G., and Thomson, J. A. 2003. High-level sustained transgene expression in human embryonic stem cells using lentiviral vectors. Stem Cells. 21:111-117.

MacKay, C. R. 1984. Ethical issues in research design and conduct: developing a test to detect carriers of Huntington's disease. IRB. 6(4):1–5.

Mann, J. R., Gadi, I., Harbison, M. L., Abbondanzo, S. J. and Stewart, C. L. 1990. Androgenetic mouse embryonic stem cells are pluripotent and cause skeletal defects in chimeras: implications for genetic imprinting. Cell. 62:251-60.

Martin, G. R. 1981. Isolation of a pluripotent cell line from early mouse embryos cultured in medium conditioned by teratocarcinoma stem cells. PNAS. 78: 7634-7638.

Martin, M. J., Muotri, A., Gage, F., and Varki, A. 2005. Human embryonic stem cells express an imunogenic nonhuman sialic acid. Nature Medicine. 11(2):228-232.

Matsui, Y., Zsebo, K., and Hogan, B. L. M. 1992. Derivation of pluripotential embryonic stem cells from murine primordial germ cells in culture. Cell. 70:841-847.

McMahon C. A., Gibson F. L., Leslie G. I., Saunders D. M., Porter K. A., and Tennant C. C. 2003. Embryo donation for medical research: attitudes and concerns of potential donors. Human Reproduction. 18(4):871-877.

Melton, D. A., Daley, G. Q., and Jennings, C. G. 2004. Altered nuclear transfer in stem-cell research—a flawed proposal. New England Journal of Medicine. 351:2791-2792.

Medical Research Council (MRC). 2004. Code of Practice for the Use of Human Stem Cell Lines (interim). United Kingdom. [Online]. Available: http://www.mrc.ac.uk/index/public-interest/public-consultation/public-stem-cell-consultation.htm.

Moutel, G., Gregg, E., Meningaud, J. P., and Herve, C. 2002. Developments in the storage of embryos in France and the limitations of the laws of bioethics. Analysis of procedures in 17 storage centres and the destiny of stored embryos. Medicine and Law. 21(3):587-604.

Mummery, C., Ward, D., van den Brink, C. E., Bird, S. D., Doevendans, P. A., Opthof, T., Brutel de la Riviere, A., Tertoolen, L., van der Heyden, M., and Pera, M. 2002. Cardiomyocyte differentiation of mouse and human embryonic stem cells. J. Anat. 200:233-242.

Munsie, M. J., Michalska, A. E., O'Brien, C. M., Trounson, A. O., Pera, M. F., and Mountford, P. S. 2000. Isolation of pluripotent embryonic stem cells from reprogrammed adult mouse somatic cell nuclei. Current Biology 10:989-992.

Nagy, A., Gocza, E., Diaz, E. M., Prideaux, V. R., Ivanyi, E., Markkula, M., and Rossant, J. 1990. Embryonic stem cells alone are able to support fetal development in the mouse. Development. 110:815-821.

Nagy, A., Rossant, J., Nagy, R., Abramow-Newerly, W., and Roder, J. C. 1993. Derivation of completely cell culture-derived mice from early-passage embryonic stem cells. PNAS. 90:8424-8428.

National Bioethics Advisory Commission (NBAC). 1997. Cloning Human Beings. 2 vols. Rockville, Md.: U.S. Government Printing Office.

NBAC. 1999a. Ethical Issues in Human Stem Cell Research. Volume I. Report and Recommendations. Rockville, Md.: U.S. Government Printing Office.

NBAC. 1999b. Ethical Issues in Human Stem Cell Research. Volume III. Religious Prespectives. Rockville, Md.: U.S. Government Printing Office.

National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research. 1975. Research on the Fetus: Report and Recommendations. Washington, D.C.: U.S. Government Printing Office.

NIH (National Institutes of Health). Human Embryo Research Panel. 1994. *Report of the Human Embryo Research Panel*. 2 volumes. Bethesda, M.D.

NIH). Office of the Director (OD). 1999. "Fact Sheet on Stem Cell Research." Bethesda, MD.

National Research Council (NRC). 2002a. Stem Cells and the Future of Regenerative Medicine. Washington, D.C.: National Academy Press.

NRC. 2002b. Scientific and Medical Aspects of Human Reproductive Cloning. Washington, D.C.: National Academy Press.

Nisbet, M. C. 2004. Public Opinion about stem cell research and human cloning. Public Opinion Quarterly 68(1):131-154.

O'Brien, M. J., Pendola, J. K., and Eppig, J. J. 2003. A revised protocol for in vitro development of mouse oocytes from primordial follicles dramatically improves their developmental competence. Biology of Reproduction. 68:682-1686.

Office of the White House Press Secretary, Statement by the President, December 2, 1994.
Orvieto, R. 2005. Can we eliminate severe ovarian hyperstimulation syndrome? Human Reproduction. 20(2):20-322.
Pera, M. F., and Trounson, A. O. 2004. Human embryonic stem cells: prospects for development. Development. 131:5515-5525.
Pfeifer, A., Ikawa, M., Dayn, Y., and Verma, I. M. 2002. Transgenesis by lentiviral vectors: lack of gene silencing in mammalian embryonic stem cells and preimplantation embryos. PNAS. 99:2140-2145.
President's Council on Bioethics. 2002. Human Cloning and Human Dignity: An Ethical Inquiry. Washington, D.C.
Resnick, J. L., Bixler, L. S., Cheng, L., and Donovan, P. J. 1992. Long-term proliferation of mouse primordial germ cells in culture. Nature. 359:550-552.
Reubinoff, B. E., Pera, M. F., Fong, C. Y., Trounson, A., and Bongso, A. 2000. Embryonic stem cell lines from human blastocysts: somatic differentiation in vitro. Nat. Biotechnol. 18:399-404.
Reubinoff, B. E., Itsykson, P., Turetsky, T., Pera, M. F., Reinhartz, E., Itzik, A., and Ben-Hur, T. 2001. Neural progenitors from human embryonic stem cells. Nature Biotechnology. 19:1134-1140.
Rideout, W. M., 3rd, Hochedlinger, K., Kyba, M., Daley, G. Q., and Jaenisch, R. 2002. Correction of a genetic defect by nuclear transplantation and combined cell and gene therapy. Cell. 109:17-27.
Robert, J. S., and Baylis F. 2003. Crossing species boundaries. American Journal of Bioethics. 3(3):1-13.
Shamblott, M. J., Axelman, J., Wang, S., Bugg, E. M., Littlefield, J. W., Donovan, P. J., Blumenthal, P. D., Huggins, G. R., and Gearhart, J. D. 1998. Derivation of pluripotent stem cells from cultured human primordial germ cells. PNAS. 95:13726-13731.
Smith, A. G., Heath, J. K., Donaldson, D. D., Wong, G. G., Moreau, J., Stahl, M., and Rogers, D. 1988. Inhibition of pluripotential embryonic stem cell differentiation by purified polypeptides. Nature. 336:688-690.
Stewart, C. L. 1993. Production of Chimeras between Embryonic Stem Cells and Embryos, Vol. 225 (San Diego, Academic Press).
Szabo, P., and Mann, J. R. 1994. Expression and methylation of imprinted genes during in vitro differentiation of mouse parthenogenetic and androgenetic embryonic stem cell lines. Development. 120:1651-1660.
Thomas, K. R., and Capecchi, M. R. 1987. Site-directed mutagenesis by gene targeting in mouse embryo-derived stem cells. Cell. 51:503-512.
Thomson, J. A., Itskovitz-Eldor, J., Shapiro, S. S., Waknitz, M. A., Swiergiel, J. J., Marshall, V. S., and Jones, J. M. 1998. Embryonic stem cell lines derived from human blastocysts. Science. 282:1145-1147.
U.S. Congress, Office of Technology Assessment. 1988. Infertility: Medical and Social Choices. OTA-BA-358. Washington, D.C.: U.S. Government Printing Office.
Veatch, R. M. 1981. A Theory of Medical Ethics. New York: Basic Books.
Verlinsky, Y., Strelchenko, N., Kukharenko, V., Rechitsky, S., Veriinsky, O., Galat, V., and Kuliev, A. 2005. Human embryonic stem cell lines with genetic disorders. Reproductive BioMedicine Online. 10(I):105-110.
Wade, N. 1998. Researchers claim embryonic cell mix of human and cow. NY Times. Nov 12:A1, A26.
Wagers, A. J., and Weissman, I. L. 2004. Plasticity of adult stem cells. Cell. 116(5):639-648.
Wakayama, T., Tabar, V., Rodriguez, I., Perry, A. C., Studer, L., and Mombaerts, P. 2001. Differentiation of embryonic stem cell lines generated from adult somatic cells by nuclear transfer. Science. 292:740-743.
Walters, L. 2004. Human embryonic stem cell research: an intercultural perspective. Kennedy Institute of Ethics Journal. 14(1):3-38.
Wichterle, H., Lieberam, I., Porter, J. A., and Jessell, T. M. 2002. Directed differentiation of embryonic stem cells into motor neurons. Cell. 110(3):385-97.
Wiles, M. V. 1993. Embryonic Stem Cell Differentiation in Vitro, Vol. 225 (San Diego, Academic Press).

Williams, R. L., Hilton, D. J., Pease, S., Willson, T. A., Stewart, C. L., Gearing, D. P., Wagner, E. F., Metcalf, D., Nicola, N. A., and Gough, N. M. 1988. Myeloid leukaemia inhibitory factor maintains the developmental potential of embryonic stem cells. Nature. 336:684-687.

Windom, R.E. 1988. Memorandum to James B. Wyngaarden, March 22, 1988. Report of the Human Fetal Tissue Transplantation Research Panel. Vol. 2, A3. Bethesda, Md.: NIH.

Wright, V. C., Schieve, L.A., Reynolds, M. A., Jeng, G., and Kissin, D. 2004. Assisted reproductive technology surveillance—United States, 2001. Morbidity and Mortality Weekly Report. April 30: 1-20.

Xu, R. H., Chen, X., Li, D. S., Li, R., Addicks, G. C., Glennon, C., Zwaka, T. P., and Thomson, J. A. 2002. BMP4 initiates human embryonic stem cell differentiation to trophoblast. Nature Biotechnology. 20:1261-1264.

Xu, R. H., Peck, R. M., Li, D. S., Feng, X., Ludwig, T., and Thomson, J. A. 2005. Basic FGF and suppression of BMP signaling sustain undifferentiated proliferation of human ES cells. Nature Methods. 2(3):185-190

Zhang, S. C., Wernig, M., Duncan, I. D., Brustle, O., and Thomson, J. A. 2001. In vitro differentiation of transplantable neural precursors from human embryonic stem cells. Nature Biotechnology. 19:1129-1133.

Zwaka, T. P., and Thomson, J. A. 2003. Homologous recombination in human embryonic stem cells. Nature Biotechnology. 21:319-321.

Glossary

Adult stem cell—An undifferentiated cell found in a differentiated tissue that can renew itself and (with limitations) differentiate to yield the specialized cell types of the tissue from which it originated.

Androgenesis—Development in which the embryo contains only paternal chromosomes.

Autologous transplant—Transplanted tissue derived from the intended recipient of the transplant. Such a transplant helps to avoid complications of immune rejection.

Blastocoel—The cavity in the center of a blastocyst.

Blastocyst—A preimplantation embryo of 50–250 cells depending on age. The blastocyst consists of a sphere made up of an outer layer of cells (the trophectoderm), a fluid-filled cavity (the blastocoel), and a cluster of cells on the interior (the inner cell mass).

Blastomere—A single cell from a morula or early blastocyst, before the differentiation into trophectoderm and inner cell mass.

Bone marrow—The soft, living tissue that fills most bone cavities and contains hematopoietic stem cells, from which all red and white blood cells evolve. The bone marrow also contains mesenchymal stem cells from which a number of cell types arise, including chondrocytes, which produce cartilage, and fibroblasts, which produce connective tissue.

Chimera—An organism composed of cells derived from at least two genetically different cell types. The cells could be from the same or separate species.

Differentiation—The process whereby an unspecialized early embryonic cell acquires the features of a specialized cell, such as a heart, liver, or muscle cell.

DNA—Deoxyribonucleic acid, a chemical found primarily in the nucleus of cells. DNA carries the instructions for making all the structures and materials the body needs to function.

Ectoderm—The outermost of the three primitive germ layers of the embryo; it gives rise to skin, nerves, and brain.

Egg cylinder—An asymmetric embryonic structure that helps to determine the body plan of the mouse.

Electroporation— Method of introducing DNA into a cell.

Embryo—An animal in the early stages of growth and differentiation that are characterized by cleavage, laying down of fundamental tissues, and the formation of primitive organs and organ systems; especially the developing human individual from the time of implantation to the end of the eighth week after conception, after which stage it becomes known as a fetus.*

Embryoid bodies (EBs)—Clumps of cellular structures that arise when embryonic stem cells are cultured. Embryoid bodies contain tissue from all three germ layers: endoderm, mesoderm, and ectoderm. Embryoid bodies are not part of normal development and occur only in vitro.

Embryonic disk—A group of cells derived from the inner cell mass of the blastocyst, which later develops into an embryo. The disk consists of three germ layers known as the endoderm, mesoderm, and ectoderm.

Embryonic germ (EG) cells—Cells found in a specific part of the embryo or fetus called the gonadal ridge that normally develop into mature gametes. The germ cells differentiate into the gametes (oocytes or sperm).

Embryonic stem (ES) cells—Primitive (undifferentiated) cells derived from the early embryo that have the potential to become a wide variety of specialized cell types.

Endoderm—Innermost of the three primitive germ layers of the embryo; it later gives rise to the lungs, liver, and digestive organs.

Enucleated cell—A cell whose nucleus has been removed.

Epidermis—The outer cell layers of the skin.

---

* http://www.nlm.nih.gov/medlineplus/mplusdictionary.html. In common parlance, "embryo" is used more loosely and variably to refer to all stages of development from fertilization until some ill-defined stage when it is called a fetus. There are strictly defined scientific terms such as "zygote," "morula," and "blastocyst" that refer to specific stages of preimplantation development (see Chapter 2). In this report, we have used the more precise scientific terms where relevant but have used the term "embryo" where more precision seemed likely to confuse rather than clarify.

Epigenetic— Refers to modifications in gene expression that are controlled by heritable but potentially reversible changes in DNA methylation or chromatin structure without involving alteration of the DNA sequence.

Epithelium—Layers of cells in various organs, such as the epidermis of the skin or the lining of the gut. These cells serve the general functions of protection, absorption, and secretion, and play a specialized role in moving substances through tissue layers. Their ability to regenerate is excellent; the cells of an epithelium may replace themselves as frequently as every 24 hours from the pools of specialized stem cells.

Feeder cell layer—Cells that are used in culture to maintain pluripotent stem cells. Feeder cells usually consist of mouse embryonic fibroblasts.

Fertilization—The process whereby male and female gametes unite to form a zygote (fertilized egg).

Fibroblasts—Cells from many organs that give rise to connective tissue.

Gamete—A mature male or female germ cell, that is, sperm or oocyte, respectively.

Gastrulation—The procedure by which an animal embryo at an early stage of development produces the three primary germ layers: ectoderm, mesoderm, and endoderm.

Gene—A functional unit of heredity that is a segment of DNA located in a specific site on a chromosome. A gene usually directs the formation of an enzyme or other protein.

Gene targeting— A procedure used to produce a mutation in a specific gene.

Genital ridge—Anatomic site in the early fetus where primordial germ cells are formed.

Genome—The complete genetic material of an organism.

Genotype— Genetic constitution of an individual.

Germ cell—A sperm or egg or a cell that can become a sperm or egg. All other body cells are called somatic cells.

Germ layer—In early development, the embryo differentiates into three distinct germ layers (ectoderm, endoderm, and mesoderm), each of which gives rise to different parts of the developing organism.

Ger line—The cell lineage from which the oocyte and sperm are derived.

Gonadal ridge—Anatomic site in the early fetus where primordial germ cells (PGCs) are formed.

Gonads—The sex glands—testis and ovary.

Hematopoietic—Blood-forming.

Hematopoietic stem cell (HSC)—A stem cell from which all red and white blood cells evolve and that may be isolated from bone marrow or umbilical cord blood for use in transplants.

Hepatocyte—Liver cell.

Heterologous—From genetically different individuals.
hES cell—Human embryonic stem cell; a type of pluripotent stem cell.
Histocompatibility antigens—Glycoproteins on the surface membranes of cells that enable the body's immune system to recognize a cell as native or foreign and that are determined by the major histocompatibility complex.
Homologous recombination—Recombining of two like DNA molecules, a process by which gene targeting produces a mutation in a specific gene.
Hybrid— An organism that results from a cross between gametes of two different genotypes.

Immune system cells—White blood cells, or leukocytes, that originate in the bone marrow. They include antigen-presenting cells, such as dendritic cells, T and B lymphocytes, macrophages, and neutrophils, among many others.
Immunodeficient mice—Genetically altered mice used in transplantation experiments because they usually do not reject transplanted tissue.
Immunogenic—Related to or producing an immune response.
Immunosuppressive— Suppressing a natural immune response.
Implantation—The process in which a blastocyst implants into the uterine wall, where a placenta forms to nurture the growing fetus.
Inner cell mass—The cluster of cells inside the blastocyst that give rise to the embryonic disk of the later embryo and, ultimately, the fetus.
Interspecific—Between species.
In utero—In the uterus.
In vitro—Literally, "in glass," in a laboratory dish or test tube; in an artificial environment.
***In vitro* fertilization (IVF)**—An assisted reproductive technique in which fertilization is accomplished outside the body.
In vivo—In the living subject; in a natural environment.

Karyotype—The full set of chromosomes of a cell arranged with respect to size, shape, and number.

Leukemia inhibitory factor (LIF)—A growth factor necessary for maintaining mouse embryonic stem cells in a proliferative, undifferentiated state.

Mesenchymal stem cells—Stem cells found in bone marrow and elsewhere from which a number of cell types can arise, including chondrocytes, which produce cartilage, and fibroblasts, which produce connective tissue.
Mesoderm—The middle layer of the embryonic disk, which consists of a group of cells derived from the inner cell mass of the blastocyst; it is formed at gastrulation and is the precursor to bone, muscle, and connective tissue.
Morula—A solid mass of 16–32 cells that resembles a mulberry and results from the cleavage (cell division without growth) of a zygote (fertilized egg).

Mouse embryonic fibroblast (MEF)—Cells used as feeder cells in culturing pluripotent stem cells.
Neural stem cell (NSC)—A stem cell found in adult neural tissue that can give rise to neurons, astrocytes, and oligodendrocytes.
Nuclear transfer (NT)—Replacing the nucleus of one cell with the nucleus of another cell.

Oocyte—Developing egg; usually a large and immobile cell.
Ovariectomy— Surgical removal of an ovary.

Parthenogenesis—Development in which the embryo contains only maternal chromosomes.
Passage—A round of cell growth and proliferation in culture.
Phenotype—Visible properties of an organism produced by interaction of genotype and environment.
Placenta—The oval or discoid spongy structure in the uterus from which the fetus derives its nourishment and oxygen.
Pluripotent cell—A cell that has the capability of developing into cells of all germ layers (endoderm, ectoderm, and mesoderm).
Precursor cells—In fetal or adult tissues, partly differentiated cells that divide and give rise to differentiated cells. Also known as progenitor cells.
Preimplantation genetic diagnosis (PGD)—A procedure applied to IVF embryos to determine which ones carry deleterious mutations predisposing to hereditary diseases.
Primary germ layers—The three initial embryonic germ layers—endoderm, mesoderm, and ectoderm—from which all other somatic tissue types develop.
Primordial germ cell—A cell appearing during early development that is a precursor to a germ cell.
Primitive streak—The initial band of cells from which the embryo begins to develop. The primitive streak establishes and reveals the embryo's head-tail and left-right orientations.
Pseudopregnant—Refers to a female primed with hormones to accept a blastocyst for implantation.

Somatic cells—Any cell of a plant or animal other than a germ cell or germ cell precursor.
Somatic cell nuclear transfer (SCNT)—The transfer of a cell nucleus from a somatic cell into an egg (oocyte) whose nucleus has been removed.
Stem cell—A cell that has the ability to divide for indefinite periods in vivo or in culture and to give rise to specialized cells.

Teratoma—A tumor composed of tissues from the three embryonic germ layers. Usually found in ovary or testis. Produced experimentally in animals by injecting pluripotent stem cells to determine the stem cells' abilities to differentiate into various types of tissues.

Tissue culture—Growth of tissue *in vitro* on an artificial medium for experimental research.

Transfection—A method by which experimental DNA may be put into a cultured cell.

Transgene—A gene that has been incorporated into a cell or organism and passed on to successive generations.

Transplantation—Removal of tissue from one part of the body or from one individual and its implantation or insertion into another, especially by surgery.

Trophectoderm—The outer layer of the developing blastocyst that will ultimately form the embryonic side of the placenta.

Trophoblast—The extraembryonic tissue responsible for negotiating implantation, developing into the placenta, and controlling the exchange of oxygen and metabolites between mother and embryo.

Undifferentiated—Not having changed to become a specialized cell type.

Xenograft or xenotransplant—A graft or transplant of cells, tissues, or organs taken from a donor of one species and grafted into a recipient of another species.

Zygote—A cell formed by the union of male and female germ cells (sperm and egg, respectively).

Abbreviations

ART	assisted reproductive technology
ASRM	American Society for Reproductive Medicine
CFR	Code of Federal Regulations
CGTP	current good tissue practices
CLIA	Clinical Laboratoy Improvement Amendments
DHEW	Department of Health, Education, and Welfare
DHHS	Department of Health and Human Services
EAB	Ethics Advisory Board
ES cell	embryonic stem cell
ESCRO	Embryonic Stem Cell Research Oversight
FDA	Food and Drug Administration
FDCA	Food, Drug, and Cosmetic Act
GLP	good laboratory practice
HCT/Ps	human cells, tissues, and cellular and tissue-based products
HERP	Human Embryo Research Panel
hEG cells	human embryonic germ cells
hES cells	human embryonic stem cells
HFEA	Human Fertilisation and Embryology Authority (United Kingdom)

HIPAA	Health Insurance Portability and Accountability Act
IACUC	Institutional Animal Care and Use Committee
IBC	Institutional Biosafety Committee
IDE	investigational device exemption
IND	investigational new drug
IRB	Institutional Review Board
IVF	*in vitro* fertilization
LIF	leukemia inhibitory factor
mES	mouse embryonic stem cells
NAS	National Academy of Sciences
NBAC	National Bioethics Advisory Commission
NIH	National Institutes of Health
NRC	National Research Council
NT	nuclear transfer
OHRP	Office for Human Research Protections
OHSS	ovarian hyperstimulation syndrome
PCB	President's Council on Bioethics
PGD	preimplantation genetic diagnosis
PHI	personal health information
PHS	Public Health Service
P.L.	Public law
RAC	Recombinant DNA Advisory Committee
rDNA	recombinant DNA
REB	Research Ethics Board (Canada)
SCNT	somatic cell nuclear transfer
USC	United States Code

Appendix A

Compilation of Recommendations

RECOMMENDATIONS FROM CHAPTER 3

Recommendation 1:
To provide local oversight of all issues related to derivation and research use of hES cell lines and to facilitate education of investigators involved in hES cell research, all institutions conducting hES cell research should establish an Embryonic Stem Cell Research Oversight (ESCRO) committee. The committee should include representatives of the public and persons with expertise in developmental biology, stem cell research, molecular biology, assisted reproduction, and ethical and legal issues in hES cell research. The ESCRO committee would not substitute for an Institutional Review Board but rather would provide an additional level of review and scrutiny warranted by the complex issues raised by hES cell research. The committee would also serve to review basic hES cell research using preexisting anonymous cell lines that does not require consideration by an Institutional Review Board.

Recommendation 2:
Through its Embryonic Stem Cell Research Oversight (ESCRO) committee, each research institution should ensure that the provenance of hES cells is documented. Documentation should include evidence that the procurement process was approved by an Institutional Review Board to ensure adherence to the basic ethical and legal principles of informed consent and protection of confidentiality.

Recommendation 3:
Embryonic Stem Cell Research Oversight (ESCRO) committees or their equivalents should divide research proposals into three categories in setting limits on research and determining the requisite level of oversight:

(a) Research that is permissible after notification of the research institution's ESCRO committee and completion of the reviews mandated by current requirements. Purely *in vitro* hES cell research with pre-existing coded or anonymous hES cell lines in general is permissible provided that notice of the research, documentation of the provenance of the cell lines, and evidence of compliance with any required Institutional Review Board, Institutional Animal Care and Use Committee, Institutional Biosafety Committee, or other mandated reviews is provided to the ESCRO committee or other body designated by the investigator's institution.

(b) Research that is permissible only after additional review and approval by an ESCRO committee or other equivalent body designated by the investigator's institution.

(i) The ESCRO committee should evaluate all requests for permission to attempt derivation of new hES cell lines from donated blastocysts, from *in vitro* fertilized oocytes, or by nuclear transfer. The scientific rationale for the need to generate new hES cell lines, by whatever means, should be clearly presented, and the basis for the numbers of blastocysts or oocytes needed should be justified. Such requests should be accompanied by evidence of Institutional Review Board approval of the procurement process.

(ii) All research involving the introduction of hES cells into nonhuman animals at any stage of embryonic, fetal, or postnatal development should be reviewed by the ESCRO committee. Particular attention should be paid to the probable pattern and effects of differentiation and integration of the human cells into the nonhuman animal tissues.

(iii) Research in which personally identifiable information about the donors of the blastocysts, gametes, or somatic cells from which the hES cells were derived is readily ascertainable by the investigator also requires ESCRO committee review and approval.

(c) Research that should not be permitted at this time:

(i) Research involving *in vitro* culture of any intact human embryo, regardless of derivation method, for longer than 14 days or until formation of the primitive streak begins, whichever occurs first.

(ii) Research in which hES cells are introduced into nonhuman primate blastocysts or in which any ES cells are introduced into human blastocysts.

In addition:

(iii) No animal into which hES cells have been introduced at any stage of development should be allowed to breed.

***Recommendation 4*:**
Through its Embryonic Stem Cell Research Oversight (ESCRO) committee, each research institution should establish and maintain a registry of investigators conducting hES cell research and record descriptive information about the types of research being performed and the hES cells in use.

***Recommendation 5*:**
If a U.S.-based investigator collaborates with an investigator in another country, the Embryonic Stem Cell Research Oversight (ESCRO) committee may determine that the procedures prescribed by the foreign institution afford protections equivalent with these guidelines and may approve the substitution of some or all of the foreign procedures for its own.

***Recommendation 6*:**
A national body should be established to assess periodically the adequacy of the guidelines proposed in this document and to provide a forum for a continuing discussion of issues involved in hES cell research.

***Recommendation 7*:**
The hES cell research community should ensure that there is sufficient genetic diversity among cell lines to allow for potential translation into health-care services for all groups in our society.

RECOMMENDATIONS FROM CHAPTER 4

***Recommendation 8*:**
Regardless of the source of funding and the applicability of federal regulations, an Institutional Review Board or its equivalent should review the procurement of gametes, blastocysts, or somatic cells for the purpose of generating new hES cell lines, including the procurement of blastocysts in excess of clinical need from *in vitro* fertilization clinics, blastocysts made through *in vitro* fertilization specifically for research purposes, and oocytes, sperm, and somatic cells donated for development of hES cell lines derived through nuclear transfer.

***Recommendation 9*:**
Institutional Review Boards may not waive the requirement for obtaining informed consent from any person whose somatic cells, gametes, or blastocysts are used in hES research.

Recommendation 10:
Investigators, institutions, Institutional Review Boards, and privacy boards should ensure that authorizations are received from donors, as appropriate and required by federal human subjects protections and the Health Insurance Portability and Accountability Act for the confidential transmission of personal health information to repositories or to investigators who are using hES cell lines derived from donated materials.

Recommendation 11:
Investigators and institutions involved in hES cell research should conduct the research in accordance with all applicable laws and guidelines pertaining to recombinant DNA research and animal care. Institutions should consider adopting Good Laboratory Practice standards for some or all of their basic hES cell research.

Recommendation 12:
hES cell research leading to potential clinical application must be in compliance with all applicable Food and Drug Administration (FDA) regulations. If FDA requires that a link to the donor source be maintained, investigators and institutions must ensure that the confidentiality of the donor is protected, that the donor understands that a link will be maintained, and that, where applicable, federal human subjects protections and Health Insurance Portability and Accountability Act or other privacy protections are followed.

RECOMMENDATIONS FROM CHAPTER 5

Recommendation 13:
When donor gametes have been used in the *in vitro* fertilization process, resulting blastocysts may not be used for research without consent of all gamete donors.

Recommendation 14:
To facilitate autonomous choice, decisions related to the production of embryos for infertility treatment should be free of the influence of investigators who propose to derive or use hES cells in research. Whenever it is practicable, the attending physician responsible for the infertility treatment and the investigator deriving or proposing to use hES cells should not be the same person.

Recommendation 15:
No cash or in kind payments may be provided for donating blastocysts in excess of clinical need for research purposes.

Recommendation 16:
Women who undergo hormonal induction to generate oocytes specifically for research purposes (such as for nuclear transfer) should be reimbursed only for direct expenses incurred as a result of the procedure, as determined by an Institutional Review Board. No cash or in kind payments should be provided for donating oocytes for research purposes. Similarly, no payments should be made for donations of sperm for research purposes or of somatic cells for use in nuclear transfer.

Recommendation 17:
Consent for blastocyst donation should be obtained from each donor at the time of donation. Even people who have given prior indication of their intent to donate to research any blastocysts that remain after clinical care should nonetheless give informed consent at the time of donation. Donors should be informed that they retain the right to withdraw consent until the blastocysts are actually used in cell line derivation.

Recommendation 18:
In the context of donation of gametes or blastocysts for hES cell research, the informed consent process, should, at a minimum, provide the following information:

a. **A statement that the blastocysts or gametes will be used to derive hES cells for research that may include research on human transplantation.**
b. **A statement that the donation is made without any restriction or direction regarding who may be the recipient of transplants of the cells derived, except in the case of autologous donation.**
c. **A statement as to whether the identities of the donors will be readily ascertainable to those who derive or work with the resulting hES cell lines.**
d. **If the identities of the donors are retained (even if coded), a statement as to whether donors wish to be contacted in the future to receive information obtained through studies of the cell lines.**
e. **An assurance that participants in research projects will follow applicable and appropriate best practices for donation, procurement, culture, and storage of cells and tissues to ensure, in particular, the traceability of stem cells. (Traceable information, however, must be secured to ensure confidentiality.)**
f. **A statement that derived hES cells and/or cell lines might be kept for many years.**
g. **A statement that the hES cells and/or cell lines might be used in research involving genetic manipulation of the cells or the mixing of human and nonhuman cells in animal models.**

h. Disclosure of the possibility that the results of study of the hES cells may have commercial potential and a statement that the donor will not receive financial or any other benefits from any future commercial development;
i. A statement that the research is not intended to provide direct medical benefit to the donor(s) except in the case of autologous donation.
j. A statement that embryos will be destroyed in the process of deriving hES cells.
k. A statement that neither consenting nor refusing to donate embryos for research will affect the quality of any future care provided to potential donors.
l. A statement of the risks involved to the donor.

Recommendation 19:
Consenting or refusing to donate gametes or embryos for research should not affect or alter in any way the quality of care provided to prospective donors. That is, clinical staff must provide appropriate care to patients without prejudice regarding their decisions about disposition of their embryos.

Recommendation 20:
Clinical personnel who have a conscientious objection to hES cell research should not be required to participate in providing donor information or securing donor consent for research use of gametes or blastocysts. That privilege should not extend to the care of a donor or recipient.

Recommendation 21:
Researchers may not ask members of the infertility treatment team to generate more oocytes than necessary for the optimal chance of reproductive success. An infertility clinic or other third party responsible for obtaining consent or collecting materials should not be able to pay for or be paid for the material obtained (except for specifically defined cost-based reimbursements and payments for professional services).

Recommendation 22:
Institutions that are banking or plan to bank hES cell lines should establish uniform guidelines to ensure that donors of material give informed consent through a process approved by an Institutional Review Board, and that meticulous records are maintained about all aspects of cell culture. Uniform tracking systems and common guidelines for distribution of cells should be established.

Recommendation 23:
Any facility engaged in obtaining and storing hES cell lines should consider the following standards:

(a) Creation of a committee for policy and oversight purposes and creation of clear and standardized protocols for banking and withdrawals.
(b) Documentation requirements for investigators and sites that deposit cell lines, including

(i) A copy of the donor consent form.
(ii) Proof of Institutional Review Board approval of the procurement process.
(iii) Available medical information on the donors, including results of infectious-disease screening.
(iv) Available clinical, observational, or diagnostic information about the donor(s).
(v) Critical information about culture conditions (such as media, cell passage, and safety information).
(vi) Available cell line characterization (such as karyotype and genetic markers).

A repository has the right of refusal if prior culture conditions or other items do not meet its standards.

(c) A secure system for protecting the privacy of donors when materials retain codes or identifiable information, including but not limited to

(i) A schema for maintaining confidentiality (such as a coding system).
(ii) A system for a secure audit trail from primary cell lines to those submitted to the repository.
(iii) A policy governing whether and how to deliver clinically significant information back to donors.

(d) The following standard practices:

(i) Assignment of a unique identifier to each sample.
(ii) A process for characterizing cell lines.
(iii) A process for expanding, maintaining, and storing cell lines.
(iv) A system for quality assurance and control.
(v) A website that contains scientific descriptions and data related to the cell lines available.
(vi) A procedure for reviewing applications for cell lines.
(vii) A process for tracking disbursed cell lines and recording their status when shipped (such as number of passages).
(viii) A system for auditing compliance.
(ix) A schedule of charges.

(x) A statement of intellectual property policies.
(xi) When appropriate, creation of a clear Material Transfer Agreement or user agreement.
(xii) A liability statement.
(xiii) A system for disposal of material.

(e) Clear criteria for distribution of cell lines, including but not limited to evidence of approval of the research by an Embryonic Stem Cell Research Oversight committee or equivalent body at the recipient institution.

Appendix B

Committee Biographies

Richard O. Hynes, PhD, (Co-Chair), (NAS, IOM) is the Daniel K. Ludwig Professor of Cancer Research at the MIT Center for Cancer Research and Department of Biology, and a Howard Hughes Medical Institute investigator. He was formerly head of the Biology Department and then director of the Center for Cancer Research. His research focuses on fibronectins and integrins and the molecular basis of cellular adhesion, both in normal development and in pathological situations, such as cancer, thrombosis, and inflammation. Dr. Hynes's current interests are cancer invasion and metastasis, angiogenesis, and animal models of human disease states. In 1997, he received the Gairdner International Foundation Award. In 2000, he served as president of the American Society for Cell Biology and testified before Congress about the need for federal support and oversight of embryonic stem cell research.

Jonathan D. Moreno, PhD, (Co-Chair), is the Emily Davie and Joseph S. Kornfeld Professor of Biomedical Ethics and director of the Center for Biomedical Ethics at the University of Virginia. He is a past president of the American Society for Bioethics and Humanities and is a member of the Council on Accreditation of the Association of Human Research Protection Programs. Dr. Moreno is also a member of the Board on Health Sciences Policy of the Institute of Medicine. Among Dr. Moreno's books are *In the Wake of Terror: Medicine and Morality in a Time of Crisis*, and *Undue Risk: Secret State Experiments on Humans*. Dr. Moreno also serves as a commentator and columnist for ABCNews.com and is a frequent guest on various news programs, including NBC Nightly News with Tom Brokaw. Dr. Moreno was a senior consultant for the National Bioethics Advisory Commission

and a senior staff member of the Advisory Committee on Human Radiation Experiments during the Clinton administration.

Elizabeth Price Foley, JD, LLM, is a professor of law at Florida International University (FIU) College of Law. Before joining the FIU College of Law in 2002 as one of its founding faculty, she was a professor of law at Michigan State University (MSU) College of Law and an Adjunct Professor in the Center for Ethics and Humanities of the MSU College of Human Medicine. Dr. Foley's scholarship focuses on bioethics and the intersection of health care law and constitutional law, and her articles have been cited in more than 100 law journals. She is a frequent commentator on health law and bioethics issues for national and international media such as CNN, Fox News, the Washington Post, and the Wall Street Journal. Before teaching law, Dr. Foley served as a judicial clerk on the U.S. Court of Appeals for the Fifth Circuit. She also spent a number of years on Capitol Hill, serving as senior legislative aide to Representative Ron Wyden (D-OR), legislative aide for the District of Columbia office of the Health Insurance Plan of Greater New York, and legislative aide for Representative Michael Andrews (D-TX). Dr. Foley received her BA from Emory University, her JD from the University of Tennessee College of Law and her LLM from Harvard Law School.

Norman Fost, MD, MPH, is a professor of pediatrics and director of the Program in Bioethics, which he founded in 1973. He is chair of the Health Sciences Institutional Review Board, chair of the University of Wisconsin Hospital Ethics Committee, chair of the university's Bioethics Advisory Committee, and director of the Child Protection Team. He was a member of Hillary Clinton's Health Care Task Force and numerous other federal and state committees. He received his AB from Princeton, his MD from Yale, and his MPH from Harvard. He has been awarded the Nellie Westerman Prize in Research Ethics, and the William Bartholome Award for Excellence in Ethics from the American Academy of Pediatrics. His research interests include regulation of human subjects research, ethical and policy issues in access to human growth hormone, and the use of interactive computers in genetic counseling.

H. Robert Horvitz, PhD, (NAS, IOM) is the David H. Koch Professor of Biology in the Department of Biology at MIT and a Howard Hughes Medical Institute investigator. He is also a member of the McGovern Institute for Brain Research at MIT and a member of the MIT Center for Cancer Research. Dr. Horvitz's research interests include molecular and cellular biology, developmental and behavioral genetics, apoptosis, human neurological disease, neural development, morphogenesis, cell lineage, cell fate, micro-RNAs, signal transduction, transcriptional repression, and chromatin remodeling. Dr. Horvitz has served as a member of the Advisory Council of the National Human Genome Research Institute of the National Institutes of Health and was co-chair of the Working Group on Preclinical Models for

Cancer of the National Cancer Institute. He was President of the Genetics Society of America in 1995. Dr. Horvitz received the Charles A. Dana Award for Pioneering Achievements in Health (1995), the General Motors Cancer Research Foundation Alfred P. Sloan, Jr. Prize (1998), the Gairdner Foundation International Award (1999), and the Bristol-Myers Squibb Award for Distinguished Achievement in Neuroscience (2001). In 2002, he received the Nobel Prize in Physiology or Medicine for his studies of the genetic regulation of organ development and programmed cell death.

Marcia Imbrescia is the current owner of Peartree Design, a landscape firm, and was previously the media director for Drumbeater, a high technology advertising agency. She holds BA degrees in marketing and journalism, and a graduate certificate in landscape design. Ms. Imbrescia has a passion for health advocacy and helping people with illness and disability. She is a member of the Board of Trustees of the Arthritis Foundation (AF), for which she has participated as a volunteer at the chapter and national levels. She served as member (1996-1998, 2001) and chairperson (2002-2003) of AF's American Juvenile Arthritis Organization. In 1992, she received the Volunteer of the Year Award from the Massachusetts Chapter of AF. Her volunteer efforts include program development, conference planning, public speaking, fundraising, and advocacy.

Terry Magnuson, PhD, is Sarah Graham Kenan Professor and chair of the Department of Genetics at the University of North Carolina. He also directs the Carolina Center for Genome Sciences, and is the program director of cancer genetics at the Lineberger Comprehensive Cancer Center. Dr. Magnuson's research interests include mammalian genetics, genomics, and development. His laboratory has developed a high-throughput system to study the effects of mutations on mouse development with mouse embryonic stem cells. He is particularly interested in the role of murine polycomb-group genes on the processes of autosomal imprinting, X-inactivation, and anterior-posterior patterning of axial structures in mammals. He is a member of the Board of Directors of the Genetics Society of America and of the Society for Developmental Biology.

Cheryl Mwaria, PhD, is professor of anthropology and director of African studies at Hofstra University. Her fieldwork as a medical anthropologist in Kenya, Botswana, Namibia, the Caribbean, and the United States has focused on women's health, race relations, and differential access to health care. She has served on the Executive Boards of the American Ethnological Society, the Society for the Study of Anthropology of North America, and the Association of Feminist Anthropology. She is currently director of the Africa Network, a nonprofit consortium of liberal arts colleges committed to literacy about and concern for Africa in American higher education. Dr. Mwaria is a member of the Center for Urban Bioethics at the New York Academy of Medicine and has served as a consultant in community values in

end-of-life care for North General Hospital in New York City and the New York Academy of Medicine Center for Urban Bioethics. Her most recent fieldwork (2002-2003) was conducted at a major cancer research center and focused on minority group access to cancer-related clinical trials. Her publications pertaining to biomedical ethics include "Biomedical Ethics, Gender and Ethnicity: Implications for Black Feminist Anthropology" in *Black Feminist Anthropology: Theory, Praxis, Politics and Poetics* (Irma McClaurin, ed., 2001).

Janet Rossant, PhD, is the co-head of the Fetal Health and Development Program at Mount Sinai Hospital, professor at the University of Toronto, and director of the Center for Modelling Human Disease. Dr. Rossant studies lineage determination in the developing embryo. She has received numerous prizes for her work in establishing the fates of early developing cells in the mouse embryo, including the McLaughlin Medal from the Royal Society of Canada, the Canadian Institute of Health Research (CIHR) Distinguished Scientist Award, and the Robert L. Noble Prize from the National Cancer Institute of Canada. She is a member of the Board of Directors of the International Society for Stem Cell Research and participated in the development of the CIHR guidelines for embryonic stem cell research, which do not permit the use of somatic cell nuclear transfer to create stem cells.

Janet D. Rowley, MD, (NAS, IOM) is the Blum-Riese Distinguished Service Professor in the Departments of Medicine, Molecular Genetics and Cell Biology, and Human Genetics at the University of Chicago. She has contributed significantly to advances in understanding of genetic changes in cancer. She focused on chromosomal abnormalities in human leukemia and lymphoma and in 1972, using new techniques of chromosome identification, discovered the first consistent chromosomal translocation in human cancer. She has identified more than a dozen recurring translocations. Her laboratory is analyzing the gene expression pattern of recurring translocations to identify unique markers of leukemias for diagnosis and potentially as therapeutic targets. With Felix Mitelman, she cofounded and is coeditor of *Genes, Chromosomes and Cancer*, the premier cancer cytogenetics journal. She is a member of the President's Council on Bioethics.

Liaison from the Board on Life Sciences

R. Alta Charo, JD, is the Elizabeth S. Wilson-Bascom Professor of Law and Bioethics at the University of Wisconsin Law and Medical Schools, and associate dean for research and faculty development at the University of Wisconsin Law School at Madison. She is the author of over 75 articles, book chapters, and government reports on such topics as voting rights, environmental law, reproductive rights, medical genetics law, reproductive technology policy, and science policy. She serves on the expert advisory boards of several organizations with an interest

in stem cell research, including the Juvenile Diabetes Research Foundation, WiCell, and the Wisconsin Stem Cell Research Program. She is also a consultant to the California Institute for Regenerative Medicine. In 1994, Dr. Charo served on the National Institutes of Health Human Embryo Research Panel. From 1996 to 2001, she was a member of the National Bioethics Advisory Commission and participated in the writing of its reports on research ethics and cloning. Since 2001, she has been a member of the National Academies Board on Life Sciences.

Appendix C

Workshop Agenda and Speaker Biographies

Board on Life Sciences
The National Academies
and
Board on Health Sciences Policy
Institute of Medicine

Guidelines for Human Embryonic Stem Cell Research

Public Workshop

Agenda, Tuesday, October 12, 2004
Main Auditorium
National Academy of Sciences
2101 Constitution Ave., NW Washington, D.C.

8:30 a.m. **Welcome**: Bruce Alberts, President, National Academy of Sciences
Harvey Fineberg, President, Institute of Medicine

8:45 a.m. **Introduction and Mandate of the Committee on Guidelines for Human Embryonic Stem Cell Research**:

Richard Hynes, Massachusetts Institute of Technology and Co-Chair, Committee on Guidelines for Human Embryonic Stem Cell Research

9:00 a.m. **Overview of the Human Embryonic Stem Cell Science and Policy Issues**

Moderator: Richard Hynes

- Stem Cell Science—Where Have We Come From, Where Are We Going?
  Martin Raff, University College London
- Overview of Policies and Rules—An International Perspective
  LeRoy Walters, Georgetown University
- Discussant: Anne McLaren, Centre for Medical Genetics and Policy, University of Cambridge

9:50 a.m. **Q & A**

10:15 a.m. Break

10:30 a.m. **Derivation and Use of Human Embryonic Stem Cells—General Issues**
Moderator: Janet Rossant, Mount Sinai Hospital, Toronto

Panel: George Daley, Harvard Medical School
Fred (Rusty) Gage, the Salk Institute

Discussants: James Battey, National Institutes of Health
Leonard Zon, Harvard Medical School

11:30 p.m. **Q & A**

12:00 p.m. Lunch

1:00 p.m. **Stem Cells and Somatic Cell Nuclear Transfer**

Moderator: H. Robert Horvitz, MIT and Howard Hughes Medical Institute

Panel: Rudolf Jaenisch, Whitehead Institute
Davor Solter, Max Planck Institute of Immunobiology

Discussant: Kevin Eggan, Harvard University

1:50 p.m. **Q&A**

2:20 p.m. Break

2:35 p.m. **Interspecies Mixing and Chimeras**

Moderator: Terry Magnusson, University of North Carolina

Panel: Irving Weissman, Stanford University School of Medicine
David Garbers, University of Texas Southwestern Medical Center at Dallas

Discussant: Brigid Hogan, Duke University

3:25 p.m. **Q&A**

3:55 p.m. **Current Legal and Regulatory Requirements That May Affect Human Embryonic Stem Cell Research**

Panel: Alta Charo, University of Wisconsin School of Law
Michael Malinowski, Louisiana State University School of Law

4:35 p.m. **Q&A**

5:00 p.m. Public Comment

5:30 p.m. **Adjourn**

Agenda, Wednesday, October 13, 2004
Lecture Room
National Academy of Sciences
2101 Constitution Ave., NW Washington, D.C.

8:30 a.m. **Opening Remarks**: Jonathan Moreno, University of Virginia, and Co-Chair, Committee on Guidelines for Human Embryonic Stem Cell Research

8:45 a.m. **Informed Consent and Procurement**

Moderator: Jonathan Moreno

Presentation: Ruth Faden, Phoebe R. Berman Bioethics Institute, Johns Hopkins University

Discussants: Alison Murdoch, Department of Reproductive Medicine, International Centre for Life
Catherine Racowsky, Brigham and Women's Hospital, Division of Reproductive Medicine

9:25 a.m. **Q&A**

9:40 a.m. **Derivation of Stem Cell Lines—Ethics and Policy Concerns**

Moderator: Janet Rowley, University of Chicago

- Panel on SCNT for human embryonic stem cell research
  Dan Brock, Harvard Medical School
  Leon Kass, President's Council on Bioethics

- Panel on species mixing/chimeras for human embryonic stem cell research
  Henry Greely, Stanford Law School
  Cynthia Cohen, Georgetown University
  William Hurlbut, Stanford University (20 minutes)

11:20 a.m. **Q&A**

11:45 a.m. **Patenting, Licensing, and Material Transfer Agreements in Relation to Human Embryonic Stem Cell Research**

Moderator: Elizabeth Price Foley, Florida International University College of Law

Presentation: Carl Gulbrandsen, Wisconsin Alumni Research Foundation

12:15 p.m. **Q&A**

12:30 p.m. Lunch

1:05 p.m. **Mechanisms for Oversight of Human Embryonic Stem Cell Research**

Moderator: Norman Fost, University of Wisconsin

Panel: Laurie Zoloth, Center for Genetic Medicine, Northwestern University
Franco Furger, Executive Director, Human Biotechnology Governance Forum, Johns Hopkins University

1:40 p.m. **Q&A**

1:55 p.m. **Industry Perspective: What Is Industry's Role in Monitoring the Ethics of Human Embryonic Stem Cell Research?**

Moderator: Marcia Imbrescia, Arthritis Foundation Board of Trustees

Presentation: Michael Werner, Chief of Policy, Biotechnology Industry Organization

2:15 p.m. **Q&A**

2:30 p.m. **Serving the Public Interest: Conducting Human Embryonic Stem Cell Research in a Democratic Society**

Moderator: Cheryl Mwaria, Hofstra University

- Panel: Dan Hausman, University of Wisconsin
  Robert Goldstein, Juvenile Diabetes Research Foundation
  Bruce Jennings, The Hastings Institute

3:30 p.m. **Q&A**

3:45 p.m. Public Comment

4:15 p.m. **Summary and Concluding Remarks**:
Jonathan Moreno and Richard Hynes

4:30 p.m. Adjourn

SPEAKER BIOGRAPHIES

James F. Battey, Jr., MD, PhD, received his BS in physics from the California Institute of Technology in 1974 and his MD and PhD in biophysics from Stanford University School of Medicine in 1980. After receiving training in pediatrics, Dr. Battey pursued a postdoctoral fellowship in genetics at Harvard Medical School under the mentorship of Philip Leder. Since completing his postdoctoral fellowship in 1983, he has held a variety of positions at the National Institutes of Health, serving in the National Cancer Institute, the National Institute of Neurological Disorders and Stroke, and the National Institute on Deafness and Other Communication Disorders, of which he is currently the director. Until recently he also served as the chair of the NIH Stem Cell Task Force.

Dan W. Brock, PhD, is a former senior scientist and member of the Department of Clinical Bioethics at the National Institutes of Health and former professor of philosophy and biomedical ethics at Brown University, where he was also the Charles C. Tillinghast, Jr. University Professor, professor of philosophy and biomedical ethics, and director of the Center for Biomedical Ethics through June 2002. He is professor of medical ethics in the Department of Social Science at Harvard Medical School. Dr. Brock works on such subjects as genes and justice, health care resource prioritization and rationing, and end of life care and euthanasia. He has published numerous papers in bioethics and in moral and political philosophy. His most recent works include "Priority to the Worst Off in Health Care Resource Prioritization" and "Broadening the Bioethics Agenda." He is also the author of *Deciding For Others: The Ethics of Surrogate Decision Making* (with Allen E. Buchanan, 1989); *Life and Death: Philosophical Essays in Biomedical Ethics* (1993); and *From Chance to Choice: Genetics and Justice* (with Allen Buchanan, Norman Daniels, and Daniel Wikler, 2000).

R. Alta Charo, JD, is the Elizabeth S. Wilson-Bascom Professor of Law and Bioethics at the University of Wisconsin Law and Medical Schools, and Associate Dean for Research and Faculty Development at the University of Wisconsin Law School at Madison. Professor Charo is the author of over 75 articles, book chapters, and government reports on topics including voting rights, environmental law, reproductive rights, medical genetics law, reproductive technology policy, and science policy. She serves on the expert advisory boards of several organizations with an interest in stem cell research, including the Juvenile Diabetes Research Foundation, WiCell, and the Wisconsin Stem Cell Research Program. She is also a consultant to the California Institute for Regenerative Medicine. In 1994, Professor Charo served on the NIH Human Embryo Research Panel, and from 1996-2001 she was a member of the presidential National Bioethics Advisory Commission, where she participated

in writing its reports on research ethics and cloning. Since 2001 she has been a member of the National Academies' Board on Life Sciences.

Cynthia Cohen, PhD, JD, is a faculty affiliate of the Kennedy Institute of Ethics at Georgetown University in Washington, D.C., and a fellow at the Hastings Center in Garrison, New York. She is the former executive director of the National Advisory Board on Ethics in Reproduction in Washington, DC, associate for ethical studies at the Hastings Center, associate to the legal counsel of the University of Michigan Hospitals, and chair of the Philosophy Department at the University of Denver. She is a member of the Canadian Stem Cell Oversight Committee and has served as a consultant to such groups as the National Institutes of Health, the American Association for the Advancement of Science, and the Stem Cell Network. Dr. Cohen has written or edited eight books and some 150 articles on ethical issues, including stem cell research, genetic testing, reproductive and therapeutic cloning, the new reproductive technologies, organ transplantation, mandatory drug testing, and religion and public policy.

George Q. Daley, MD, PhD, is an associate professor of biological chemistry and molecular pharmacology at Harvard Medical School. He received a bachelor's degree (1982) from Harvard University, his PhD (1989) in biology from the Massachusetts Institute of Technology (MIT), and his MD (1991) from Harvard Medical School through the Harvard-MIT Division of Health Sciences and Technology. Dr. Daley's laboratory studies stem cell development and differentiation, emphasizing derivation of functional hematopoietic and germ cell elements from embryonic stem cells and the genetic mechanisms that predispose to malignancy. Dr. Daley is Board Certified in Internal Medicine and Hematology, and is a staff physician in Hematology/Oncology at the Children's Hospital, the Dana Farber Cancer Institute, and the Brigham and Women's Hospital in Boston. He has been elected to the American Society for Clinical Investigation and has received research awards from Harvard Medical School, the National Institutes of Health, the New England Cancer Society, the Burroughs Wellcome Fund, the Edward Mallinckrodt, Jr. Foundation, and the Leukemia and Lymphoma Society of America. Dr. Daley was recently named a recipient of the NIH Director's Pioneer Award, an unrestricted grant to pursue highly innovative avenues of research.

Kevin Eggan, PhD, is a junior fellow in the Harvard Society of Fellows at Harvard University, having recently completed postdoctoral studies in the laboratory of Rudy Jaenisch at the Whitehead Institute for Biomedical Research. At Harvard, Dr. Eggan is establishing an independent research group to study the molecular and genetic control of mouse preimplantation development, investigate epigenetic reprogramming after somatic cell nuclear transfer, and derive disease-specific human embryonic stem cell lines from diabetic and Parkinson's disease patients by nuclear transfer. Dr. Eggan has been invited to present his work at numerous symposia and

workshops. He received a BS degree from the University of Illinois and a PhD from the Massachusetts Institute of Technology.

Ruth Faden, MPH, PhD, (IOM) is the Philip Franklin Wagley Professor of Biomedical Ethics and executive director of the Phoebe R. Berman Bioethics Institute at Johns Hopkins University. She is also a senior research scholar at the Kennedy Institute of Ethics, Georgetown University. Dr. Faden is the author and editor of numerous books and articles on biomedical ethics and health policy, including *A History and Theory of Informed Consent* (with Tom L. Beauchamp), *AIDS, Women and the Next Generation* (Ruth Faden, Gail Geller, and Madison Powers, eds.), and *HIV, AIDS and Childbearing: Public Policy, Private Lives* (Ruth Faden and Nancy Kass, eds.). She is a fellow of the Hastings Center and the American Psychological Association. She has served on several national advisory committees and commissions including the President's Advisory Committee on Human Radiation Experiments, which she chaired. Dr. Faden holds a BA from the University of Pennsylvania, an MA in general studies in humanities from the University of Chicago, and an MPH and PhD (Program in Attitudes and Behavior) from the University of California, Berkeley.

Franco Furger, PhD, is the executive director of the Human Biotechnology Governance Forum at the Foreign Policy Institute of the Paul H. Nitze School of Advanced International Studies at Johns Hopkins University. The 2-year project is exploring options for controlling research in and applications of "reprogenetics," research activities that focus on the beginning of life and procedures aimed at preventing the inheritance of genetic diseases. Such research activities include research cloning, stem cell research, and preimplantation genetic diagnosis. Before joining Johns Hopkins, he was a member of the faculty of George Mason University's School of Public Policy. Dr. Furger received an MS in electrical engineering in 1982 and a PhD in environmental sciences in 1992 from the Federal Institute of Technology in Zurich.

Fred H. Gage, PhD, (NAS) is a professor in the Laboratory of Genetics at the Salk Institute in La Jolla, California, and a professor of neuroscience at the University of California, San Diego. Dr. Gage received his undergraduate degree from the University of Florida and a PhD from Johns Hopkins University and is known for his discovery of structural and functional plasticity in the adult mammalian brain. His research focuses on the development of strategies to induce recovery of function after central nervous system damage and on the unexpected plasticity and adaptability that remain throughout the life of all mammals. His work may lead to methods of replacing brain tissue lost to stroke or Alzheimer's disease and repairing spinal cords damaged by trauma. Dr. Gage's laboratory showed that, contrary to years of dogma, human beings are capable of growing new nerve cells throughout life. Dr. Gage is a past president of the Society for Neuroscience. Among the awards

he has received are the Charles A. Dana Award for Pioneering Achievements in Health and Education (1993), the Christopher Reeve Research Medal (1997), and the Max Planck Research Prize (1999).

David Garbers, PhD, is professor of pharmacology at the University of Texas Southwestern Medical Center in Dallas, Texas, and director of the Cecil H. and Ida Green Center for Reproductive Biology Sciences. He is also a Howard Hughes Medical Institute investigator. His laboratory explores how cells communicate with each other, particularly the mechanisms by which mammalian sperm detect signals from the egg. His research includes the development of technology to produce germ cells in vitro and to understand the mechanisms by which the mammalian egg is capable of reprogramming a somatic cell nucleus. He is a member of the American Academy of Arts and Sciences and has served on the editorial boards of various scientific journals, including the *Journal of Biological Chemistry*, *Biology of Reproduction*, and *Biochemical Journal and Endocrine Reviews*. Dr. Garbers received his bachelor's, master's, and PhD degrees in science from the University of Wisconsin. In 2001, he received the Endocrine Society's Edwin B. Astwood award.

Robert A Goldstein, MD, PhD, is the chief scientific officer of the Juvenile Diabetes Research Foundation International, where he is responsible for developing and guiding the research agenda. Before joining the foundation in 1997, he was director of the Division of Allergy, Immunology and Transplantation at the National Institute of Allergy and Infectious Diseases. He received his undergraduate degree from Brandeis University, his MD from Jefferson Medical College, his PhD in microbiology and immunology from George Washington University, and an MBA from the Stern School of Business, New York University. He recently testified before Congress on stem cell research.

Henry T. Greely, JD, is the Deane F. and Kate Edelman Johnson Professor of Law and a professor, by courtesy, of genetics at Stanford University. He specializes in legal and social issues arising from advances in the biological sciences and in health law and policy. He has written on genetic testing, human cloning, the ethics of human genetics research, legal issues in neuroscience, and policy issues in the health care financing system. He directs the Stanford Center for Law and the Biosciences, chairs the steering committee of the Stanford University Center for Biomedical Ethics, and co-directs the Stanford Program on Genomics, Ethics, and Society. Dr. Greely graduated from Stanford in 1974 and from Yale Law School in 1977. He joined the Stanford faculty in 1985.

Carl Gulbrandsen, PhD, JD, is the managing director of the Wisconsin Alumni Research Foundation (WARF) at the University of Wisconsin, Madison. He received his undergraduate degree from St. Olaf College in Northfield, Minnesota, a PhD in physiology from the University of Wisconsin, Madison, and a JD degree

from the University of Wisconsin Law School. In 1992, after 9 years of private practice law focusing on intellectual property rights, Dr. Gulbrandsen joined Madison, WI companies Lunar Corporation and Bone Care International, Inc. as general counsel. He joined WARF in October 1997 as director of patents and licensing and in 2000 he became the managing director. He is a member of the Association of University Technology Managers, the Licensing Executive Society, the American Intellectual Property Law Association, the Wisconsin State Bar, and the American Bar Association. He is also a director of the WiCell Research Institute, the Cornell Research Foundation, and the Wisconsin Biotechnology Association.

Dan Hausman, PhD, is Herbert A. Simon Professor in the Department of Philosophy of the University of Wisconsin. After graduating from Harvard in 1969, where he studied biochemistry and then English history and literature, he taught public school in New York City and received a Master of Arts in Teaching from New York University. He then received a BA in philosophy from Cambridge University and a PhD from Columbia University in 1978. His dissertation (later published as *Capital, Profits and Prices*) addressed questions in the philosophy of science raised by economics, and a large portion of his research has focused on economic methodology. Partly as a result of editing the journal *Economics and Philosophy* (in 1984-1994, jointly with Michael McPherson), he has worked on issues in ethics and economics and foundational questions concerning the nature of rationality. His interest in economic methodology has led to a long and continuing research interest concerning the nature of causation.

Brigid Hogan, PhD, (IOM) is the George Barth Geller Professor and chair of the Department of Cell Biology, Duke University Medical Center. Before joining Duke, Dr. Hogan was a Howard Hughes Medical Institute investigator and Hortense B. Ingram Professor in the Department of Cell Biology at Vanderbilt University Medical Center. Dr. Hogan earned her PhD in biochemistry at the University of Cambridge. She was then a postdoctoral fellow in the Department of Biology at MIT. Before moving to the United States in 1988, Dr. Hogan was head of the Molecular Embryology Laboratory at the National Institute for Medical Research in London. Her research focuses on the genetic control of embryonic development and morphogenesis, using the mouse as a model system. Her laboratory developed methods for deriving mouse pluripotent embryonic germ cell lines. She was co-chair for science of the 1994 National Institutes of Health Human Embryo Research Panel and a member of the National Academies Panel on Scientific and Medical Aspects of Human Cloning. In the past few years, Dr. Hogan has been elected to the Royal Society of London, the American Academy of Arts and Sciences, and the Institute of Medicine.

William Hurlbut, MD, is a physician and consulting professor in the Program on Human Biology at Stanford University, where he has cotaught integrative courses

with Luca Cavelli-Sforza on human genetic diversity and with Nobelist Baruch Blumberg on epidemics, evolution, and ethics. Dr. Hurlbut's main interests involve ethical issues associated with advancing biotechnology and neuroscience and the integration of philosophy of biology with Christian theology. His recent work has focused on the evolutionary origins of religious, spiritual, and moral awareness. In 2002, Dr. Hurlbut was appointed to the President's Council on Bioethics. He is a member of the Chemical and Biological Warfare working group of Stanford's Center for Security and International Cooperation. Dr. Hurlbut received his MD from Stanford and later conducted theological studies at Stanford and the Institute Catholique, Paris. His recent writings include *From Biology to Biography: The Science of the Human Person,* a chapter in Blankenhorn, D., Benson, I.T. and O'Hara, M. (eds.) *Who are We?: Essays on the Nature of the Human Person* (in press, 2004).

Rudolf Jaenisch, MD, (NAS) is a founding member of the Whitehead Institute and professor of biology at the Massachusetts Institute of Technology. Born in Germany, he received his MD from the University of Munich in 1967 and was a postdoctoral fellow first at the Max Planck Institute for Biochemistry, Munich, and then at Princeton University. After a period as a visiting fellow at the Institute for Cancer Research in Philadelphia, Dr. Jaenisch joined the Salk Institute in La Jolla, California, where he remained from 1972 to 1977, rising from assistant to associate research professor. In 1977 he returned to Germany, where until 1984 (when he joined the Whitehead Institute) he was head of the Department of Tumor Virology at the Heinrich Pette Institute for Experimental Virology and Immunology at the University of Hamburg. Dr. Jaenisch is a pioneer in transgenic science (making mouse models of human disease) whose methods have been used to explore the role of DNA modification, genomic imprinting, and X chromosome inactivation, which are important topics in the study of cancer, developmental processes, and neurological and connective tissue disorders. Dr. Jaenisch has made major contributions to the study of genomic reprogramming that occurs during nuclear cloning. In addition to receiving many awards for his work, he was elected to the U.S. National Academy of Sciences in 2003.

Bruce Jennings, MA, is senior research scholar at the Hastings Center. From 1991 through 1999, he served as the Center's executive vice president. He has directed several research projects on the care of the dying, health policy, chronic illness and long-term care, and ethical issues in human genetics. He served as associate director of a project that produced the widely cited and influential *Guidelines on the Termination of Life-Sustaining Treatment and the Care of the Dying*. With Mildred Z. Solomon of the Education Development Center in Newton, Massachusetts, he is cofounder of the Decisions Near the End of Life Program, a hospital-based educational program for physicians and other health professionals that has been used in over 200 hospitals in 30 states. Mr. Jennings has served as a consultant to several

government and private organizations, including the American Hospital Association, the Education Development Center, the Robert Wood Johnson Foundation, the New York Academy of Medicine, the Prudential Foundation, and Eli Lilly and Company. He serves on the boards of directors of such organizations as the National Hospice and Palliative Care Organization, American Health Decisions, the American Association of Bioethics (1994-1997), and the Association of Politics and the Life Sciences. Mr. Jennings also serves on bioethics advisory committees for the Alzheimer's Association, the Episcopal Church of the United States, and the National Hospice and Palliative Care Organization. In addition to his work with the Hastings Center, Mr. Jennings teaches at the Yale University School of Medicine in the Department of Epidemiology and Public Health.

Leon Kass, MD, PhD, is Hertog Fellow in Social Thought at the American Enterprise Institute and is the Addie Clark Harding Professor at the College and the Committee on Social Thought at the University of Chicago (on leave of absence). He earned his BS and MD degrees at the University of Chicago (1958 and1962) and his PhD in biochemistry at Harvard (1967). After conducting molecular biology research at the National Institutes of Health while serving in the U.S. Public Health Service, Dr. Kass turned to the ethical and philosophical issues raised by biomedical advances and, more recently, to broader moral and cultural issues. From 1970 to 1972, Dr. Kass served as executive secretary of the Committee on the Life Sciences and Social Policy of the National Research Council, whose report *Assessing Biomedical Technologies* provided one of the first overviews of the emerging moral and social questions posed by biomedical advance. He taught at St. John's College, Annapolis, MD, and served as Joseph P. Kennedy Sr. Research Professor in Bioethics at the Kennedy Institute of Ethics at Georgetown University before returning in 1976 to the University of Chicago. His widely reprinted essays on biomedical ethics have dealt with issues raised by *in vitro* fertilization, cloning, genetic screening and genetic technology, organ transplantation, aging research, euthanasia and assisted suicide, and the moral nature of the medical profession. In 2001, Dr. Kass was appointed by President Bush to chair the President's Council on Bioethics.

Michael Malinowski, JD, is the Ernest and Iris Eldred Professor of Law, and associate director of the Program in Law, Science, and Public Health at the Paul M. Hebert Law Center at Louisiana State University. He is cofounder of the Program in Law, Medicine, and BioScience and chair of the Health and Human Services Committee of the American Bar Association (ABA). He is a member of the ABA President's Special Committee on Bioethics, Phi Beta Kappa, and Oxford University's 21st Century Trust. In 1999-2000, Dr. Malinowski was a SmithKline Beecham Distinguished Fellow in Law and Genetics at the Center for the Study of Law, Science and Technology and a visiting professor of law at the Arizona State University College of Law. Previously, he was counsel to the law firm of Foley, Hoag & Eliot LLP in Boston, where his practice focused on biotechnology and health care.

He received a BA from Tufts University and a JD from Yale Law School. After law school, he clerked for a year for the Honorable Emilio M. Garza and a year for the Honorable Carolyn Dineen King, both federal appellate judges on the U.S. Court of Appeals for the Fifth Circuit. While clerking for Judge King, he was an adjunct professor of law in the Health Law Institute at the University of Houston Law Center. Dr. Malinowski has served as a member of the Special Committee on Genetic Information Policy of the Commonwealth of Massachusetts; the Grant Advisory Committee for the Ethical, Legal, and Social Issues Joint Working Group for the Human Genome Project; and the Biotechnology Industry Organization's Bioethics Committee and Working Group on Biomedical Information. He has published extensively on the commercialization of biotechnology and related health care issues, including a recent piece, "Choosing the Genetic Makeup of Children: Our Eugenics Past, Present, and Future?" (36 Connecticut L. Rev. 125-224, 2003), and lectured on these topics throughout the United States, Europe, and Canada.

Anne McLaren, DBE, PhD, FRS, is a principal research associate at the Wellcome Trust/Cancer Research UK Gurdon Institute at the University of Cambridge and a member of the European Molecular Biology Organization (EMBO). Before joining the Institute in 1992, she spent 19 years as director of the Medical Research Council's Mammalian Development Unit in London. For the previous 15 years, she worked for the Agriculture Research Council in C. H. Waddington's Institute of Animal Genetics in Edinburgh. Dr. McLaren's research interests include developmental biology, reproductive biology, and genetics, including molecular genetics. Her primary model is the laboratory mouse and she is working on the development of mouse primordial germ cells and the pluripotent stem cells derived from them. Dr. McLaren was a member of the UK government's Warnock Committee on Human Fertilisation and Embryology and until the end of 2001 was a member of the UK Human Fertilisation and Embryology Authority, which regulates *in vitro* fertilization and human embryo research in the UK. She chaired the Scientific and Technical Advisory Group of the World Health Organization's Human Reproduction Programme and was a member of the Nuffield Foundation's Bioethics Council. She is a member of the European Group on Ethics, which advises the European Commission on social and ethical implications of new technologies. Dr. McLaren, who completed her undergraduate and graduate work at Oxford University, was elected a fellow of the Royal Society in 1975 and she has served as the Society's Foreign Secretary and Vice-President. She is a founding member of Academia Europaea and of the recently established Academy of Medical Sciences. In 2002, she was awarded (jointly with A. K. Tarkowski) the Japan Prize for Developmental Biology.

Alison Murdoch, MD, FRCOG, is a consultant gynecologist and professor of reproductive medicine and the head of the Newcastle Fertility Centre for Life of the International Centre for Life at Newcastle University. Dr. Murdoch received her

BSc in medical science from Edinburgh University in 1972, followed by an MBChB (Bachelor of Medicine, Bachelor of Surgery) in 1975, an MD degree in 1987, and an FRCOG (Fellow of the Royal College of Obstetricians and Gynecologists) in 2001. Dr. Murdoch has been a speaker at such prestigious events as the International Conference on IVF in Chennai in 2001, the Stem Cell Research BFS/RCOG Ethics Meeting in 2002, and the Indian Medical Association Conference in Mangalore in September 2002. She was a guest lecturer at the medical staff rounds at Hammersmith Hospital in February 2003, a speaker at the British Council Symposium at the International Centre for Life in March 2003, the Updates in Infertility Conference in Florida in 2004, and she was the Keynote speaker at the British Congress of Obstetrics and Gynecology in Glasgow in 2004. In addition to her work at the Fertility Centre for Life, Dr. Murdoch is the chair of the British Fertility Society, an inspector for the Human Fertilisation and Embryology Authority, and a member of a panel that gave evidence to the House of Lords Select Committee on Stem Cell Research.

Catherine Racowsky, PhD, is the director of Assisted Reproductive Technologies (ART) Laboratory in the Department of Obstetrics, Gynecology and Reproductive Biology at the Center for Reproductive Medicine, Brigham and Women's Hospital. She is also an associate professor at Harvard University. Dr. Racowsky received her BA from the University of Oxford and her PhD from the University of Cambridge. Before joining Harvard and Brigham and Women's, her academic appointments included the University of Arizona Department of Animal Sciences, Department of Physiology, and Center of Toxicology. She served as the director of research in the Department of Obstetrics, Gynecology and Reproductive Biology in the College of Medicine at the University of Arizona and also director of the ART Laboratory. She is a full member of the Canadian Andrology and Fertility Society. From 1997 through 2001, she was a Member of the Reproductive Toxicology Editorial Board. She received the 2000 Partners Healthcare Excellence Award in Leadership and Innovation. Her research focuses on the effects of caffeine and smoking on human fertility. She has recently spoken at such diverse places as the Jones Institute in Norfolk, Virginia, on the topic "Embryo Selection: Can It Be Improved?" and the Taiwanese Society for Reproductive Medicine in Taipei, Taiwan, on the topics "Quality Management of the IVF Laboratory" and "Embryo Selection and Its Impact on How Many Embryos to Transfer."

Martin Raff, MD, (NAS) is a professor in the Department of Biology of the Medical Research Council MRC Laboratory for Molecular and Cell Biology at University College London. He received his BSc and MD from McGill University. He then pursued residencies in medicine at the Royal Victoria Hospital in Montreal and in neurology at Massachusetts General Hospital in Boston. Dr. Raff completed his postdoctoral training in immunology at the National Institute for Medical Research in London, after which he moved to University College London and has been a

professor of biology since 1979. He is a Fellow of the Royal Society and of Academia Europaea, a foreign member of the American Academy of Arts and Sciences, and past president of the British Society of Cell Biology. His research interests span immunology, cell biology, and developmental neurobiology. Using the retina and optic nerve as model systems, he discovered that animal cells live, grow, differentiate, or proliferate depending on a combination of cell-cell interactions and cell-intrinsic programs. Dr. Raff is a foreign associate of the U.S. National Academy of Sciences.

Davor Solter, MD, PhD, is the director and a member of the Max Planck Institute of Immunobiology. He is also a senior staff scientist at the Jackson Laboratory in Bar Harbor, Maine, and an adjunct professor at the Wistar Institute in Philadelphia. Dr. Solter received his MSc, MD, and PhD from the University of Zagreb. He serves as a member of numerous editorial and advisory boards and is the European editor of *Genes and Development*. He is a member of the American Academy of Arts and Sciences, the European Molecular Biology Organization, and Academia Europea. In 1998, he received the March of Dimes Prize in Developmental Biology for pioneering the concept of imprinting, and in 1999, he was distinguished as a J. W. Jenkinson Memorial Lecturer at Oxford University. Dr. Solter has contributed to many fields of mammalian developmental biology, including the differentiation of germ layers, the role of cell surface molecules in regulating early development, the biology and genetics of teratocarcinoma, the biology of embryonic stem cells, and imprinting and cloning. His current research focuses on genetic and molecular control of genome reprogramming and of activation of the embryonic genome.

LeRoy Walters, PhD, is the Joseph P. Kennedy, Sr. Professor of Christian Ethics at the Kennedy Institute of Ethics, Georgetown University, and a professor of philosophy at Georgetown. He is coauthor with Julie Gage Palmer of *The Ethics of Human Gene Therapy* (1997), coeditor with Tom L. Beauchamp of an anthology titled *Contemporary Issues in Bioethics* (6th ed., 2003) and coeditor with Tamar Joy Kahn and Doris M. Goldstein of the annual *Bibliography of Bioethics* (1975-present). From 1965 through 1967, he studied at the University of Heidelberg and the Free University of Berlin. In 1971, he received his PhD from Yale University. Since 1999, Dr. Walters has had an active interest in human embryonic stem cell research. He served as a consultant to the National Bioethics Advisory Committee in 1999 and discussed ethical issues in human embryonic stem cell research at a National Academy of Sciences workshop in June 2001. In August 2001, he was consulted by President Bush on public policies for stem cell research. His most recent article, published in the March 2004 issue of the *Kennedy Institute of Ethics Journal*, was "Human Embryonic Stem Cell Research: An Intercultural Perspective."

Irving L. Weissman, MD, PhD, (NAS, IOM) is the Karel and Avice Bekhuis Professor of Cancer Biology and professor of pathology and developmental biology at Stanford University. He is cofounder and director of StemCells, Inc., a company focused on adult stem cell biology. Dr. Weissman's research interests encompass developmental biology, self-renewal, homing, and functions of the cells that make up the blood-forming and immune systems. His main focus for the last several years has been the purification, biology, transplantation, and evolution of stem cells. The isolation of mouse hematopoietic stem cells (HSC) in his laboratory was followed by the isolation of human HSCs by Dr. Weissman and his colleagues at SyStemix, Inc., of which he was a founder. Purified human HSCs have been successfully used to provide cancer-free autologous stem cell transplants for patients receiving otherwise lethal chemotherapy and radiotherapy for cancer. His laboratory has gone on to identify the stages of development between stem cells and mature blood cells. Dr. Weissman is the recipient of several awards, including the Leukemia Society of America de Villier's International Achievement Award, the E. Donnall Thomas Prize from the American Society of Hematology, and the Montana Conservationist of the Year Award.

Michael J. Werner is chief of policy for the Biotechnology Industry Organization (BIO), overseeing all policy development, legislative, regulatory, bioethics, and legal department activities. Before becoming chief of policy, Mr. Werner was BIO's vice president of bioethics. In that capacity, he led BIO's efforts to develop policies, programs, and activities that promote responsible and ethical uses of biotechnology. His work has explored a variety of bioethics issues, including, confidentiality of medical information, use of genetic information, gene therapy, cloning, stem cell research, xenotransplantation, protection of human subjects in research, and global health. Mr. Werner has over 17 years of experience in health law and policy in Washington, DC. Before joining BIO, he spent 6 years as counsel for legislation and policy for the American College of Physicians-American Society of Internal Medicine, performing legal analysis, policy development, and congressional and regulatory advocacy on a variety of issues, including end of life care, Medicare reform, liability reform, and integration and delivery system re-structuring. Mr. Werner also served as a senior health adviser to US Senate Majority Leader George Mitchell and as senior adviser to Maryland Governor William Donald Schaefer.

Laurie Zoloth, PhD, is professor of medical ethics and humanities and of religion at the Feinberg School of Medicine of Northwestern University. Her research projects include work on emerging issues in medical and research genetics, ethical issues in stem cell research, and distributive justice in health care. Dr. Zoloth chairs the Howard Hughes Medical Institute's Bioethics Advisory Board and served as president of the American Society for Bioethics and Humanities in 2001. She is a member of numerous advisory boards including, the National Aeronautics and Space Administration National Advisory Council; the Executive Committee of the

International Society for Stem Cell Research; the American Association of the Advancement of Science's (AAAS) Dialogue on Science, Ethics and Religion; the Geron Ethics Advisory Board; the Data Safety Monitoring Board for the National Institutes of Health International AIDS Clinical Trials Group; the AAAS Working Group on Human Germ-Line Interventions and on Stem Cell Research; and the Ethics Section of the American Academy of Religion. In 1999, she was invited to give testimony to the National Bioethics Advisory Board on Jewish philosophy and stem cell research. In 2001, she was named principal investigator for the International Project on Judaism and Genetics, cosponsored by the AAAS and supported by the Haas Foundation and the Greenwall Foundation. Dr. Zoloth received a BA in Women's Studies and History from the University of California at Berkeley, a BSN from the State University of New York, an MA in English from San Francisco State University, and an MA in Jewish studies and PhD in social ethics from the Graduate Theological Union in Berkeley.

Leonard I. Zon, MD, is professor of pediatrics and a Howard Hughes Medical Institute investigator at Children's Hospital in Boston. He received a BS in chemistry and natural sciences from Muhlenberg College and an MD from Jefferson Medical College. He did an internal medicine residency at New England Deaconess Hospital and a fellowship in medical oncology at Dana-Farber Cancer Institute. His postdoctoral research was in the laboratory of Stuart Orkin. Dr. Zon's research focuses on the zebrafish, a new genetic and developmental model system for understanding blood formation. His laboratory has characterized over 26 mutant groups that can live with decreased blood or no blood at all. Several of them represent models of human disease. Dr. Zon is the president of the International Society for Stem Cell Research.

Index

A

Abnormalities, found in cloned animals, 34
Accountability issues, 51
Adult stem cells, 17, 115
Advanced Cell Technology, 15
Alzheimer's disease, 33
American Society for Reproductive Medicine (ASRM), 27
  Ethics Committee, 27, 52
American Type Culture Collection, 93
Androgenesis, 37, 100, 103, 115
  diploid androgenetic mES cells, 36
Animal care and use, 70–71
  handling chimeras with human-like characteristics, 50
Animal cells, mixing with, disclosure that cells and cell lines could be used in, 91, 102, 127
Animal feeder cells, 18
Animal Welfare Act, 71
ART. *See* Assisted reproductive technology
Asilomar Conference, 26–27, 70
ASRM. *See* American Society for Reproductive Medicine
Assisted human reproduction agency, in Canada, 27
Assisted reproductive technology (ART), 81, 91–92
  services offering, 10
Australia, 76–77
  national body established in, 59
  procurement practices in, 66
Authorizations, 68–69, 126
Autologous transplantation, 9, 34, 44, 84, 115
Autonomy, 9, 58, 85, 101

B

Banking and distribution of hES cell lines, 92–95, 103–105
  facilities for implementing specific recommended standards, 94–95, 104–105, 129–130
  institutions establishing uniform guidelines and record-keeping processes approved by an IRB, 94, 103–104, 128
  recommendations for, 12–13
Benefits
  of hES cell research, just distribution of, 60
  personal, informing donors there will be none, 9, 84
Best Practices for the Licensing of Genomic Inventions, 64
Bioethics Advisory Committee
  in Israel, 78
  in Singapore, 78
Biohazards, 59

Biological therapies, 3
Biomedical research, 21–22, 31
Blastocoel, 29–30, 115
Blastocysts, 1–2, 29–31, 34–36, 47–48, 66, 83, 115. *See also* Mouse blastocysts
  discarding, 10, 82
  donors of, 4, 7–9, 57, 100, 106
  excess, 42
  implanting, 30
  increasing the yield of, 36
  produced by donor gametes used in IVF, 83, 101, 126
  safe handling, storage, and transportation of, 4, 90, 101–102, 127
  safeguards against misuse of, 4, 26
Bone marrow, 17, 115
Brown, Louise, 22
Bush, George H. W., 23
Bush, George W., 2, 18, 21

C

Cadaveric fetal tissue, derivation of stem cells from, 16–17
California Institute for Regenerative Medicine, 76
Canada, 78–79, 84
  national body established in, 59
  procurement practices in, 66
Canadian Assisted Human Reproduction Agency, 27
Canadian Institute for Health Research, 78
Cash payments, not offering to donors, 9–10, 85
Categories of research proposals, 7–8, 57–58, 98–99, 124–125
  research not permissible at this time, 8, 57–58, 99, 124–125
  research permissible after notification of the ESCRO committee, 7, 57, 99, 124
  research permissible only after additional review and approval of the ESCRO committee, 7–8, 57, 124
Cell-based therapies, 20, 44
Cell lines
  banking and distribution of, 92–95
  ensuring sufficient genetic diversity among, 60, 125
Cells restricted to specific developmental fates, development of hES cells down particular pathways to generate, 43–44
CFR. *See* Code of Federal Regulations
CGTP. *See* Current good tissue practices
Chimeras, 6–8, 17, 30, 39–41, 116
  preventing from breeding, 40, 55, 58, 106
Choice. *See* Autonomy
CLIA. *See* Clinical Laboratory Improvement Amendments
Clinical care. *See* Standards of clinical care
Clinical Laboratory Improvement Amendments (CLIA), 90
Clinton, William J., 20, 23
Cloning
  of Dolly the sheep, 2, 16, 34
  human reproductive, 5
  producing abnormalities, 34
  therapeutic, 19
Cloning-for-biomedical-research now, 21
Cloning Human Beings, 20
Code of Federal Regulations (CFR), 64–66, 68
Code of Practice for the Use of Human Stem Cell Lines, 52
Collaborations, substituting equivalent foreign procedures in, 58, 106, 125
Common Rule, 54n, 64n
Compliance
  imposing sanctions to ensure, 14, 106–107
  recommendations for, 11–12, 71, 126
  with relevant FDA regulations, 74, 126
Confidentiality, 4, 56, 82
Conflict of interest, 107
Conscience, personnel objecting to hES cell research for reasons of, 11, 92, 102, 128
Consent. *See* Informed consent of donors
Contamination, concerns about, 18
Contraception, developing better techniques for, 77
Coriell, 93
"Council of Europe Recommendation R(97)5 on the Protection of Medical Data," 74n
Cryopreserved embryos, 81
Culture conditions, not including mouse feeder cells and bovine serum, 43
Current good tissue practices (CGTP), 72

D

Department of Health, Education, and Welfare (DHEW), 22–23
Department of Health and Human Services (DHHS), 24, 54n, 64
  Office for Human Research Protections, 65–67

Derivation
of hES cell lines, 102–103
of new hES cell lines, permissible only after additional review and approval of the ESCRO committee, 7, 57, 99, 124
of oocytes from nonreproductive material, 38
DHEW. *See* Department of Health, Education, and Welfare
DHHS. *See* Department of Health and Human Services
Diabetes. *See* Type I diabetes
Dickey-Wicker amendment, 24
Differentiation, 32–33, 43
assaying, 116
Diploid androgenetic mES cells, 36
Disease development and progression
treating, 50
understanding, 2, 17
DNA, 36, 79, 116, 117
Documentation, 6, 55, 68, 99
of the provenance of hES cells, 6–7, 56, 105, 123
Dolly the sheep, cloning of, 2, 16, 34
Donors. *See also* Informed consent of donors
advertising for, 85
anonymous, 73, 75
of blastocysts, 4, 6, 57
disclosure of whether identities will be readily ascertainable to researchers, 90, 101, 127
disclosure that research could have commercial potential without benefit to, 91, 102, 128
disclosure that research is not intended to directly benefit, 91, 102, 128
ensuring authorizations comply with the HIPAA, 68–69, 126
of gametes, 4, 6, 57
physical interactions with, 65
protecting, 82, 106
suitability rules for, 68, 83
Drugs. *See also* Investigational new drugs; Targeting
immunosuppressive, 34, 118

E

EAB. *See* Ethics Advisory Board
Ectoderm, 31, 116
Ectopic sites, 44
Electroporation, 33, 116
Embryoid bodies (EBs), 31–32, 116
Embryonic disk, 29, 116
Embryonic Stem Cell Research Oversight (ESCROs) committees, 5–6, 12, 14, 53–59, 79, 98–100, 102, 105–106
establishing, 5–6, 56, 100, 123
institutional, 100
Embryonic stem cells (ES cells), 1–2, 29–33, 41, 116
derived from mouse blastocysts, 1
Embryos, 116
buying and selling of forbidden, 75, 78
cryopreserved, 81
disclosure that these will be destroyed in deriving hES cells, 91, 102, 128
respect for donors of human, 49
special status of human, 48–49
Endoderm, 31, 116
Endogenous genes, 31, 42
Endothelial cells, 31–32
Epigenetics, 43, 117
ES cells. *See* Embryonic stem cells
ESCRO. *See* Embryonic Stem Cell Research Oversight
Ethical, Legal and Social Issues in Human Stem Cell Research, Reproductive and Therapeutic Cloning, 78
Ethical and scientific concerns addressed through oversight, 1–2, 28, 41, 47–61, 70, 106. *See also* Moral issues
ensuring sufficient genetic diversity among cell lines, 60, 125
institutional oversight of hES cell research, 53–58
just distribution of the benefits of hES cell research, 60
national body needed to assess adequacy of guidelines proposed and provide a forum for a continuing discussion of issues, 59–60, 126
need for a national perspective, 58–60
need for an oversight system, 51–53
objections to the use of NT for reproductive purposes, 51
recommendations regarding, 53–60
respect for donors of human embryos and gametes, 49
special status of the human embryo, 48–49
transferring hES cells into nonhuman animals, 49–50
Ethical Issues in Human Stem Cell Research, 20
Ethics Advisory Board (EAB), 22–23
Ethics Committee (ASRM), 27, 52

Ethics review bodies, 52
EU Data Protection Directive, 74n
European Commission, 84
European Union, collaborations with members of, 74
Excess oocytes and unfertilized eggs, from IVF procedures, 37
Exclusion criteria, medical, 4
Exogenous genes, delivering, 33
Expertise, calling upon suitable, 107
Extracellular matrices, 32

F

Facilities obtaining and storing hES cell lines, implementing specific recommended standards, 94–95, 104–105, 129–130
FDA. *See* Food and Drug Administration
FDCA. *See* Food, Drug, and Cosmetic Act
Federal legislation, 2, 18, 23–24, 63–79, 86
Feeder cell layer, 18, 30, 32, 43, 73, 117
Fertility clinics, 84
Fertilization, 29, 51, 117
Fetal tissue, derivation of stem cells from cadaveric, 16–17
Fetuses, 30–31
Fibroblasts, 30, 32, 117
Financial incentives, 4. *See also* Cash payments; In kind payments
Food, Drug, and Cosmetic Act (FDCA), 72
Food and Drug Administration (FDA), 3, 6, 12, 19, 39, 51, 63–64, 72, 74, 82–83
  letter to investigators/sponsors, 20n, 51n
Forum for a continuing discussion of issues, national body needed to provide, 59–60, 126
Funding sources for hES cell research, 14, 18–19, 59, 106
  nonfederal, 106
  public, 19, 106

G

Gametes, 30, 117
  donors of, 4, 7–9, 57, 100, 106
  respect for donors of, 49
Gearhart, John, 15
Gene therapy, 42, 79
Generation
  of additional hES cell lines, 42
  of hES cells of defined genetic backgrounds, 42
Genetic disease, experiments exploring underpinnings of, 16, 77
Genetic diversity among cell lines, ensuring sufficient, 60, 125
Genetic manipulation, disclosure that cells and cell lines could be used in, 42, 91, 102, 127
Genetically altered nuclei, 36
Genital ridges, 31, 117
Genotype, 42–43, 117
Germline cells, 39–40, 44, 117
GLP. *See* Good Laboratory Practice regulations
Glycolipids, antigenic, 18
Gonadal ridge, 117
  derivation of stem cells from primordial, 16
Good Laboratory Practice (GLP) regulations, 12, 71
Graft rejection, averting, 20
Guidance on Research Involving Coded Private Information or Biological Specimens, 93
Guidelines for Research Involving Recombinant DNA Molecules, 69
Guidelines for research on human embryonic stem cells, 97–107
  banking and distribution of hES cell lines, 103–105
  blastocysts made for reproductive purposes and later obtained for research from IVF clinics, 4
  blastocysts made specifically for research using IVF, 4
  coverage of, 4–5, 98
  derivation of hES cell lines, 102–103
  dividing research proposals into categories, 98–99
  establishment of institutional ESCRO committees, 100
  international collaboration, 106
  national body needed to assess adequacy of, 59–60, 126
  need for, 18–22
  obligations of investigators and institutions, 99–100
  procurement of gametes, blastocysts, or cells for hES generation, 100–102
  research use of hES cell lines, 105–106
  somatic cell nuclear transfer (NT) into oocytes, 4
"Guidelines for the Security of Information Systems," 74n

H

Handling of cells and tissues, assurance that all researchers will follow best practices in, 90, 101–102, 127
HCT/Ps. *See* Human cells, tissues, and cellular and tissue-based products
Health Insurance Portability and Accountability Act (HIPAA), 11–12, 68, 74, 82
  Standards for Privacy of Individually Identifiable Health Information (Privacy Rule), 68, 73, 89–90
hEG cells. *See* Human embryonic germ cells
Hematopoietic stem cells (HSCs), 31–32, 40–41, 117
HERP. *See* Human Embryo Research Panel
hES cells. *See* Human embryonic stem cells
HFEA. *See* Human Fertilisation and Embryology Authority (United Kingdom)
HIPAA. *See* Health Insurance Portability and Accountability Act
Histocompatibility antigens, 45, 118
  immune rejection due to, 44–45
History, of U.S. discussions and policies regarding research involving human embryos, 22–25
Homologous recombination, 42, 118
Honoraria, paying, 86
Hormonal induction, 10
HSCs. *See* Hematopoietic stem cells
Human brain cells, implanting into nonhuman animals, 54
Human cells, tissues, and cellular and tissue-based products (HCT/Ps), 72
Human Cloning Act, in Australia, 76
Human Cloning and Human Dignity: An Ethical Inquiry, 21
Human dignity, protecting, 48–49, 55, 76
Human disease. *See* Disease development and progression
Human Embryo Research Panel (HERP), 23–24, 52
Human embryonic germ cells (hEG cells), 31
Human Embryonic Stem Cell Registry, 18
Human embryonic stem cell research
  clearinghouse for proposals, 5, 54
  current regulation of, 28, 63–79
  prerequisites to, 4, 26
  public sponsorship of, 19
Human embryonic stem cells (hES cells), 1, 32, 118
  genetic modification of, 42
  knowing the provenance of, 54
  maintaining a registry of, 8
  self-renewing capacity of, 17, 32, 43
  using already derived, 6, 56, 72
Human embryos, special status of, 49
Human Fertilisation and Embryology Authority (HFEA), in the U.K., 27, 52–53, 77
Human reproductive cloning, 5. *See also* Cloning
Human subjects protection system, 9
Human transplantation, disclosure regarding possible use of derived cells for, 90, 101, 127
Hwang, Woo Suk, 35

I

IACUC. *See* Institutional Animal Care and Use Committee
IBC. *See* Institutional Biosafety Committee
Iceland, collaborations with scientists in, 74
IDE. *See* Investigational device exemption
Immune rejection, due to histocompatibility problems, 44–45
Immune system cells, 44, 118
Immunogenicity, reducing, 34, 118
Immunosuppressive drugs, 34, 118
Imprinted genes, 73
In kind payments, not offering to donors, 9–10, 85
In vitro experiments, 6, 31–32, 37, 40, 44, 55, 57–58, 78, 118
  culture of any intact human embryo past 14 days, 8, 57
  growing hES cells, 32
In vitro fertilization (IVF), 2, 4, 10–11, 16, 21, 37, 42–43, 76, 81–83, 85, 87, 98, 100–101, 118
  commercial practices regarding, 2, 4, 10–11, 16, 21, 37, 43, 76, 81–83, 85, 87, 100–101, 118
  researchers not having any influence over IVF decisions, 85, 101, 126
In vivo experiments, 44–45
  differentiation, 32, 118
Incentives. *See* Financial incentives; Nonfinancial incentives
Incorporation of hES cells or cells derived from them
  into nonhuman blastocysts, 40–41
  into postgastrulation stages of another species, 40
  into postnatal animals of another species, 39–40

IND. *See* Investigational new drugs
Infertility treatments, 1, 11, 48, 77
Influence, 85–86, 101, 126
Informed consent of donors, 4, 18, 56, 66, 88–91, 101–102, 125, 127–128
  obtaining from each donor at time of donation, 88, 101, 127
  and the potential discovery of clinically significant information, 89–91
  requiring an invitation, if donors' identities are retained, to be notified in the future of what was learned from studying their cell lines, 90, 101, 127
  requiring assurance that all researchers will follow best practices in their handling of cells and tissues, 90, 101–102, 127
  requiring disclosure of whether donors' identities will be readily ascertainable to researchers, 90, 101, 127
  requiring disclosure regarding possible uses of cells derived for human transplantation, 90, 101, 127
  requiring disclosure that cells and cell lines could be used in genetic manipulation or mixing with animal cells, 91, 102, 127
  requiring disclosure that cells and cell lines may be kept for many years, 90, 102, 127
  requiring disclosure that embryos will be destroyed in deriving hES cells, 91, 102, 128
  requiring disclosure that no restriction or direction can be made regarding possible recipients, 90, 101, 127
  requiring disclosure that research could have commercial potential, without benefit to the donors, 91, 102, 128
  requiring disclosure that research is not intended to directly benefit the donors, 91, 102, 128
  requiring statement of risks involved to donors, 91, 102, 128
  requiring statement that neither consenting nor refusing to donate embryos for research will affect quality of future care provided potential donors, 91, 102, 128
  voluntary, 83
  written, 10
Informed refusal by donors, 82, 104
Inner cell masses, 30–31, 40, 118
Institutional Animal Care and Use Committee (IACUC), 6–7, 54, 57, 71, 99, 105
Institutional Biosafety Committee (IBC), 6–7, 54, 57, 70, 99
Institutional oversight of hES cell research, 53–58
  in collaborations, substituting equivalent foreign procedures, 58, 106, 125
  establishing uniform guidelines and record-keeping processes approved by an IRB, 94, 103–104, 128
  oversight of, 53–58
  registry of investigators conducting hES cell research and records of research being performed and cell types used, 58, 105, 125
Institutional Review Boards (IRBs), 5–12, 39, 49, 54–56, 64–69, 82–87, 93–94, 99–102, 104–106
Intellectual property issues, 26
International collaboration, 12, 106
International regulations, 4, 26
Interpretation of genetic information, 4
Interspecies mixing, 38–41
  incorporation of hES cells into nonhuman blastocysts, 40–41
  incorporation of hES cells or cells derived from them into postgastrulation stages of another species, 40
  incorporation of hES cells or cells derived from them into postnatal animals of another species, 39–40
  use of nonhuman oocytes as recipients of human somatic nuclei in NT, 41
Introduction of hES cells into nonhuman animals, research permissible only after additional review and approval of the ESCRO committee, 7, 57, 99, 105–106, 124
Investigational device exemptions (IDEs), 72
Investigational new drugs (INDs), 72
IRBs. *See* Institutional Review Boards
Islam, views on the human embryo, 48
Israel
  buying and selling of embryos forbidden in, 78, 116
  national body established in, 59
  procurement practices in, 66
Israel Academy of Sciences and Humanities, Bioethics Advisory Committee, 78
Issues to Consider in Research Use of Stored Data or Tissues, 93
IVF. *See In vitro* fertilization

J

Johns Hopkins University, 15
Joint Commission for the Accreditation of Health Care Organizations, 27
Journals. *See* Scholarly journals
Judaism, views on the human embryo, 48
Just distribution, of the benefits of hES cell research, oversight of, 60

K

Karyotypes, 32, 118
Korean scientists, 16, 35–36

L

Laboratory practice, 71
Legal issues, 1, 26. *See also* Federal legislation; State legislation
Leukemia inhibitory factor (LIF), 30–31, 118
Liechtenstein, collaborations with scientists in, 74
LIF. *See* Leukemia inhibitory factor
Life sciences, scientific self-regulation in, 26
Long-term cultures, ensuring stability of genotype, epigenetic status, and phenotypic properties of ES cells grown in, 43

M

Manipulation of life, 49. *See also* Genetic manipulation
Markers, 32
Material Transfer Agreement, 105
Matrigel, 32
Medical exclusion criteria, 4
Medical Research Council, in the U.K., 77, 84, 87
Medical risks, considered unacceptable, 16
Medicare reimbursement, 27
MEF. *See* Mouse embryonic fibroblast
mES. *See* Mouse embryonic stem cells
Mesoderm, 31, 118
Mexico City conference, calling for a global ban on NT for human reproduction, 21
Mitochondria, 34
Mixing with animal cells, disclosure that cells and cell lines could be used in, 91, 102, 127
Monkey virus experiment, 26
Moral issues, 60–61, 85
Moratoria to delay scientific research, voluntary, 19, 34
Morula, 29–30, 36, 118
Mouse blastocysts, embryonic stem cells derived from, 1
Mouse embryonic fibroblast (MEF), 30, 32, 119
Mouse embryonic stem cell (mES), 30–32, 34
  diploid androgenetic, 36
Mouse gonads, 31

N

National Academy of Sciences (NAS), 3, 5, 19–21, 26, 51, 64, 97
  guidelines for research on human embryonic stem cells, 97–107
National Bioethics Advisory Commission (NBAC), 20–21, 48, 52
National body needed
  to assess adequacy of guidelines proposed, 59–60, 126
  to provide a forum for a continuing discussion of issues, 59–60, 126
National Cancer Institute, 93
National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research, 22
National Health and Medical Research Council Licensing Committee, 77
National Heart, Lung and Blood Institute, 93
National Institutes of Health (NIH), 18, 20, 22–26, 64, 69–70, 84, 95
  efforts to encourage the sharing and dissemination of important research resources, 95
  Human Embryo Research Panel, 52
  Revitalization Act, 23–24
  Stem Cell Task Force, 25
National perspective, 58–60
  oversight of, 58–60
  recommendations for a national policy review, 13
National Science Foundation, 20
NBAC. *See* National Bioethics Advisory Commission
Neural stem cells (NSCs), 39, 119
Neurodegenerative diseases, 40
Neuronal progenitors, 31, 39–40
New York Times, 16

NIH. *See* National Institutes of Health
Nonfinancial incentives, avoiding all, 9
Nonhuman animals
  implanting human brain cells into, 54
  transferring hES cells into, 49–50
Nonhuman oocytes, 38
  using as recipients of human somatic nuclei in NT, 41
  using for NT, 43
Nonhuman primate ES cells, 41
"Nonpersonalizing" data, 74
Normal preimplantation development, compared with nuclear transfer, 35
Norway, collaborations with scientists in, 74
NRC. *See* National Research Council
NSCs. *See* Neural stem cells
NT. *See* Nuclear transfer
Nuclear genomes, 2
Nuclear transfer (NT), 2, 4, 16, 34, 47, 75, 84–85, 91, 119
  calls for a global ban on using for human reproduction, 21
  fears of its use for producing a child, 2
  to generate stem cells, 33–37
  normal preimplantation development compared with, 35
  reproductive uses of, 4
Nuclei, genetically altered, 36

O

Objections to the use of NT for reproductive purposes, 51. *See also* Personnel objecting to hES cell research
Obligations
  informing donors they have none, 9
  of investigators and institutions, 99–100
Office for Human Research Protections (OHRP), 65–67, 93
Office of Technology Assessment, 22
OHRP. *See* Office for Human Research Protections
OHSS. *See* Ovarian hyperstimulation syndrome
Oocytes, 30, 34–38, 43, 48, 66, 83, 119. *See also* Sources of oocytes for NT ES cells
  donors of, 37–38
  excess, 37
  matured from ovariectomies or fetal ovaries from pregnancy terminations, 37
  risks associated with retrieval, 4
Oophorectomy, 85
Organ donation, 37
Organ transplants. *See* Transplantation
Ovarian hyperstimulation syndrome (OHSS), 87
Ovariectomy, 37, 119
Oversight system for hES cell research, 14, 21, 51, 58–59, 78, 106
  need for, 51–53

P

Parkinson's disease, 50
Parthenogenesis, 36–37, 100, 103, 119
Patient advocacy groups, 22
Payments, not offering to donors, 9–10, 85–86
PCB. *See* President's Council on Bioethics
Penalties, informing donors there are none, 9
Personal health information (PHI) about donors, 11, 68–69
  being readily ascertainable, 7–8, 57, 99, 124
  "deidentification" of, 68–69
Personnel objecting to hES cell research, for reasons of conscience, 92, 102, 128
PGD. *See* Preimplantation genetic diagnosis procedures
Pharmaceuticals. *See* Drugs
PHI. *See* Personal health information
PHS. *See* Public Health Service
Placenta, 29–31, 119
Pluripotent cells, 15, 17, 29–30, 34–35, 39, 119
Policy issues, 1, 10. *See also* National perspective
Political independence of researchers, 107
Poverty issues, 86
Pregnancy terminations, 37
Preimplantation development, 30
Preimplantation genetic diagnosis (PGD) procedures, 33, 42, 119
President's Council on Bioethics (PCB), 21, 53
Priorities for hES cell research, 41–45
  alternative sources of human oocytes, 43
  developing culture conditions that do not include mouse feeder cells and bovine serum, 43
  directing development of hES cells down particular pathways to generate cells restricted to specific developmental fates, 43–44
  ensuring stability of genotype, epigenetic status, and phenotypic properties of ES cells grown in long-term cultures, 43
  generating additional hES cell lines, 42
  generating hES cells of defined genetic backgrounds, 42

genetic manipulation of hES cells, 42
immune rejection due to histocompatibility problems, 44–45
maintaining the self-renewing capacity of hES cells over long-term culture and expansion, 43
separating progenitors of restricted developmental potential from hES cells, 44
testing the potential of the derived cells to contribute usefully when implanted, 44
testing therapeutic drugs, 45
using nonhuman oocytes for NT, 43
Privacy Rule, HIPAA, 68–69, 73, 89–90
Procedural requirements, 52
Procurement process. *See also* Sources of oocytes for NT ES cells
recommendations for review of, 8–9, 66, 100–102, 125
Professional societies
overseeing hES cell research, 14, 59, 106
regulations from, 4, 26
Profit motive, 38
Progenitors of restricted developmental potential, separating from hES cells, 44
Proposition 71, in California, 75, 87
Protestant denominations, views on the human embryo, 48
Pseudopregnant females, 30, 119
Public concern, 22, 58, 100, 106
Public Health Service (PHS), 64
Policy on Humane Care and Use of Laboratory Animals, 71
Public Health Service Act, 72
Section 361, 73

R

RAC. *See* Recombinant DNA Advisory Committee
Radiation safety committees, 54
rDNA. *See* Recombinant DNA
REB. *See* Research Ethics Board (Canada)
Recombinant DNA Advisory Committee (RAC), 20, 26–27, 70, 79
guidelines from, 27
Recombinant DNA (rDNA) research, 27, 47, 69–70
Recommendations, 66
for addressing ethical and scientific concerns through oversight, 53–60
for banking of hES cell lines, 12–13
compilation of, 123
for compliance with all relevant FDA regulations, 74, 126
for compliance with all relevant regulations, 11–12, 71, 126
for ensuring authorizations received from donors comply with the HIPAA, 68–69, 126
for a national policy review, 13
for review of the procurement process, 66, 125
"Recommendations of the Council of the OECD concerning Guidelines on the Protection of Privacy and Trans-border flows of Personal Data," 74n
Recommendations regarding banking and distribution of cell lines
facilities obtaining and storing hES cell lines implementing specific recommended standards, 94–95, 104–105, 129–130
institutions establishing uniform guidelines and record-keeping processes approved by an IRB, 94, 103–104, 128
Recommendations regarding informed consent of donors, 9–10, 66, 90–91, 101, 125, 127–128
assurance that all researchers will follow best practices in their handling of cells and tissues, 90, 101–102, 127
disclosure of whether donors' identities will be readily ascertainable to researchers, 90, 101, 127
disclosure regarding possible use of cells derived for human transplantation, 90, 101, 127
disclosure that cells and cell lines could be used in genetic manipulation or mixing with animal cells, 91, 102, 127
disclosure that cells and cell lines may be kept for many years, 90, 102, 127
disclosure that embryos will be destroyed in deriving hES cells, 91, 102, 128
disclosure that no restriction or direction can be made regarding possible recipients, 90, 101, 127
disclosure that research could have commercial potential, without benefit to the donors, 91, 102, 128
disclosure that research is not intended to directly benefit the donors, 91, 102, 128

invitation, if donors' identities are retained, to be notified in the future of what was learned from studying their cell lines, 90, 101, 127
statement of risks involved to donors, 91, 102, 128
statement that neither consenting nor refusing to donate embryos for research will affect quality of future care provided potential donors, 91, 102, 128
Recommendations regarding institutional oversight of hES cell research, 5–8, 53–58
dividing research proposals into categories, 7–8, 57–58, 98–99, 124–125
Recommendations regarding standards of clinical care, 10–11
consenting or refusing to donate gametes or embryos for research not affecting the care potential donors receive, 91, 128
personnel objecting to hES cell research for reasons of conscience not being required to participate, 11, 92, 102, 128
researchers not pressuring or paying any third party to obtain oocytes for them, 11, 92, 102, 128
Recommendations regarding the procurement process, 8–9, 66, 100–102, 125
blastocysts produced by donor gametes used in IVP never being used without consent of all gamete donors, 83, 101, 126
hES researchers not having any influence over IVF decisions, 85, 101, 126
no cash or in kind payments being made for donating excess blastocysts, 85, 101, 126
women undergoing hormonal induction to generate oocytes being reimbursed only for direct expenses, 87, 101, 127
Recommendations regarding timing of decision to donate excess blastocysts, consent for blastocyst donation being obtained from each donor at time of donation, 88, 101, 127
Records
need for maintaining meticulous, 12
of research being performed and cell types used, 58, 105, 125
Recruiting donors, 28, 81–96
adherence to standards of clinical care, 91–92
banking and distribution of cell lines, 92–95
informed consent requirements, 88–91, 101–102, 127–128
review of the procurement and informed consent process, 83–87
timing of decision to donate excess blastocysts, 88
Refusal, right of, 82, 104
Regenerative medicine, 2, 30–31, 60
Registry, of investigators conducting hES cell research, 58, 105, 125
Regulation of human embryonic stem cell research, 28, 63–79
of clinical research with cell lines and differentiated tissue, 71–74
of hES cell and NT research in other countries, 76–79
implications of the privacy rule and human subjects protections in research with biological materials for hES cell research, 67–69
patchwork of existing, 1
of procurement of gametes, somatic cells, and blastocysts, 64–66
professional and international, 4
U.S. state law on hES cell research, 74–76
of *in vitro* and animal studies using hES cell lines, 69–71
Regulation of *in vitro* and animal studies using hES cell lines, 69–71
animal care and use, 70–71
compliance with all relevant regulations, 71, 126
laboratory practice, 71
recombinant DNA research, 69–70
Regulatory oversight
gaps in, 63
through public funding of research, 19
Reimbursement, only for direct expenses incurred, 9, 11
Reproductive technology, 20
Research
funding, 14, 18
institutions conducting hES cell research, 14, 59, 106
meritorious, 8
not permissible at this time, 8, 57–58, 99, 124–125
permissible after notification of the ESCRO committee, 7, 57, 99, 124
using hES cell lines, 105–106
voluntary moratoria to delay, 20
Research collaborations, substituting equivalent foreign procedures in, 58, 106, 125
Research ethics boards (REBs), in Canada, 78–79

Research Involving Human Embryos Act, in Australia, 76
Research permissible only after additional review and approval of the ESCRO committee, 7–8, 57, 124
  derivation of new hES cell lines, 7, 57, 99, 124
  introduction of hES cells into nonhuman animals, 7, 57, 99, 105–106, 124
  where personally identifiable information about the donors is readily ascertainable, 7–8, 57, 99, 124
Research proposals, categories of, 98–99
Researchers
  following best practices in their handling of cells and tissues, 90, 101–102, 127
  not pressuring or paying any third party to obtain oocytes for them, 11, 92, 102, 128
Respect for donors of human embryos and gametes, 49, 58
Review of the procurement and informed consent process, 66, 83–87, 125
  blastocysts produced by donor gametes used in IVP never used without consent of all gamete donors, 83, 101, 126
  ensuring donations are voluntary, 84–85
  hES researchers should have no influence over IVF decisions, 85, 101, 126
  no cash or in kind payments may be made for donating excess blastocysts, 85, 101, 126
  recruiting and paying gamete donors for research purposes, 85–87
  women undergoing hormonal induction to generate oocytes should be reimbursed only for direct expenses, 87, 101, 127
Right of refusal, 104
Risks
  associated with retrieval of oocytes, 4
  involved to donors, statement of, 87, 91, 102, 128

S

Sanctions, imposing to ensure compliance, 14, 106–107
Scholarly journals. *See* Scientific journals
Science, social investment in, 60
Scientific and Medical Aspects of Human Reproductive Cloning, 4, 20, 98
Scientific background of human embryonic stem cell research, 1, 28–45
  interspecies mixing, 38–41
  nuclear transfer to generate stem cells, 33–37
Scientific concerns. *See* Ethical and scientific concerns addressed through oversight
Scientific journals
  reporting on hES cell research, 14, 59, 106
  requiring evidence of compliance before publication of results, 14, 59
Scientific self-regulation
  in the life sciences, 26, 106
  precedents for, 26–27
SCNT. *See* Somatic cell nuclear transfer
Self-regulation. *See* Scientific self-regulation
Self-renewing capacity of hES cells, 17, 32
  maintaining over long-term culture and expansion, 43
Sensitivities, 47
Serum-free growth media, 32
Singapore, 78
  forbidding all NT, 78
  national body established in, 59
  procurement practices in, 66
Single-gene defects, 33
Skin cell transplants, 17
Society for Assisted Reproductive Technologies, 27
Somatic cell nuclear transfer (SCNT), 2, 16, 43, 119. *See also* Nuclear transfer
Somatic cells, 66, 119
  donors of, 4, 7–9, 57, 100, 106
Sources of oocytes for NT ES cells, 37–38
  alternative, 43
  derivation of oocytes from nonreproductive material, 38
  excess oocytes and unfertilized eggs from IVF procedures, 37
  oocyte donation, 37–38
  oocytes matured from ovariectomies or fetal ovaries from pregnancy terminations, 37
  use of nonhuman oocytes, 38
Spinal-cord injuries, combating, 40
Stability of genotype, epigenetic status, and phenotypic properties, of ES cells grown in long-term cultures, ensuring, 43
Standards for Privacy of Individually Identifiable Health Information (Privacy Rule), 68, 73, 89–90
Standards of clinical care, 91–92
  consenting or refusing to donate gametes or embryos for research not affecting care potential donors receive, 91, 128
  personnel objecting to hES cell research for reasons of conscience not being required to participate, 11, 92, 102, 128
  recommendations for adherence to, 10–11

researchers not pressuring or paying any third party to obtain oocytes for them, 11, 92, 102, 128
State legislation, 75–76, 106
Statistical data, 66, 68
Statutory bans, 16
Stem Cell Bank, in the UK, 44, 77, 92
Stem Cell Oversight Committee, 78
Stem Cell Task Force, 25
Stem cells, 15, 50, 119
derivation of, 16
safe handling and storage of, 4, 90, 101–102, 127
standardization of and validation of results, 4
Stem Cells and the Future of Regenerative Medicine, 19
Stored cells
confidence in the value of, 12
disclosure that cells and cell lines may be kept for many years, 90, 102, 127

T

Targeting
in the development of drugs, 2
in genes, 117
Taxonomies, 49
Teratomas, 31–32, 36, 120
Testing
potential of derived cells to contribute usefully when implanted, 44
therapeutic drugs, 45
Therapeutic potentials, 50, 76
involving cloning, 19
Thomson, James, 1, 15
Timing of consent to donate excess blastocysts, 88
obtaining from each donor at time of donation, 88, 101, 127
Tissue culture, 30–33, 120
Tissue rejection, overcoming, 34
Tracking, 72
Transfection, 33, 120
Transgenes, 42, 120
Transparency, 51
Transplantation uses, 34, 42, 44–45, 67, 72–73, 105, 120
Trophectoderm, 29–31, 120
Type I diabetes, 33

U

U.N. General Assembly, 74n, 76
Undifferentiated cells, 32, 120
United Kingdom, 77
national body established in, 59
procurement practices in, 66
United States Code (USC), 72
University of Wisconsin, 15
U.S. state law on hES cell research, 74–76
USC. *See* United States Code
Uterus, 29–30, 34

V

Varmus, Harold, 23
Viral infections, 33
Voluntary donations, ensuring to all donors, 84–85

W

Web-based primer, 56
Women
possible exploitation of, 48
undergoing hormonal induction to generate oocytes, 87, 101, 127

X

Xenograft or xenotransplant, 39, 73, 120

Z

Zygote, 29–30, 120

2007 AMENDMENTS

THE NATIONAL ACADEMIES' GUIDELINES FOR HUMAN EMBRYONIC STEM CELL RESEARCH

Human Embryonic Stem Cell Research Advisory Committee

Board on Life Sciences
Division on Earth and Life Studies

Board on Health Sciences Policy
Institute of Medicine

NATIONAL RESEARCH COUNCIL *AND*
INSTITUTE OF MEDICINE
OF THE NATIONAL ACADEMIES

THE NATIONAL ACADEMIES PRESS
Washington, D.C.
www.nap.edu

THE NATIONAL ACADEMIES PRESS 500 Fifth Street, N.W. Washington, DC 20001

NOTICE: The project that is the subject of this report was approved by the Governing Board of the National Research Council, whose members are drawn from the councils of the National Academy of Sciences, the National Academy of Engineering, and the Institute of Medicine. The members of the committee responsible for the report were chosen for their special competences and with regard for appropriate balance.

This study was supported by The Ellison Medical Foundation, The Greenwall Foundation, and the Howard Hughes Medical Institute. Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the author(s) and do not necessarily reflect the views of the organizations or agencies that provided support for the project.

International Standard Book Number-13: 978-0-309-10559-0
International Standard Book Number-10: 0-309-10559-5

Suggested Citation: National Research Council (NRC) and Institute of Medicine (IOM). 2007. *2007 Amendments to the National Academies' Guidelines for Human Embryonic Stem Cell Research*. Washington, DC: The National Academies Press.

Cover: A cluster of motor neurons and neural fibers derived from human embryonic stem cells in the lab of University of Wisconsin-Madison stem cell researcher and neurodevelopmental biologist Su-Chan-Zhang. The motor neurons are shown in red, neural fibers appear green, and the blue specks indicate DNA in cell nuclei. These motor neurons were developed from one of James Thomson's original human embryonic stem cell lines. Copyright for the photograph is held by the University of Wisconsin's Board of Regents.

Additional copies of this report are available from the National Academies Press, 500 Fifth Street, N.W., Lockbox 285, Washington, DC 20055; (800) 624-6242 or (202) 334-3313 (in the Washington metropolitan area); Internet, http://www.nap.edu.

Printed in the United States of America

THE NATIONAL ACADEMIES

Advisers to the Nation on Science, Engineering, and Medicine

The **National Academy of Sciences** is a private, nonprofit, self-perpetuating society of distinguished scholars engaged in scientific and engineering research, dedicated to the furtherance of science and technology and to their use for the general welfare. Upon the authority of the charter granted to it by the Congress in 1863, the Academy has a mandate that requires it to advise the federal government on scientific and technical matters. Dr. Ralph J. Cicerone is president of the National Academy of Sciences.

The **National Academy of Engineering** was established in 1964, under the charter of the National Academy of Sciences, as a parallel organization of outstanding engineers. It is autonomous in its administration and in the selection of its members, sharing with the National Academy of Sciences the responsibility for advising the federal government. The National Academy of Engineering also sponsors engineering programs aimed at meeting national needs, encourages education and research, and recognizes the superior achievements of engineers. Dr. Wm. A. Wulf is president of the National Academy of Engineering.

The **Institute of Medicine** was established in 1970 by the National Academy of Sciences to secure the services of eminent members of appropriate professions in the examination of policy matters pertaining to the health of the public. The Institute acts under the responsibility given to the National Academy of Sciences by its congressional charter to be an adviser to the federal government and, upon its own initiative, to identify issues of medical care, research, and education. Dr. Harvey V. Fineberg is president of the Institute of Medicine.

The **National Research Council** was organized by the National Academy of Sciences in 1916 to associate the broad community of science and technology with the Academy's purposes of furthering knowledge and advising the federal government. Functioning in accordance with general policies determined by the Academy, the Council has become the principal operating agency of both the National Academy of Sciences and the National Academy of Engineering in providing services to the government, the public, and the scientific and engineering communities. The Council is administered jointly by both Academies and the Institute of Medicine. Dr. Ralph J. Cicerone and Dr. Wm. A. Wulf are chair and vice chair, respectively, of the National Research Council.

www.national-academies.org

HUMAN EMBRYONIC STEM CELL RESEARCH ADVISORY COMMITTEE

R. ALTA CHARO (*Co-Chair*), University of Wisconsin, Madison
RICHARD O. HYNES (*Co-Chair*), Massachusetts Institute of Technology, Cambridge
ELI Y. ADASHI, Brown University, Providence, Rhode Island
BRIGID L.M. HOGAN, Duke University Medical Center, Durham, North Carolina
MARCIA IMBRESCIA, Arthritis Foundation, Lynnfield, Massachusetts
TERRY MAGNUSON, University of North Carolina, Chapel Hill
LINDA B. MILLER, Volunteer Trustees Foundation, Washington, D.C.
JONATHAN D. MORENO, University of Pennsylvania, Philadelphia
STUART H. ORKIN,[1] Harvard Medical School and Dana Farber Cancer Institute, Boston, Massachusetts
PILAR N. OSSORIO, University of Wisconsin, Madison
E. ALBERT REECE, University of Maryland, Baltimore
JOSHUA R. SANES, Harvard University, Cambridge, Massachusetts
HAROLD T. SHAPIRO, Princeton University, Princeton, New Jersey
JOHN E. WAGNER, Jr., University of Minnesota, Minneapolis

Staff

ADAM P. FAGEN, Study Co-Director, Board on Life Sciences
BRUCE M. ALTEVOGT, Study Co-Director, Board on Health Sciences Policy
FRANCES E. SHARPLES, Director, Board on Life Sciences
ANDREW M. POPE, Director, Board on Health Sciences Policy
ANNE F. JURKOWSKI, Senior Program Assistant, Board on Life Sciences
RIMA L. ADLER, Christine Mirzayan Science & Technology Policy Graduate Fellow, Board on Life Sciences
SARAH L. HANSON, Research Associate, Board on Health Sciences Policy

[1]Resigned from committee effective December 18, 2006.

Acknowledgments

The Committee would like to acknowledge the input received from members of the stem cell research and oversight communities as well as the speakers and participants in its meetings.

This report has been reviewed in draft form by individuals chosen for their diverse perspectives and technical expertise, in accordance with procedures approved by the National Research Council's Report Review Committee. The purpose of this independent review is to provide candid and critical comments that will assist the institution in making its published report as sound as possible and to ensure that the report meets institutional standards for objectivity, evidence, and responsiveness to the study charge. The review comments and draft manuscript remain confidential to protect the integrity of the deliberative process. We wish to thank the following individuals for their review of this report:

Robert Cook-Deegan, Duke University
William H. Danforth, Washington University
Norman Fost, University of Wisconsin–Madison
Larry Goldstein, University of California, San Diego
Henry T. Greely, Stanford Law School
Bernard Lo, University of California, San Francisco

Gail Martin, University of California, San Francisco
P. Pearl O'Rourke, Partners HealthCare System, Inc.
Steven Peckman, University of California, Los Angeles
Catherine Racowsky, Harvard Medical School
Brock C. Reeve, Harvard Stem Cell Institute
Susan L. Solomon, The New York Stem Cell Foundation
Clive Svendsen, University of Wisconsin–Madison

Although the reviewers listed above have provided many constructive comments and suggestions, they were not asked to endorse the conclusions or recommendations nor did they see the final draft of the report before its release. The review of this report was overseen by **Floyd E. Bloom**, The Scripps Research Institute, and **Janet D. Rowley**, University of Chicago. Appointed by the National Research Council, they were responsible for making certain that an independent examination of this report was carried out in accordance with institutional procedures and that all review comments were carefully considered. Responsibility for the final content of this report rests entirely with the authoring committee and the institution.

Contents

Introduction 1

Clarifying the Phrase "Provenance of the Cell Lines" 4

1.2(a) hES Cell Research Permissible After Currently Mandated Reviews 5

Use of NIH-Approved hES Cell Lines 5

1.4 Use of NIH-Approved hES cell lines 6

Importation of hES Cell Lines into an Institution or Jurisdiction 7

1.5 Acceptability of Research Using hES Cell Lines Imported from Other Institutions or Jurisdictions 8

ESCRO Committees Serving Multiple Institutions 8

2.0 Establishment of an Institutional Embryonic Stem Cell Research Oversight Committee 10

Frozen IVF Blastocysts Derived from Anonymous Sperm Donors: Absence of Informed Consent 11

Considering the Science in hES Cell Research Proposals: Advice for ESCRO Committees 12

Sample Questions for Reviewing hES Cell Research 13

Appendixes

A National Academies' Guidelines for Human Embryonic Stem Cell Research, Amended as of February 2007 15

B Committee Biographical Sketches 29

2007 Amendments to the National Academies' Guidelines for Human Embryonic Stem Cell Research

INTRODUCTION

The National Academies' report *Guidelines for Human Embryonic Stem Cell Research* was developed by the Committee on Guidelines for Human Embryonic Stem Cell Research and released in April 2005. The body of the report provided the background and rationale for the choices involved in formulating the guidelines, which were compiled in its final chapter. Because human embryonic stem (hES) cell research touches on many ethical, legal, scientific, and policy issues that are of concern to some people, the Guidelines are intended to make explicit how research with hES cells can be pursued most responsibly. While the Guidelines are primarily intended to address researchers in the United States, they may have applicability internationally as well.

The 2005 publication of the Guidelines offered a common set of ethical standards for a field that, due to the absence of comprehensive federal funding, was lacking national standards for research. Many have found the guidelines useful, but several constituencies identified sections of the Guidelines that they believe should be clarified. In addition, numerous scientific organizations and individuals encouraged the National Academies to establish an advisory committee to keep the Guidelines up to date, given the rapid pace of scientific developments in the field of stem cell research. Further,

Statement of Task of the Human Embryonic Stem Cell Research Advisory Committee

The Advisory Committee will meet two to three times per year over a period of 36 months to (1) monitor and review scientific developments and changing ethical, legal, and policy issues related to human embryonic stem cell research, (2) discuss the need for revisions to the Guidelines for Human Embryonic Stem Cell Research, and (3) prepare periodic reports to update the Guidelines as needed. Minimal but necessary changes may be issued as letter reports, but more extensive modifications may necessitate the preparation of traditional reports to fully provide the rationale for the changes.

Sources of information that will be considered by the Advisory Committee will include public symposia organized by the Committee to review developments in stem cell science and how these impact the ethical and policy issues surrounding hES cell research.

they urged the National Academies to consider correcting or clarifying aspects of the Guidelines in the light of experience.

Responding to these requests for revision and ongoing monitoring, the Human Embryonic Stem Cell Research Advisory Committee was established in 2006 with support from The Ellison Medical Foundation, The Greenwall Foundation, and the Howard Hughes Medical Institute.

The Human Embryonic Stem Cell Research Advisory Committee has engaged in a number of efforts to gather information about the need, if any, for revision of the Guidelines. The Committee met for the first time in July 2006 and heard from a number of invited guests representing organizations and academic institutions that are actively involved in stem cell research. In addition, in early November 2006, the Committee organized a symposium at which invited speakers reviewed the latest scientific developments, described how these developments might affect the analysis of associated ethical issues, and identified possible effects on the workability or justifiability of the current Guidelines. The Committee also hosted a panel discussion at the symposium for representatives of seven Embryonic Stem Cell Research Oversight (ESCRO) committees.[1] This panel shared their experiences in working with the content and procedures of the Guidelines.

[1]The 2005 Guidelines called for institutions involved in hES cell research to establish ESCRO committees to provide institutional oversight on all issues related to derivation and use of hES cell lines and to facilitate education of investigators involved in hES cell research.

As an ongoing effort, the Committee is also monitoring discussions of the Guidelines held by others, such as the April 2006 Association of American Medical Colleges (AAMC) meeting for medical school administrators to discuss the conduct and management of stem cell research at their institutions, a discussion which encompassed a review and critique of the Guidelines, and which was summarized for the Committee at its July meeting.[2] The Committee has also established a listserv for ESCRO committee members and staff to communicate and share questions and answers, and will be hosting a series of regional meetings in the spring of 2007 to bring together ESCRO committee members and staff, receive input from ESCRO committees, and clarify the Guidelines. In addition, members of the Committee have been actively soliciting input from their colleagues and receiving comments via a Web site[3] established for this purpose.

The Committee identified issues that appeared to merit consideration of revisions of the Guidelines. This report addresses issues that are both in need of amendment and amenable to prompt solution. The Committee is issuing this first set of amendments primarily to clarify or re-emphasize earlier recommendations and conclusions. Because the changes being made are minor and affect only Sections 1 and 2 of the Guidelines, this brief letter report is the best method of communicating these changes. Future deliberations of the Committee will deal with items for which additional information gathering and more extensive debate and discussion will probably be necessary. For example, the Committee has received numerous comments both praising and disputing the current policy on no compensation for oocyte donors. Similarly, some commenters have expressed dissatisfaction with the current restrictions on research using chimeras or have asked for further guidance on how to evaluate such research. More time will be required for the Committee to give adequate consideration to these and other issues and it will report on its findings in the future.

Four changes to the Guidelines are discussed herein:

1. clarifying the phrase "provenance of the cell lines" (changes to Section 1.2);
2. use of the hES cells approved for use in federally-funded research (addition of Section 1.4);

[2]A summary of the AAMC meeting was subsequently published as "Human Embryonic Stem Cell Research: Regulatory and Administrative Challenges." This AAMC monograph is available at <http://www.aamc.org/publications>.

[3]<http://www.nationalacademies.org/stemcells>.

3. importation of hES cell lines into an institution or jurisdiction (addition of Section 1.5); and
4. allowing ESCRO committees to serve multiple institutions (changes to Section 2.0 and addition of Section 2.1).

These amended Guidelines supersede those issued in 2005 by the Committee on Guidelines for Human Embryonic Stem Cell Research. It is important that these clarifications be interpreted in context with the complete set of amended Guidelines, which is included at the end of this report. It is also worth noting that these Guidelines continue to use the word "blastocyst" to refer to the stage of embryonic development from which hES cells are obtained. Both the public and the scientific community are engaged in conversation about the best terminology by which to describe this field of research, and the Committee will be attentive to those discussions as they develop.

This report also discusses two additional issues that do not result in formal changes to the Guidelines: (a) the lack of informed consent from sperm donors for some frozen in vitro fertilization (IVF) blastocysts and (b) advice for ESCRO committees in establishing criteria for considering the science in hES cell research proposals.

CLARIFYING THE PHRASE "PROVENANCE OF THE CELL LINES"

The National Academies' Human Embryonic Stem Cell Research Advisory Committee has received many comments from the scientific community questioning the meaning of the phrase "provenance of the cell lines," which occurs in Sections 1.2(a) and 6.1, to describe documentation of the derivation of stem cell lines. The wording of Section 1.2(a) is confusing due to unintended redundancy. It asks for documentation of the provenance of cell lines, documentation of appropriate informed consent in their derivation, and evidence of compliance with required review by an Institutional Review Board (IRB) and other committees, all of which address approximately the same issue. This makes it appear that "documenting the provenance of the cell lines" is something other than documenting informed consent and IRB approval. In order to resolve this confusion, the text of Section 1.2(a) is rewritten (see underlined wording) to read:

1.2(a) hES Cell Research Permissible After Currently Mandated Reviews

Purely in vitro hES cell research that uses previously derived hES cell lines is permissible provided that the ESCRO committee or equivalent body designated by the investigator's institution (see Section 2.0) receives documentation of the provenance of the cell lines including: (i) documentation of the use of an acceptable informed consent process that was approved by an Institutional Review Board (IRB) or foreign equivalent for their derivation (consistent with Section 3.6); and (ii) documentation of compliance with any additional required review by an Institutional Animal Care and Use Committee (IACUC), Institutional Biosafety Committee (IBC), or other institutionally mandated review.

USE OF NIH-APPROVED hES CELL LINES

The National Academies' Guidelines were issued a few years after a limited number of cell lines were deemed as useable in federally funded research in the United States.[4] As of the publication of this report in early 2007, these "NIH-approved cell lines" are the only hES cell lines that may be used in federally funded research.

NIH-approved cell lines were derived before August 2001 under protocols that predated the issuance of the National Academies' Guidelines in 2005. Nonetheless, NIH's agreement to fund research using these lines was premised on confirmation that all the cell lines in question were derived from blastocysts that were donated without payment, with voluntary, informed consent, and pursuant to an IRB-approved protocol. The precise details of the consent process for the NIH-approved cell lines may not have included each element called for in the National Academies' Guidelines. In particular, the Guidelines require informed consent from all embryo, gamete, and somatic cell donors, even anonymous gamete donors. For the

[4]"President Discusses Stem Cell Research," August 9, 2001. <http://www.whitehouse.gov/news/releases/2001/08/20010809-2.html>.

NIH-approved cell lines, the presence or absence of anonymously donated gametes cannot be confirmed, thus rendering impossible a determination of whether consent was obtained from all gamete donors. The NIH-approved cell lines were, however, derived from embryos that were donated under protocols that were substantially similar to those contemplated by the Guidelines.

Norms and procedures evolve, but it would be unnecessarily rigid to discourage institutions that follow the National Academies' Guidelines from working on the cell lines that are eligible for federal funding. The protocols under which the NIH-approved cell lines were derived were consistent with ethical norms then in place, were substantially similar to those now adopted in these Guidelines, and were adequately documented. The Committee considers the NIH-approved cell lines to be a special category because they are governed by a unique set of federal pronouncements (presidential statement and NIH rules). The intention of "grandfathering" the NIH-approved cell lines is to avoid precluding hES cell research that would otherwise be rendered difficult or impossible for investigators using NIH funding who wish to follow the National Academies' Guidelines. The clarification is not intended to "encourage" the use of these cell lines, either inside or outside the United States. For these reasons, retroactive application of the Guidelines is not warranted in this circumstance.

Therefore, the Guidelines are amended by adding a new Section 1.4:

1.4 Use of NIH-Approved hES Cell Lines

(a) It is acceptable to use hES cell lines that were approved in August 2001 for use in U.S. federally funded research.

(b) ESCRO committees should include on their registry a list of NIH-approved cell lines that have been used at their institution in accord with the requirement in Section 2.0 of the Guidelines.

(c) Presence on the list of NIH-approved cell lines constitutes adequate documentation of provenance, as per Section 6.1 of the Guidelines.

IMPORTATION OF hES CELL LINES INTO AN INSTITUTION OR JURISDICTION

Institutions following the National Academies' Guidelines may find themselves considering proposals for the importation of cell lines derived according to different rules, such as those from the United Kingdom, Canada, and the California Institute for Regenerative Medicine. These cell lines, while meeting all legal requirements of the respective jurisdictions for cell line derivation, may not have been derived in a manner that accords in every detail with the National Academies' Guidelines. For example, hES cell line derivations in the United Kingdom are managed through a licensing procedure that differs from the IRB and ESCRO committee review processes recommended in the Guidelines. Within the United States, state laws may vary from the Guidelines. California's laws, regulations, and guidelines, for example, though consistent with the Guidelines, apply some additional requirements concerning the details of the consent form, conflict-of-interest disclosures, and management of adverse medical events that may result from the donation of oocytes. As other states regulate such research, some state laws may differ from the Guidelines in some details but be sufficiently similar to be substantially equivalent.

Section 7.0 of the National Academies' Guidelines anticipates this problem in the international context. Section 7.0 specifically contemplates acceptance of cell lines derived under the extant legal and ethical regimes of another country provided that those regimes are substantially equivalent to the regime laid out in the Guidelines. This deference facilitates collaboration among institutions and shows proper respect for the diversity of authority in this area. This is analogous to the technique by which the U.S. federal government determines whether to accept the ethical and procedural norms of foreign research ethics review bodies as acceptable proxies for domestic IRB review.

Section 7.0 of the current National Academies' Guidelines reads: "If a U.S.-based investigator collaborates with an investigator in another country, the ESCRO committee may determine that the procedures prescribed by the foreign institution afford protections consistent with these guidelines, and the ESCRO committee may approve the substitution of some of or all of the foreign procedures for its own."

Therefore, without in any way suggesting that the addition of a new section should be construed by ESCRO committees to revoke any of the requirements of these Guidelines with respect to new donations or cell line derivations undertaken at their own institutions, the Guidelines are amended

by adding a new Section 1.5. This section applies to cell lines derived both before and after release of the Guidelines. ESCRO committees can review pre-2005 derivations and determine whether or not they are acceptable, following the guidance in new Section 1.5.

1.5 Acceptability of Research Using hES Cell Lines Imported from Other Institutions or Jurisdictions

(a) Before approving use of hES cell lines imported from other institutions or jurisdictions, ESCRO committees should consider whether such cell lines have been "acceptably derived."

(b) "Acceptably derived" means that the cell lines were derived from gametes or embryos for which

(1) the donation protocol was reviewed and approved by an IRB or, in the case of donations taking place outside the United States, a substantially equivalent oversight body;
(2) consent to donate was voluntary and informed;
(3) donation was made with reimbursement policies consistent with these Guidelines; and
(4) donation and derivation complied with the extant legal requirements of the relevant jurisdiction.

(c) ESCRO committees should include on their registry a list of cell lines that have been imported from other institutions or jurisdictions and information on the specific guidelines, regulations, or statutes under which the derivation of the imported cell lines was conducted. This is in accord with the requirement in Section 2.0 of the Guidelines that calls for ESCRO committees to maintain registries listing the cell lines in use at their institutions.

ESCRO COMMITTEES SERVING MULTIPLE INSTITUTIONS

The report *Guidelines for Human Embryonic Stem Cell Research* laid out a series of recommendations pertaining to the composition and role of ESCRO committees. Based on feedback from the community, it appears that

some of these recommendations need clarification. Although the text of Chapter 3 contains the statement that "In some cases, smaller institutions may wish to avail themselves of the services of larger facilities that have ESCRO committees," the idea that it is acceptable for institutions to use a nonlocal (external) ESCRO committee was unintentionally omitted from the wording of Section 2.0 of the Guidelines. Furthermore, since the Guidelines were issued in April 2005, it has become clear that there are other models for establishing ESCRO committees consistent with the principles of the Guidelines. New alternatives for the organization of IRB reviews are currently emerging that can serve as models for ESCRO review.

For example, the National Cancer Institute (NCI) has established a "Central IRB Initiative"[5] that is "designed to help reduce the administrative burden on local IRBs and investigators while continuing a high level of protection for human research participants." The NCI states that a local IRB's use of the Central IRB would facilitate the review of clinical trial protocols. The initiative is sponsored by NCI in consultation with the Department of Health and Human Services' Office for Human Research Protections (OHRP). OHRP's current guidance in the form of "Frequently Asked Questions" on its Web site[6] addresses institutions that do not have internal IRBs and provides options that include negotiating agreements with other institutions to have research reviewed as well as the use of commercial or independent IRBs. Finally, a November 2005 workshop summary report on "Alternative Models of IRB Review"[7] sponsored by the National Institutes of Health, OHRP, AAMC, and the American Society for Clinical Oncology explored the use of up to 10 alternative models, such as sharing materials among local IRBs, institutions relying on review by the IRB of another institution, and sites forming consortia to use a single IRB in a collaborative process. Although acceptance of the use of such alternative models for IRBs has not yet been indicated in updated guidance from OHRP or the Food and Drug Administration, the trend toward collaborative efforts is a topic that is actively under discussion and offers the possibility of more efficient and timely IRB (and, by analogy, ESCRO committee) review. The Tri-Institutional ESCRO Committee established by Rockefeller University, Memorial-Sloan Kettering Cancer Center, and Weill Medical College of Cornell University is an example of a single committee serving three research

[5]See <http://www.ncicirb.org/> for more information about the initiative.
[6]<http://www.hhs.gov/ohrp/faq.html>.
[7]<http://www.hhs.gov/ohrp/sachrp/documents/AltModIRB.pdf>.

institutions. Although the Committee on Guidelines for Human Embryonic Stem Cell Research quite clearly intended to allow for the use of shared or central ESCRO committees, it failed to state that explicitly. Therefore, Section 2.0 of the Guidelines is amended. (New wording is underlined.)

For projects involving more than one institution, there have also been concerns about the difficulty of multiple ESCRO committee reviews. Section 2.1 is added to explicitly allow—but not require—that multi-institution collaborations can be reviewed by a single ESCRO committee.

2.0 ESTABLISHMENT OF AN INSTITUTIONAL EMBRYONIC STEM CELL RESEARCH OVERSIGHT COMMITTEE

To provide oversight of all issues related to derivation and use of hES cell lines and to facilitate education of investigators involved in hES cell research, each institution should have activities involving hES cells overseen by an Embryonic Stem Cell Research Oversight (ESCRO) committee. This committee could be internal to a single institution or established jointly with one or more other institutions. Alternatively, an institution may have its proposals reviewed by an ESCRO committee of another institution, or by an independent ESCRO committee. An ESCRO committee should include independent representatives of the lay public as well as persons with expertise in developmental biology, stem cell research, molecular biology, assisted reproduction, and ethical and legal issues in hES cell research. It must have suitable scientific, medical, and ethical expertise to conduct its own review and should have the resources needed to coordinate the management of the various other reviews required for a particular protocol. A preexisting committee could serve the functions of the ESCRO committee provided that it has the recommended expertise and representation to perform the various roles described in this report. For example, an institution might elect to constitute an ESCRO committee from among some members of an IRB. But the ESCRO committee should not be a subcommittee of the IRB, as its responsibilities extend beyond human subject protections. Furthermore, much hES cell research does not require IRB review. The ESCRO committee should

(1) provide oversight over all issues related to derivation and use of hES cell lines,
(2) review and approve the scientific merit of research protocols,
(3) review compliance of all in-house hES cell research with all relevant regulations and these guidelines,
(4) maintain registries of hES cell research conducted at the institution and hES cell lines derived or imported by institutional investigators, and
(5) facilitate education of investigators involved in hES cell research.

An institution that uses an external ESCRO committee should nevertheless ensure that the registry and educational functions of an internal ESCRO committee are carried out by the external ESCRO committee on its behalf or internally by other administrative units.

2.1 For projects that involve more than one institution, review of the scientific merit, justification, and compliance status of the research may be carried out by a single ESCRO committee if all participating institutions agree to accept the results of the review.

FROZEN IVF BLASTOCYSTS DERIVED FROM ANONYMOUS SPERM DONORS: ABSENCE OF INFORMED CONSENT

Members of the scientific community raised concerns that the National Academies' Guidelines require that donors of all embryos, gametes, and somatic cells give informed consent for the use of their tissues for the derivation of human embryonic stem cell lines. Specifically, Section 3.3 of the Guidelines states that "When donor gametes have been used in the IVF process, resulting blastocysts may not be used for research without consent of all gamete donors." This requirement might preclude the use of frozen blastocysts from IVF clinics, which do not customarily request informed consent from sperm donors. The Committee, therefore, was asked to consider the effects this requirement might have on the available supply of

blastocysts for hES cell research and whether the population of frozen blastocysts now residing at IVF clinics needs to be "grandfathered" or exempt from the requirement for sperm donor consent.

To evaluate these effects, the Committee contacted the Society for Assisted Reproductive Technology (SART), which is actively involved in the collection of data on outcomes from its member IVF clinics. SART works closely with the Centers for Disease Control and Prevention in compliance with the Fertility Clinic Success Rate and Certification Act of 1992 (Wyden Act) to reflect accurately outcomes of the procedures commonly used in IVF practices.[8] The information returned in response to the Committee's request indicated that the number of blastocysts created with anonymous donor sperm in SART member practices is only about 3.5 percent.[9]

Given this small number, it is the Committee's view that maintaining the requirement for sperm donor consent in cases where human embryonic stem cell lines are to be derived from excess IVF clinic blastocysts should not significantly affect the availability of blastocysts for donation to research. The Committee, therefore, has concluded that it is not necessary to modify the Guidelines by "grandfathering" the frozen embryo population in IVF clinics and exempting them from the informed consent requirement for sperm donors. In light of the inability to determine whether any of these donors would have foregone sperm donation had they known of possible nonreproductive uses of the resulting blastocysts, the existing Guidelines reasonably balance respect for the gamete donors' expectations with the needs of the research community.

CONSIDERING THE SCIENCE IN hES CELL RESEARCH PROPOSALS: ADVICE FOR ESCRO COMMITTEES

It has been brought to the Committee's attention that some ESCRO committees would appreciate additional guidance on how to evaluate research proposals that are submitted for ESCRO committee review. In several places, the Guidelines emphasize the need to consider the scientific rationale for an experiment as part of the ethical analysis of the experiment. Although this section of this report does not amend the Guidelines, the

[8]See <http://www.sart.org/WhatIsSART.html> for more information about this data collection effort.

[9]2004 SART CORS© database.

Committee has compiled a list of questions that ESCRO committees may wish to consider when evaluating the scientific aspects of proposals for research involving hES cells. Many of these questions are contained in the 2005 report *Guidelines for Human Embryonic Stem Cell Research* but are distributed throughout the report. Not all of these questions will be applicable to every situation. Neither will answers to these questions necessarily be definitive with respect to the acceptability of the proposed research. Their goal is to ensure that the relevant scientific and ethical issues are considered.

Sample Questions for Reviewing hES Cell Research

- What is the scientific question being asked by the proposed research involving hES cells? Does the underlying hypothesis address an important scientific question? Could the question reasonably be addressed in any other way?
- Does the research team have the appropriate expertise and training in deriving or culturing either human or nonhuman stem cells? If training is the primary purpose of the proposal, is the training being conducted under the supervision of appropriate experts?
- Has the investigator articulated a compelling rationale for using human stem cells instead of nonhuman stem cells?
- Has the investigator articulated a compelling rationale for using hES cells instead of other types of stem cells?
- Has the investigator justified the selection of the stem cell line(s) to be used?
- Has the investigator articulated a rationale for creating a new stem cell line or could the proposed research be conducted with existing cell lines? If more than one cell line is to be derived, has the investigator justified the number he/she proposes to make?

Additional questions arise in considering protocols involving introduction of hES cells or cellular derivatives thereof into an animal host to form a chimera. Some of those questions were addressed in the 2005 *Guidelines for Human Embryonic Stem Cell Research*, and the committee intends to revisit these issues in future discussions.

Appendix A
National Academies' Guidelines for Human Embryonic Stem Cell Research, Amended as of February 2007[1]

1.0 Introduction
2.0 Establishment of an Institutional Embryonic Stem Cell Research Oversight Committee
3.0 Procurement of Gametes, Blastocysts, or Cells for hES Generation
4.0 Derivation of hES Cell Lines
5.0 Banking and Distribution of hES Cell Lines
6.0 Research Use of hES Cell Lines
7.0 International Collaboration
8.0 Conclusion

1.0 INTRODUCTION

In this chapter we collect all the recommendations made throughout the report and translate them into a series of formal guidelines. These guidelines focus on the derivation, procurement, banking, and use of human embryonic stem (hES) cell lines. They provide an oversight process that will help to ensure that research with hES cells is conducted in a responsible and ethically sensitive manner and in compliance with all regulatory requirements

[1]New or modified wording is indicated by underlining.

pertaining to biomedical research in general. The National Academies are issuing these guidelines for the use of the scientific community, including researchers in university, industry, or other private-sector research organizations.

1.1(a) What These Guidelines Cover

These guidelines cover all derivation of hES cell lines and all research that uses hES cells derived from

(1) blastocysts made for reproductive purposes and later obtained for research from in vitro fertilization (IVF) clinics,
(2) blastocysts made specifically for research using IVF,
(3) Somatic cell nuclear transfer (NT) into oocytes.

The guidelines do not cover research that uses nonhuman stem cells.

Many, but not all, of the guidelines and concerns addressed in this report are common to other areas of human stem cell research, such as

(1) research that uses human adult stem cells,
(2) research that uses fetal stem cells or embryonic germ cells derived from fetal tissue; such research is covered by federal statutory restrictions at 42 U.S.C. 289g-2(a) and federal regulations at 45 CFR 46.210.

Institutions and investigators conducting research using such materials should consider which individual provisions of these guidelines are relevant to their research.

1.1(b) Reproductive Uses of NT

These guidelines also do not apply to reproductive uses of nuclear transfer (NT), which are addressed in the 2002 report *Scientific and Medical Aspects of Human Reproductive Cloning*, in which the National Academies recommended that "Human reproductive cloning should not now be practiced. It is dangerous and likely to fail." Although these guidelines do not specifically address human reproductive cloning, it continues to be the view of the National Academies that research aimed at the reproductive cloning of a human being should not be conducted at this time.

1.2 Categories of hES Cell Research

These guidelines specify categories of research that

(a) Are permissible after currently mandated reviews and proper notification of the relevant research institution.
(b) Are permissible after additional review by an Embryonic Stem Cell Research Oversight (ESCRO) committee, as described in Section 2.0 of the guidelines.
(c) Should not be conducted at this time.

Because of the sensitive nature of some aspects of hES cell research, these guidelines in many instances set a higher standard than is required by laws or regulations with which institutions and individuals already must comply.

1.2(a) hES Cell Research Permissible After Currently Mandated Reviews

Purely in vitro hES cell research that uses previously derived hES cell lines is permissible provided that the ESCRO committee or equivalent body designated by the investigator's institution (see Section 2.0) receives documentation of the provenance of the cell lines including (i) documentation of the use of an acceptable informed consent process that was approved by an Institutional Review Board (IRB) or foreign equivalent for their derivation (consistent with Section 3.6); and (ii) documentation of compliance with any additional required review by an Institutional Animal Care and Use Committee (IACUC), Institutional Biosafety Committee (IBC), or other institutionally mandated review.

1.2(b) hES Cell Research Permissible Only After Additional Review and Approval

(1) Generation of new lines of hES cells by whatever means.
(2) Research involving the introduction of hES cells into nonhuman animals at any stage of embryonic, fetal, or postnatal development; particular attention should be paid to the probable pattern and effects of differentiation and integration of the human cells into the nonhuman animal tissues.
(3) Research in which the identity of the donors of blastocysts, gametes, or somatic cells from which the hES cells were derived is readily ascertainable or might become known to the investigator.

1.2(c) hES Cell Research That Should Not Be Permitted at This Time

The following types of research should not be conducted at this time:

(1) Research involving in vitro culture of any intact human embryo, regardless of derivation method, for longer than 14 days or until formation of the primitive streak begins, whichever occurs first.
(2) Research in which hES cells are introduced into nonhuman primate blastocysts or in which any embryonic stem cells are introduced into human blastocysts.

In addition:

(3) No animal into which hES cells have been introduced at any stage of development should be allowed to breed.

1.3 Obligations of Investigators and Institutions

All scientific investigators and their institutions, regardless of their field, bear the ultimate responsibility for ensuring that they conduct themselves in accordance with professional standards and with integrity. In particular, people whose research involves hES cells should work closely with oversight bodies, demonstrate respect for the autonomy and privacy of those who donate gametes, blastocysts, or somatic cells, and be sensitive to public concerns about research that involves human embryos.

1.4 Use of NIH-Approved hES Cell Lines

(a) It is acceptable to use hES cell lines that were approved in August 2001 for use in U.S. federally funded research.

(b) ESCRO committees should include on their registry a list of NIH-approved cell lines that have been used at their institution in accord with the requirement in Section 2.0 of the Guidelines.

(c) Presence on the list of NIH-approved cell lines constitutes adequate documentation of provenance, as per Section 6.1 of the Guidelines.

1.5 Acceptability of Research Using hES Cell Lines Imported from Other Institutions or Jurisdictions

(a) Before approving use of hES cell lines imported from other institutions or jurisdictions, ESCRO committees should consider whether such cell lines have been "acceptably derived."

(b) "Acceptably derived" means that the cell lines were derived from gametes or embryos for which

(1) The donation protocol was reviewed and approved by an IRB or, in the case of donations taking place outside the United States, a substantially equivalent oversight body;
(2) Consent to donate was voluntary and informed;
(3) Donation was made with reimbursement policies consistent with these Guidelines; and
(4) Donation and derivation complied with the extant legal requirements of the relevant jurisdiction.

(c) ESCRO committees should include on their registry a list of cell lines that have been imported from other institutions or jurisdictions and information on the specific guidelines, regulations, or statutes under which the derivation of the imported cell lines was conducted. This is in accord with the requirement in Section 2.0 of the Guidelines that calls for ESCRO committees to maintain registries listing the cell lines in use at their institutions.

2.0 ESTABLISHMENT OF AN INSTITUTIONAL EMBRYONIC STEM CELL RESEARCH OVERSIGHT COMMITTEE

To provide oversight of all issues related to derivation and use of hES cell lines and to facilitate education of investigators involved in hES cell research, each institution should have activities involving hES cells overseen by an Embryonic Stem Cell Research Oversight (ESCRO) committee. This committee could be internal to a single institution or established jointly with one or more other institutions. Alternatively, an institution may have its proposals reviewed by an ESCRO committee of another institution, or by an independent ESCRO committee. An ESCRO committee should include independent representatives of the lay public as well as persons with expertise in developmental biology, stem cell research, molecular biology, assisted reproduction, and ethical and legal issues in hES cell research. It must have suitable scientific, medical, and ethical expertise to conduct its own review

and should have the resources needed to coordinate the management of the various other reviews required for a particular protocol. A preexisting committee could serve the functions of the ESCRO committee provided that it has the recommended expertise and representation to perform the various roles described in this report. For example, an institution might elect to constitute an ESCRO committee from among some members of an IRB. But the ESCRO committee should not be a subcommittee of the IRB, as its responsibilities extend beyond human subject protections. Furthermore, much hES cell research does not require IRB review. The ESCRO committee should

(1) provide oversight over all issues related to derivation and use of hES cell lines,
(2) review and approve the scientific merit of research protocols,
(3) review compliance of all in-house hES cell research with all relevant regulations and these guidelines,
(4) maintain registries of hES cell research conducted at the institution and hES cell lines derived or imported by institutional investigators, and
(5) facilitate education of investigators involved in hES cell research.

An institution that uses an external ESCRO committee should nevertheless ensure that the registry and educational functions of an internal ESCRO committee are carried out by the external ESCRO committee on its behalf or internally by other administrative units.

2.1 For projects that involve more than one institution, review of the scientific merit, justification, and compliance status of the research may be carried out by a single ESCRO committee if all participating institutions agree to accept the results of the review.

3.0 PROCUREMENT OF GAMETES, BLASTOCYSTS, OR CELLS FOR hES GENERATION

3.1 An IRB, as described in federal regulations at 45 CFR 46.107, should review the procurement of all gametes, blastocysts, or somatic cells for the purpose of generating new hES cell lines, including the procurement of blastocysts in excess of clinical need from infertility clinics, blastocysts made through IVF specifically for research purposes, and oocytes, sperm, and

somatic cells donated for development of hES cell lines derived through NT or by parthenogenesis or androgenesis.

3.2 Consent for donation should be obtained from each donor at the time of donation. Even people who have given prior indication of their intent to donate to research any blastocysts that remain after clinical care should nonetheless give informed consent at the time of donation. Donors should be informed that they retain the right to withdraw consent until the blastocysts are actually used in cell-line derivation.

3.3 When donor gametes have been used in the IVF process, resulting blastocysts may not be used for research without consent of all gamete donors.

3.4a No payments, cash or in-kind, may be provided for donating blastocysts in excess of clinical need for research purposes. People who elect to donate stored blastocysts for research should not be reimbursed for the costs of storage prior to the decision to donate.

3.4b Women who undergo hormonal induction to generate oocytes specifically for research purposes (such as for NT) should be reimbursed only for direct expenses incurred as a result of the procedure, as determined by an IRB. No payments, cash or in-kind, should be provided for donating oocytes for research purposes. Similarly, no payments should be made for donations of sperm for research purposes or of somatic cells for use in NT.

3.5 To facilitate autonomous choice, decisions related to the creation of embryos for infertility treatment should be free of the influence of investigators who propose to derive or use hES cells in research. Whenever it is practicable, the attending physician responsible for the infertility treatment and the investigator deriving or proposing to use hES cells should not be the same person.

3.6 In the context of donation of gametes or blastocysts for hES cell research, the informed consent process, should, at a minimum, provide the following information:

(a) A statement that the blastocysts or gametes will be used to derive hES cells for research that may include research on human transplantation.

(b) A statement that the donation is made without any restriction or direction regarding who may be the recipient of transplants of the cells derived, except in the case of autologous donation.
(c) A statement as to whether the identities of the donors will be readily ascertainable to those who derive or work with the resulting hES cell lines.
(d) If the identities of the donors are retained (even if coded), a statement as to whether donors wish to be contacted in the future to receive information obtained through studies of the cell lines.
(e) An assurance that participants in research projects will follow applicable and appropriate best practices for donation, procurement, culture, and storage of cells and tissues to ensure, in particular, the traceability of stem cells. (Traceable information, however, must be secured to ensure confidentiality.)
(f) A statement that derived hES cells and/or cell lines might be kept for many years.
(g) A statement that the hES cells and/or cell lines might be used in research involving genetic manipulation of the cells or the mixing of human and nonhuman cells in animal models.
(h) Disclosure of the possibility that the results of study of the hES cells may have commercial potential and a statement that the donor will not receive financial or any other benefits from any future commercial development.
(i) A statement that the research is not intended to provide direct medical benefit to the donor(s) except in the case of autologous donation.
(j) A statement that embryos will be destroyed in the process of deriving hES cells.
(k) A statement that neither consenting nor refusing to donate embryos for research will affect the quality of any future care provided to potential donors.
(l) A statement of the risks involved to the donor.

In addition, donors could be offered the option of agreeing to some forms of hES cell research but not others. For example, donors might agree to have their materials used for deriving new hES cell lines but might not want their

materials used, for example, for NT. The consent process should fully explore whether donors have objections to any specific forms of research to ensure that their wishes are honored.

3.7 Clinical personnel who have a conscientious objection to hES cell research should not be required to participate in providing donor information or securing donor consent for research use of gametes or blastocysts. That privilege should not extend to the care of a donor or recipient.

3.8 Researchers may not ask members of the infertility treatment team to generate more oocytes than necessary for the optimal chance of reproductive success. An infertility clinic or other third party responsible for obtaining consent or collecting materials should not be able to pay for or be paid for the material obtained (except for specifically defined cost-based reimbursements and payments for professional services).

4.0 DERIVATION OF hES CELL LINES

4.1 Requests to the ESCRO committee for permission to attempt derivation of new hES cell lines from donated embryos or blastocysts must include evidence of IRB approval of the procurement process (see Section 3.0 above).

4.2 The scientific rationale for the need to generate new hES cell lines, by whatever means, must be clearly presented, and the basis for the numbers of embryos and blastocysts needed should be justified.

4.3 Research teams should demonstrate appropriate expertise or training in derivation or culture of either human or nonhuman ES cells before permission to derive new lines is given.

4.4 When NT experiments involving either human or nonhuman oocytes are proposed as a route to generation of ES cells, the protocol must have a strong scientific rationale. Proposals that include studies to find alternatives to donated oocytes in this research should be encouraged.

4.5 Neither blastocysts made using NT (whether produced with human or nonhuman oocytes) nor parthenogenetic or androgenetic human embryos may be transferred to a human or nonhuman uterus or cultured as intact embryos in vitro for longer than 14 days or until formation of the primitive streak, whichever occurs first.

4.6 Investigators must document how they will characterize, validate, store, and distribute any new hES cell lines and how they will maintain the confidentiality of any coded or identifiable information associated with the lines (see Section 5.0 below).

5.0 BANKING AND DISTRIBUTION OF hES CELL LINES

There are several models for the banking of human biological materials, including hES cells. The most relevant is the U.K. Stem Cell Bank. The guidelines developed by this and other groups generally adhere to key ethical principles that focus on the need for consent of donors and a system for monitoring adherence to ethical, legal, and scientific requirements. As hES cell research advances, it will be increasingly important for institutions that are obtaining, storing, and using cell lines to have confidence in the value of stored cells—that is, that they were obtained ethically and with the informed consent of donors, that they are well characterized and screened for safety, and that the conditions under which they are maintained and stored meet the highest scientific standards. Institutions engaged in hES research should seek mechanisms for establishing central repositories for hES cell lines—through partnerships or augmentation of existing quality research cell line repositories and should adhere to high ethical, legal, and scientific standards. At a minimum, an institutional registry of stem cell lines should be maintained.

5.1 Institutions that are banking or plan to bank hES cell lines should establish uniform guidelines to ensure that donors of material give informed consent through a process approved by an IRB and that meticulous records are maintained about all aspects of cell culture. Uniform tracking systems and common guidelines for distribution of cells should be established.

5.2 Any facility engaged in obtaining and storing hES cell lines should consider the following standards:

(a) Creation of a committee for policy and oversight purposes and creation of clear and standardized protocols for banking and withdrawals.

(b) Documentation requirements for investigators and sites that deposit cell lines, including

(i) A copy of the donor consent form.
(ii) Proof of Institutional Review Board approval of the procurement process.
(iii) Available medical information on the donors, including results of infectious-disease screening.
(iv) Available clinical, observational, or diagnostic information about the donor(s).
(v) Critical information about culture conditions (such as media, cell passage, and safety information).
(vi) Available cell line characterization (such as karyotype and genetic markers).

A repository has the right of refusal if prior culture conditions or other items do not meet its standards.

(c) A secure system for protecting the privacy of donors when materials retain codes or identifiable information, including but not limited to
  (i) A schema for maintaining confidentiality (such as a coding system).
  (ii) A system for a secure audit trail from primary cell lines to those submitted to the repository.
  (iii) A policy governing whether and how to deliver clinically significant information back to donors.

(d) The following standard practices:
  (i) Assignment of a unique identifier to each sample.
  (ii) A process for characterizing cell lines.
  (iii) A process for expanding, maintaining, and storing cell lines.
  (iv) A system for quality assurance and control.
  (v) A Web site that contains scientific descriptions and data related to the cell lines available.
  (vi) A procedure for reviewing applications for cell lines.
  (vii) A process for tracking disbursed cell lines and recording their status when shipped (such as number of passages).
  (viii) A system for auditing compliance.
  (ix) A schedule of charges.
  (x) A statement of intellectual property policies.

(xi) When appropriate, creation of a clear Material Transfer Agreement or user agreement.
(xii) A liability statement.
(xiii) A system for disposal of material.

(e) Clear criteria for distribution of cell lines, including but not limited to evidence of approval of the research by an ESCRO committee or equivalent body at the recipient institution.

6.0 RESEARCH USE OF hES CELL LINES

Once hES cell lines have been derived, investigators and institutions, through ESCRO committees and other relevant committees (such as an IACUC, an IBC, or a radiation safety committee) should monitor their use in research.

6.1 Institutions should require documentation of the provenance of all hES cell lines, whether the cells were imported into the institution or generated locally. Notice to the institution should include evidence of IRB approval of the procurement process and of adherence to basic ethical and legal principles of procurement. In the case of lines imported from another institution, documentation that these criteria were met at the time of derivation will suffice.

6.2 In vitro experiments involving the use of already derived and coded hES cell lines will not need review beyond the notification required in Section 6.1.

6.3 Each institution should maintain a registry of its investigators who are conducting hES cell research and ensure that all registered users are kept up to date with changes in guidelines and regulations regarding the use of hES cells.

6.4 All protocols involving the combination of hES cells with nonhuman embryos, fetuses, or adult animals must be submitted to the local IACUC for review of animal welfare issues and to the ESCRO committee for consideration of the consequences of the human contributions to the resulting chimeras. (See also Section 1.2(c)(3) concerning breeding of chimeras.)

6.5 Transplantation of differentiated derivatives of hES cells or even hES cells themselves into adult animals will not require extensive ESCRO committee review. If there is a possibility that the human cells could contribute in a major organized way to the brain of the recipient animal, however, the scientific justification for the experiments must be strong, and proof of principle using nonhuman (preferably primate) cells, is desirable.

6.6 Experiments in which hES cells, their derivatives, or other pluripotent cells are introduced into nonhuman fetuses and allowed to develop into adult chimeras need more careful consideration because the extent of human contribution to the resulting animal may be higher. Consideration of any major functional contributions to the brain should be a main focus of review. (See also Section 1.2(c)(3) concerning breeding of chimeras.)

6.7 Introduction of hES cells into nonhuman mammalian blastocysts should be considered only under circumstances in which no other experiment can provide the information needed. (See also Sections 1.2(c)(2) and 1.2(c)(3) concerning restrictions on breeding of chimeras and production of chimeras with nonhuman primate blastocysts.)

6.8 Research use of existing hES cells does not require IRB review unless the research involves introduction of the hES cells or their derivatives into patients or the possibility that the identity of the donors of the blastocysts, gametes, or somatic cells is readily ascertainable or might become known to the investigator.

7.0 INTERNATIONAL COLLABORATION

If a U.S.-based investigator collaborates with an investigator in another country, the ESCRO committee may determine that the procedures prescribed by the foreign institution afford protections consistent with these guidelines, and the ESCRO committee may approve the substitution of some of or all of the foreign procedures for its own.

8.0 CONCLUSION

The substantial public support for hES cell research and the growing trend by many nonfederal funding agencies and state legislatures to support this field requires a set of guidelines to provide a framework for hES cell re-

search. In the absence of the oversight that would come with unrestricted federal funding of this research, these guidelines will offer reassurance to the public and to Congress that the scientific community is attentive to ethical concerns and is capable of self-regulation while moving forward with this important research.

To help ensure that these guidelines are taken seriously, stakeholders in hES cell research—sponsors, funding sources, research institutions, relevant oversight committees, professional societies, and scientific journals, as well as investigators—should develop policies and practices that are consistent with the principles inherent in these guidelines. Funding agencies, professional societies, journals, and institutional review panels can provide valuable community pressure and impose appropriate sanctions to ensure compliance. For example, ESCRO committees and IRBs should require evidence of compliance when protocols are reviewed for renewal, funding agencies should assess compliance when reviewing applications for support, and journals should require that evidence of compliance accompanies publication of results.

As individual states and private entities move into hES cell research, it will be important to initiate a national effort to provide a formal context in which the complex moral and oversight questions associated with this work can be addressed on a continuing basis. Both the state of hES cell research and clinical practice and public policy surrounding these topics are in a state of flux and are likely to be so for several years. Therefore, the committee believes that a national body should be established to assess periodically the adequacy of the policies and guidelines proposed in this document and to provide a forum for a continuing discussion of issues involved in hES cell research. New policies and standards may be appropriate for issues that cannot now be foreseen. The organization that sponsors this body should be politically independent and without conflicts of interest, should be respected in the lay and scientific communities, and able to call on suitable expertise to support this effort.

Appendix B

Committee Biographical Sketches

CO-CHAIRS

R. Alta Charo, J.D., is the Warren P. Knowles Professor of Law and Bioethics at the University of Wisconsin–Madison, on the faculties of both the Law School and Medical School, and, in 2006, was Visiting Professor of Law at the University of California, Berkeley, Boalt Hall School of Law. Professor Charo is the author of nearly 100 articles, book chapters, and government reports on topics including voting rights, environmental law, family planning and abortion law, medical genetics law, reproductive technology policy, science policy, and medical ethics. Professor Charo is a member of the boards of the Alan Guttmacher Institute and the Foundation for Genetic Medicine, a member of the National Medical Advisory Committee of the Planned Parenthood Federation of America, and a member of the ethics advisory boards of the International Society for Stem Cell Research, the Juvenile Diabetes Research Foundation and WiCell. In 2005, she was appointed to the ethics standards working group of the California Institute for Regenerative Medicine and was elected as a fellow of the Wisconsin Academy of Sciences, Arts, and Letters. In 1994, Professor Charo served on the NIH Human Embryo Research Panel, and from 1996 to 2001 she was a member of the presidential National Bioethics Advisory Commission where she participated in drafting its reports on *Cloning Human Beings* (1997); *Research Involving Persons with Mental Disorders That May Affect*

Decisionmaking Capacity (1998); *Research Involving Human Biological Materials: Ethical Issues and Policy Guidance* (1999); *Ethical Issues in Human Stem Cell Research* (1999); *Ethical and Policy Issues in International Research: Clinical Trials in Developing Countries*, and *Ethical and Policy Issues in Research Involving Human Participants* (2001). Since 2001, she has been a member of the National Academies' Board on Life Sciences and since 2006, she has been a member of the Institute of Medicine's Board on Population Health and Public Health Practices. Professor Charo was elected to the Institute of Medicine in 2006.

Richard O. Hynes, Ph.D., is the Daniel K. Ludwig Professor for Cancer Research at the MIT Center for Cancer Research and Department of Biology, and a Howard Hughes Medical Institute investigator. He was formerly head of the Biology Department and then director of the Center for Cancer Research at MIT. His research focuses on fibronectins and integrins and the molecular basis of cellular adhesion, both in normal development and in pathological situations, such as cancer, thrombosis, and inflammation. Dr. Hynes' current interests are cancer invasion and metastasis, angiogenesis, and animal models of human disease states. He is a member of the National Academy of Sciences and the Institute of Medicine and is a Fellow of the Royal Society of London and the American Academy of Arts and Sciences. In 1997, he received the Gairdner International Foundation Award. In 2000, he served as president of the American Society for Cell Biology and testified before Congress about the need for federal support and oversight of embryonic stem cell research. He co-chaired the 2005 National Academies' *Guidelines for Human Embryonic Stem Cell Research*.

MEMBERS

Eli Y. Adashi, M.D., M.S., FACOG, is currently the Dean of Medicine and Biological Sciences and the Frank L. Day Professor of Biology, the Warren Alpert Medical School of Brown University. Previously, Dr. Adashi served as the professor and chair of the Department of Obstetrics and Gynecology at the University of Utah Health Sciences Center. Dr. Adashi is a member of the Institute of Medicine of the National Academies, a member of the Association of American Physicians, and a fellow of the American Association for the Advancement of Science. Dr. Adashi is a former member of the Advisory Council of the National Institute of Child Health and Human Development and a former president of the Society for Reproductive Endo-

crinologists, the Society for Gynecologic Investigation, and the American Gynecological and Obstetrical Society. Dr. Adashi is also a former examiner and director of the Division of Reproductive Endocrinology of the American Board of Obstetrics and Gynecology. Finally, Dr. Adashi is a founding member and treasurer and, more recently, chair of the advisory committee of the Geneva-based Bertarelli Foundation, dedicated to promoting the welfare of the infertile couple and to addressing the current "epidemic" of high-order multiple gestations.

Brigid L.M. Hogan, Ph.D., is the George Barth Geller Professor and Chair of the Department of Cell Biology, Duke University Medical Center. Prior to joining Duke, Dr. Hogan was an Investigator of the Howard Hughes Medical Institute and Hortense B. Ingram Professor in the Department of Cell Biology at Vanderbilt University Medical Center. Dr. Hogan earned her Ph.D. in Biochemistry at the University of Cambridge. After completing her Ph.D. she was a postdoctoral fellow in the Department of Biology at MIT. Before moving to the United States in 1988, Dr. Hogan was head of the Molecular Embryology Laboratory at the National Institute for Medical Research in London. Her research focuses on the genetic control of embryonic development and morphogenesis, using the mouse as a model system. Her laboratory developed methods for deriving mouse pluripotential embryonic germ (EG) cell lines. She was Co-Chair for Science of the 1994 NIH Human Embryo Research Panel and a member of the 2001/2002 National Academies' Panel on Scientific and Medical Aspects of Human Cloning. Within the past few years, Dr. Hogan has been elected to the Royal Society of London, the American Academy of Arts and Sciences, the Institute of Medicine, and the National Academy of Sciences.

Marcia Imbrescia is the current owner of Peartree Design, a landscape design firm, and was previously the media director for Drumbeater, a high-technology advertising agency. She holds B.A. degrees in marketing and journalism, and a graduate certificate in landscape design. Ms. Imbrescia has a passion for health advocacy and helping people with illness and disability. She is a member of the Board of Trustees of the Arthritis Foundation (AF), for which she has participated as a volunteer at the chapter and national levels. She served as member (1996–1998, 2001) and chairperson (2002–2003) of AF's American Juvenile Arthritis Organization. In 1992, she received the Volunteer of the Year Award from the Massachusetts Chapter of AF. Her volunteer efforts include program development, conference planning, public speaking, fundraising, and advocacy. She served on the

National Academies' Committee on Guidelines for Human Embryonic Stem Cell Research in 2004–2005.

Terry Magnuson, Ph.D., is Sarah Graham Kenan Professor and chair of the Department of Genetics at the University of North Carolina. He also directs the Carolina Center for Genome Sciences, and is the program director of cancer genetics at the Lineberger Comprehensive Cancer Center. Dr. Magnuson's research interests include mammalian genetics, genomics, and development. His laboratory has developed a high-throughput system to study the effects of mutations on mouse development with mouse embryonic stem cells. He is particularly interested in the role of chromatin remodeling complexes in processes such as autosomal imprinting, X-inactivation, and anterior-posterior patterning of axial structures in mammals. He is a member of the Board of Directors of the Genetics Society of America and of the Society for Developmental Biology.

Linda B. Miller, OTR, M.S. in Hospital Administration, is President of the Washington, D.C.-based Volunteer Trustees Foundation, a consortium of not-for-profit hospital governing boards. She has extensive experience in trustee education, advocacy, and the legal, ethical, and policy issues facing voluntary health care institutions. Recently, she has worked closely with the states' attorneys general in developing guidelines for protecting the community interest in the sale and conversion of nonprofit hospitals, as well as in designing models for practice and legal oversight. She was elected to membership in the Institute of Medicine in 1997.

Ms. Miller has been a frequent speaker on health policy issues and has been published extensively in both the medical and popular press, including the *New England Journal of Medicine*, *Health Affairs*, *USA Today*, the *Washington Post,* and *New York Times,* among others. She served as a Special Assistant to the Secretary of Health, Education and Welfare (now HHS) and on numerous health-related policy councils and advisory committees, including the National Institutes of Health's Consensus Panel on Liver Transplantation and, most recently, the Institute of Medicine's Committee on Spinal Cord Injury. Ms. Miller currently serves on the Advisory Board of the University of Louisville-based Institute for Cellular Therapeutics, headed by Dr. Suzanne Ildstad, which does research in adult bone marrow transplant, and has been a member of several academic and health care institutions' board of governors, including Blythedale Childrens Hospital in New York, Capital Hospice in the national capital region, and Cornell University's Alumni Council, among others.

Jonathan D. Moreno, Ph.D., is the David and Lyn Silfen University Professor at the University of Pennsylvania. Until 2007, he was the Emily Davie and Joseph S. Kornfeld Professor of Biomedical Ethics at the University of Virginia where he also directed the Center for Biomedical Ethics. Dr. Moreno is a member of the Institute of Medicine of the National Academies. He is also a bioethics advisor for the Howard Hughes Medical Institute, a faculty affiliate of the Kennedy Institute of Ethics at Georgetown University, and a Fellow of the Hastings Center. During 1995–1996 he was Senior Policy and Research Analyst for the President's Advisory Committee on Human Radiation Experiments and during 1998–2000 was a senior consultant for the National Bioethics Advisory Commission. He co-chaired the 2005 National Academies' Committee on Guidelines for Human Embryonic Stem Cell Research and is a consultant to the Ethical, Social, and Cultural Program of the Bill & Melinda Gates Foundation Grand Challenges in Global Health initiative, for ethical and regulatory issues regarding stem cell research in China.

Stuart H. Orkin,[1] M.D., is the David G. Nathan Professor of Pediatrics at Harvard Medical School, Chair of the Department of Pediatric Onocology at the Dana-Farber Cancer Institute, and an Investigator with the Howard Hughes Medical Institute. His laboratory utilizes multidisciplinary approaches to understand how mammalian cells choose specific fates and how mutations in important transcriptional regulators lead to developmental defects or malignancy. Recent and ongoing work falls into several overlapping areas, including study of essential hematopoietic transcription factors, the genetic pathogenesis of two forms of leukemia, and whether some of the lessons of hematopoiesis may be applied to consideration of the pathogenesis of solid tumors. Finally, the fundamental properties of stem cells—pluripotency and self-renewal—are being addressed from a biochemical perspective in mouse embryonic stem (ES) cells. In the future, his laboratory will pursue the functions of the associated proteins in order to unravel the biochemistry of ES fate specification. This strategy may ultimately suggest how directed manipulation of somatic cells to an ES cell fate might be achieved.

Pilar N. Ossorio, Ph.D., J.D., is Associate Professor of Law and Bioethics at the University of Wisconsin–Madison, and Program Faculty in the Graduate

[1]Resigned from committee effective December 18, 2006.

Program in Population Health at UW. Prior to taking her position at UW, she was Director of the Genetics Section at the Institute for Ethics at the American Medical Association, and taught as an adjunct faculty member at the University of Chicago Law School. For the 2006 calendar year, Professor Ossorio was a visiting professor of law at the University of California, Berkeley, Boalt Hall School of Law.

Dr. Ossorio received her Ph.D. in Microbiology and Immunology in 1990 from Stanford University. She went on to complete a postdoctoral fellowship in cell biology at Yale University School of Medicine. Throughout the early 1990s, Dr. Ossorio also worked as a consultant for the federal program on the Ethical, Legal, and Social Implications (ELSI) of the Human Genome Project, and in 1994, she took a full-time position with the Department of Energy's ELSI program. In 1993, she served on the Ethics Working Group for President Clinton's Health Care Reform Task Force. Dr. Ossorio received her J.D. from the University of California at Berkeley School of Law (Boalt Hall) in 1997. While at Boalt she was elected to the legal honor society Order of the Coif and received several awards for outstanding legal scholarship.

Dr. Ossorio is a fellow of the American Association for the Advancement of Science (AAAS), on the editorial board of the *American Journal of Bioethics*, an advisor to NHGRI on ethical issues in large-scale sequencing, and a member of UW's institutional review board for health sciences research. She is a past member of AAAS's Committee on Scientific Freedom and Responsibility, a past member of the National Cancer Policy Board (Institute of Medicine), and has been a member or chair of several working groups on genetics and ethics. She has published scholarly articles in bioethics, law, and molecular biology.

E. Albert Reece, M.D., Ph.D., is currently Dean of the University of Maryland School of Medicine and Vice President for Medical Affairs at the University of Maryland, Baltimore. Previously, he was Vice Chancellor and Dean of the University of Arkansas College of Medicine. Dr. Reece received his undergraduate degree from Long Island University, his M.D. (Magna Cum Laude) from New York University, his Ph.D. degree in biochemistry from the University of the West Indies, and his M.B.A. degree from the Fox School of Business and Management of Temple University. He completed a residency in OB/GYN at Columbia University–Presbyterian Hospital, and a fellowship in maternal-fetal medicine at Yale University School of Medicine. He served on the faculty at Yale for 10 years, and was the Chairman of the Department of Obstetrics, Gynecology, and Reproductive Sciences at

Temple University. Dr. Reece has published over 400 journal articles, book chapters, and abstracts, and 9 textbooks including *Diabetes in Pregnancy*; *Medicine of the Fetus & Mother*; and *Fundamentals of Ultrasound in Obstetrics & Gynecology*. He is an editor for the *Journal of Maternal-Fetal Medicine* and a reviewer for several other scientific journals. His research focuses on diabetes in pregnancy, birth defects, and prenatal diagnosis. Dr. Reece is a member of the Institute of Medicine.

Joshua R. Sanes, Ph.D., is Professor of Molecular and Cellular Biology and the Paul J. Finnegan Family Director of the Center for Brain Science at Harvard University. He was previously Alumni Endowed Professor of Neurobiology at the Washington University School of Medicine. Dr. Sanes earned a B.A. in biochemistry and psychology at Yale and a Ph.D. in Neurobiology at Harvard. He studies the formation of the synapses that interconnect nerve cells, including pioneering work on the signals exchanged between nerve cells and their target muscles as new connections are made. He is also using the vertebrate visual system to examine how nerve cells develop and migrate to the right location in the body. He was elected a Fellow of the American Association for the Advancement of Science in 1992 and a member of the National Academy of Sciences in 2002.

Harold T. Shapiro, Ph.D., is President Emeritus of both Princeton University and the University of Michigan and is currently Professor of Economics and Public Affairs at Princeton University. His research interests include bioethics, the social role of higher education, hospital/medical center administration, university administration, econometrics, statistics, and economics. Dr. Shapiro currently chairs the Board of Trustees of the Alfred P. Sloan Foundation, is presiding director for the Dow Chemical Company, and is a member of numerous boards including the Robert Wood Johnson Medical School, HCA, the Merck Vaccine Advisory Board, the Knight Foundation Commission on Intercollegiate Athletics, U.S. Olympic Committee, and the Stem Cell Institute of New Jersey. He is a former Chair of the Association of American Universities and the National Bioethics Advisory Committee and Vice Chair of the President's Council of Advisors on Science and Technology. He has also served on the Board of Directors of the National Bureau of Economic Research, Inc. and the Board of Trustees of the Universities Research Association, Inc. He has chaired and served on numerous National Academies committees including the Committee on the Organizational Structure of the National Institutes of Health and the Committee on Particle Physics. Dr. Shapiro was awarded the 2006 American Association for the

Advancement of Science's William D. Carey Lecture for his leadership in science policy. He earned a Ph.D. in economics from Princeton University and holds 14 honorary doctorates.

John E. Wagner, Jr., M.D., is a professor of pediatrics at the University of Minnesota Medical School. He is the first recipient of the Children's Cancer Research Fund/Hageboeck Family Chair in Pediatric Oncology and also holds the Variety Club Endowed Chair in Molecular and Cellular Therapy. He is the director of the division of Pediatric Hematology/Oncology and Bone Marrow Transplantation and Scientific Director of Clinical Research of the Stem Cell Institute. Dr. Wagner is a member of numerous societies, including the American Society of Hematology, the International Society of Experimental Hematology, and the American Society of Blood and Marrow Transplantation. He is a member of several honorary societies including Alpha Omega Alpha (1980), the American Society of Clinical Investigation (2000), and the Association of American Physicians (2006). Dr. Wagner holds a patent on the isolation of the pluripotential quiescent stem cell population. Dr. Wagner holds a B.A. in Biological Sciences and a B.A. in Psychology from the University of Delaware and an M.D. from Jefferson Medical College. Dr. Wagner's research has focused on the development of novel cellular therapies for tissue repair and suppression of the immune response using subpopulations of neonatal umbilical cord blood and adult bone marrow and peripheral blood. Projects are funded by both NIH (P01 CA65493, Biology and Transplantation of the Human Stem Cell; and N01-HB-37164, Somatic Cell Therapeutics) and industry (ViaCell, Inc., on the transplantation of expanded umbilical cord blood hematopoietic stem cells; and Athersys, Inc., on the large-scale development of multipotent adult progenitor cells). In addition, Dr. Wagner pioneered the use of embryo selection to "create" a perfectly tissue matched stem cell donor in the treatment of genetic disease. Dr. Wagner has authored more than 180 articles and book chapters on the subject of hematopoietic stem cell transplantation. He currently co-chairs the Graft Sources and Manipulation Working Committee of the Center of the International Blood and Marrow Transplant Research, serves on the Scientific Board of Directors of the National Marrow Donor Program, and is a member of the Scientific and Medical Accountability Standards Working Group of the California Institute of Regenerative Medicine. Dr. Wagner has previously served as a member of the Institute of Medicine's Committee on Establishing a National Cord Blood Stem Cell Banking Program.

2007 Amendments to the National Academies'

Guidelines for Human Embryonic Stem Cell Research,

NAT'L ACADEMY OF SCIENCES (2007)

2007 AMENDMENTS

THE NATIONAL ACADEMIES' GUIDELINES FOR HUMAN EMBRYONIC STEM CELL RESEARCH

Human Embryonic Stem Cell Research Advisory Committee

Board on Life Sciences
Division on Earth and Life Studies

Board on Health Sciences Policy
Institute of Medicine

NATIONAL RESEARCH COUNCIL *AND*
INSTITUTE OF MEDICINE
OF THE NATIONAL ACADEMIES

THE NATIONAL ACADEMIES PRESS
Washington, D.C.
www.nap.edu

THE NATIONAL ACADEMIES PRESS 500 Fifth Street, N.W. Washington, DC 20001

NOTICE: The project that is the subject of this report was approved by the Governing Board of the National Research Council, whose members are drawn from the councils of the National Academy of Sciences, the National Academy of Engineering, and the Institute of Medicine. The members of the committee responsible for the report were chosen for their special competences and with regard for appropriate balance.

This study was supported by The Ellison Medical Foundation, The Greenwall Foundation, and the Howard Hughes Medical Institute. Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the author(s) and do not necessarily reflect the views of the organizations or agencies that provided support for the project.

International Standard Book Number-13: 978-0-309-10559-0
International Standard Book Number-10: 0-309-10559-5

Suggested Citation: National Research Council (NRC) and Institute of Medicine (IOM). 2007. *2007 Amendments to the National Academies' Guidelines for Human Embryonic Stem Cell Research*. Washington, DC: The National Academies Press.

Cover: A cluster of motor neurons and neural fibers derived from human embryonic stem cells in the lab of University of Wisconsin-Madison stem cell researcher and neurodevelopmental biologist Su-Chan-Zhang. The motor neurons are shown in red, neural fibers appear green, and the blue specks indicate DNA in cell nuclei. These motor neurons were developed from one of James Thomson's original human embryonic stem cell lines. Copyright for the photograph is held by the University of Wisconsin's Board of Regents.

Additional copies of this report are available from the National Academies Press, 500 Fifth Street, N.W., Lockbox 285, Washington, DC 20055; (800) 624-6242 or (202) 334-3313 (in the Washington metropolitan area); Internet, http://www.nap.edu.

Printed in the United States of America

THE NATIONAL ACADEMIES

Advisers to the Nation on Science, Engineering, and Medicine

The **National Academy of Sciences** is a private, nonprofit, self-perpetuating society of distinguished scholars engaged in scientific and engineering research, dedicated to the furtherance of science and technology and to their use for the general welfare. Upon the authority of the charter granted to it by the Congress in 1863, the Academy has a mandate that requires it to advise the federal government on scientific and technical matters. Dr. Ralph J. Cicerone is president of the National Academy of Sciences.

The **National Academy of Engineering** was established in 1964, under the charter of the National Academy of Sciences, as a parallel organization of outstanding engineers. It is autonomous in its administration and in the selection of its members, sharing with the National Academy of Sciences the responsibility for advising the federal government. The National Academy of Engineering also sponsors engineering programs aimed at meeting national needs, encourages education and research, and recognizes the superior achievements of engineers. Dr. Wm. A. Wulf is president of the National Academy of Engineering.

The **Institute of Medicine** was established in 1970 by the National Academy of Sciences to secure the services of eminent members of appropriate professions in the examination of policy matters pertaining to the health of the public. The Institute acts under the responsibility given to the National Academy of Sciences by its congressional charter to be an adviser to the federal government and, upon its own initiative, to identify issues of medical care, research, and education. Dr. Harvey V. Fineberg is president of the Institute of Medicine.

The **National Research Council** was organized by the National Academy of Sciences in 1916 to associate the broad community of science and technology with the Academy's purposes of furthering knowledge and advising the federal government. Functioning in accordance with general policies determined by the Academy, the Council has become the principal operating agency of both the National Academy of Sciences and the National Academy of Engineering in providing services to the government, the public, and the scientific and engineering communities. The Council is administered jointly by both Academies and the Institute of Medicine. Dr. Ralph J. Cicerone and Dr. Wm. A. Wulf are chair and vice chair, respectively, of the National Research Council.

www.national-academies.org

HUMAN EMBRYONIC STEM CELL RESEARCH ADVISORY COMMITTEE

R. ALTA CHARO (*Co-Chair*), University of Wisconsin, Madison
RICHARD O. HYNES (*Co-Chair*), Massachusetts Institute of Technology, Cambridge
ELI Y. ADASHI, Brown University, Providence, Rhode Island
BRIGID L.M. HOGAN, Duke University Medical Center, Durham, North Carolina
MARCIA IMBRESCIA, Arthritis Foundation, Lynnfield, Massachusetts
TERRY MAGNUSON, University of North Carolina, Chapel Hill
LINDA B. MILLER, Volunteer Trustees Foundation, Washington, D.C.
JONATHAN D. MORENO, University of Pennsylvania, Philadelphia
STUART H. ORKIN,[1] Harvard Medical School and Dana Farber Cancer Institute, Boston, Massachusetts
PILAR N. OSSORIO, University of Wisconsin, Madison
E. ALBERT REECE, University of Maryland, Baltimore
JOSHUA R. SANES, Harvard University, Cambridge, Massachusetts
HAROLD T. SHAPIRO, Princeton University, Princeton, New Jersey
JOHN E. WAGNER, Jr., University of Minnesota, Minneapolis

Staff

ADAM P. FAGEN, Study Co-Director, Board on Life Sciences
BRUCE M. ALTEVOGT, Study Co-Director, Board on Health Sciences Policy
FRANCES E. SHARPLES, Director, Board on Life Sciences
ANDREW M. POPE, Director, Board on Health Sciences Policy
ANNE F. JURKOWSKI, Senior Program Assistant, Board on Life Sciences
RIMA L. ADLER, Christine Mirzayan Science & Technology Policy Graduate Fellow, Board on Life Sciences
SARAH L. HANSON, Research Associate, Board on Health Sciences Policy

[1]Resigned from committee effective December 18, 2006.

Acknowledgments

The Committee would like to acknowledge the input received from members of the stem cell research and oversight communities as well as the speakers and participants in its meetings.

This report has been reviewed in draft form by individuals chosen for their diverse perspectives and technical expertise, in accordance with procedures approved by the National Research Council's Report Review Committee. The purpose of this independent review is to provide candid and critical comments that will assist the institution in making its published report as sound as possible and to ensure that the report meets institutional standards for objectivity, evidence, and responsiveness to the study charge. The review comments and draft manuscript remain confidential to protect the integrity of the deliberative process. We wish to thank the following individuals for their review of this report:

Robert Cook-Deegan, Duke University
William H. Danforth, Washington University
Norman Fost, University of Wisconsin–Madison
Larry Goldstein, University of California, San Diego
Henry T. Greely, Stanford Law School
Bernard Lo, University of California, San Francisco

Gail Martin, University of California, San Francisco
P. Pearl O'Rourke, Partners HealthCare System, Inc.
Steven Peckman, University of California, Los Angeles
Catherine Racowsky, Harvard Medical School
Brock C. Reeve, Harvard Stem Cell Institute
Susan L. Solomon, The New York Stem Cell Foundation
Clive Svendsen, University of Wisconsin–Madison

Although the reviewers listed above have provided many constructive comments and suggestions, they were not asked to endorse the conclusions or recommendations nor did they see the final draft of the report before its release. The review of this report was overseen by **Floyd E. Bloom**, The Scripps Research Institute, and **Janet D. Rowley**, University of Chicago. Appointed by the National Research Council, they were responsible for making certain that an independent examination of this report was carried out in accordance with institutional procedures and that all review comments were carefully considered. Responsibility for the final content of this report rests entirely with the authoring committee and the institution.

Contents

Introduction 1

Clarifying the Phrase "Provenance of the Cell Lines" 4

1.2(a) hES Cell Research Permissible After Currently Mandated Reviews 5

Use of NIH-Approved hES Cell Lines 5

1.4 Use of NIH-Approved hES cell lines 6

Importation of hES Cell Lines into an Institution or Jurisdiction 7

1.5 Acceptability of Research Using hES Cell Lines Imported from Other Institutions or Jurisdictions 8

ESCRO Committees Serving Multiple Institutions 8

2.0 Establishment of an Institutional Embryonic Stem Cell Research Oversight Committee 10

Frozen IVF Blastocysts Derived from Anonymous Sperm Donors: Absence of Informed Consent 11

Considering the Science in hES Cell Research Proposals: Advice for ESCRO Committees 12

Sample Questions for Reviewing hES Cell Research 13

Appendixes

A National Academies' Guidelines for Human Embryonic Stem Cell Research, Amended as of February 2007 15

B Committee Biographical Sketches 29

2007 Amendments to the National Academies' Guidelines for Human Embryonic Stem Cell Research

INTRODUCTION

The National Academies' report *Guidelines for Human Embryonic Stem Cell Research* was developed by the Committee on Guidelines for Human Embryonic Stem Cell Research and released in April 2005. The body of the report provided the background and rationale for the choices involved in formulating the guidelines, which were compiled in its final chapter. Because human embryonic stem (hES) cell research touches on many ethical, legal, scientific, and policy issues that are of concern to some people, the Guidelines are intended to make explicit how research with hES cells can be pursued most responsibly. While the Guidelines are primarily intended to address researchers in the United States, they may have applicability internationally as well.

The 2005 publication of the Guidelines offered a common set of ethical standards for a field that, due to the absence of comprehensive federal funding, was lacking national standards for research. Many have found the guidelines useful, but several constituencies identified sections of the Guidelines that they believe should be clarified. In addition, numerous scientific organizations and individuals encouraged the National Academies to establish an advisory committee to keep the Guidelines up to date, given the rapid pace of scientific developments in the field of stem cell research. Further,

Statement of Task of the Human Embryonic Stem Cell Research Advisory Committee

The Advisory Committee will meet two to three times per year over a period of 36 months to (1) monitor and review scientific developments and changing ethical, legal, and policy issues related to human embryonic stem cell research, (2) discuss the need for revisions to the Guidelines for Human Embryonic Stem Cell Research, and (3) prepare periodic reports to update the Guidelines as needed. Minimal but necessary changes may be issued as letter reports, but more extensive modifications may necessitate the preparation of traditional reports to fully provide the rationale for the changes.

Sources of information that will be considered by the Advisory Committee will include public symposia organized by the Committee to review developments in stem cell science and how these impact the ethical and policy issues surrounding hES cell research.

they urged the National Academies to consider correcting or clarifying aspects of the Guidelines in the light of experience.

Responding to these requests for revision and ongoing monitoring, the Human Embryonic Stem Cell Research Advisory Committee was established in 2006 with support from The Ellison Medical Foundation, The Greenwall Foundation, and the Howard Hughes Medical Institute.

The Human Embryonic Stem Cell Research Advisory Committee has engaged in a number of efforts to gather information about the need, if any, for revision of the Guidelines. The Committee met for the first time in July 2006 and heard from a number of invited guests representing organizations and academic institutions that are actively involved in stem cell research. In addition, in early November 2006, the Committee organized a symposium at which invited speakers reviewed the latest scientific developments, described how these developments might affect the analysis of associated ethical issues, and identified possible effects on the workability or justifiability of the current Guidelines. The Committee also hosted a panel discussion at the symposium for representatives of seven Embryonic Stem Cell Research Oversight (ESCRO) committees.[1] This panel shared their experiences in working with the content and procedures of the Guidelines.

[1]The 2005 Guidelines called for institutions involved in hES cell research to establish ESCRO committees to provide institutional oversight on all issues related to derivation and use of hES cell lines and to facilitate education of investigators involved in hES cell research.

As an ongoing effort, the Committee is also monitoring discussions of the Guidelines held by others, such as the April 2006 Association of American Medical Colleges (AAMC) meeting for medical school administrators to discuss the conduct and management of stem cell research at their institutions, a discussion which encompassed a review and critique of the Guidelines, and which was summarized for the Committee at its July meeting.[2] The Committee has also established a listserv for ESCRO committee members and staff to communicate and share questions and answers, and will be hosting a series of regional meetings in the spring of 2007 to bring together ESCRO committee members and staff, receive input from ESCRO committees, and clarify the Guidelines. In addition, members of the Committee have been actively soliciting input from their colleagues and receiving comments via a Web site[3] established for this purpose.

The Committee identified issues that appeared to merit consideration of revisions of the Guidelines. This report addresses issues that are both in need of amendment and amenable to prompt solution. The Committee is issuing this first set of amendments primarily to clarify or re-emphasize earlier recommendations and conclusions. Because the changes being made are minor and affect only Sections 1 and 2 of the Guidelines, this brief letter report is the best method of communicating these changes. Future deliberations of the Committee will deal with items for which additional information gathering and more extensive debate and discussion will probably be necessary. For example, the Committee has received numerous comments both praising and disputing the current policy on no compensation for oocyte donors. Similarly, some commenters have expressed dissatisfaction with the current restrictions on research using chimeras or have asked for further guidance on how to evaluate such research. More time will be required for the Committee to give adequate consideration to these and other issues and it will report on its findings in the future.

Four changes to the Guidelines are discussed herein:

1. clarifying the phrase "provenance of the cell lines" (changes to Section 1.2);
2. use of the hES cells approved for use in federally-funded research (addition of Section 1.4);

[2]A summary of the AAMC meeting was subsequently published as "Human Embryonic Stem Cell Research: Regulatory and Administrative Challenges." This AAMC monograph is available at <http://www.aamc.org/publications>.

[3]<http://www.nationalacademies.org/stemcells>.

3. importation of hES cell lines into an institution or jurisdiction (addition of Section 1.5); and
4. allowing ESCRO committees to serve multiple institutions (changes to Section 2.0 and addition of Section 2.1).

These amended Guidelines supersede those issued in 2005 by the Committee on Guidelines for Human Embryonic Stem Cell Research. It is important that these clarifications be interpreted in context with the complete set of amended Guidelines, which is included at the end of this report. It is also worth noting that these Guidelines continue to use the word "blastocyst" to refer to the stage of embryonic development from which hES cells are obtained. Both the public and the scientific community are engaged in conversation about the best terminology by which to describe this field of research, and the Committee will be attentive to those discussions as they develop.

This report also discusses two additional issues that do not result in formal changes to the Guidelines: (a) the lack of informed consent from sperm donors for some frozen in vitro fertilization (IVF) blastocysts and (b) advice for ESCRO committees in establishing criteria for considering the science in hES cell research proposals.

CLARIFYING THE PHRASE "PROVENANCE OF THE CELL LINES"

The National Academies' Human Embryonic Stem Cell Research Advisory Committee has received many comments from the scientific community questioning the meaning of the phrase "provenance of the cell lines," which occurs in Sections 1.2(a) and 6.1, to describe documentation of the derivation of stem cell lines. The wording of Section 1.2(a) is confusing due to unintended redundancy. It asks for documentation of the provenance of cell lines, documentation of appropriate informed consent in their derivation, and evidence of compliance with required review by an Institutional Review Board (IRB) and other committees, all of which address approximately the same issue. This makes it appear that "documenting the provenance of the cell lines" is something other than documenting informed consent and IRB approval. In order to resolve this confusion, the text of Section 1.2(a) is rewritten (see underlined wording) to read:

1.2(a) hES Cell Research Permissible After Currently Mandated Reviews

Purely in vitro hES cell research that uses previously derived hES cell lines is permissible provided that the ESCRO committee or equivalent body designated by the investigator's institution (see Section 2.0) receives documentation of the provenance of the cell lines including: (i) documentation of the use of an acceptable informed consent process that was approved by an Institutional Review Board (IRB) or foreign equivalent for their derivation (consistent with Section 3.6); and (ii) documentation of compliance with any additional required review by an Institutional Animal Care and Use Committee (IACUC), Institutional Biosafety Committee (IBC), or other institutionally mandated review.

USE OF NIH-APPROVED hES CELL LINES

The National Academies' Guidelines were issued a few years after a limited number of cell lines were deemed as useable in federally funded research in the United States.[4] As of the publication of this report in early 2007, these "NIH-approved cell lines" are the only hES cell lines that may be used in federally funded research.

NIH-approved cell lines were derived before August 2001 under protocols that predated the issuance of the National Academies' Guidelines in 2005. Nonetheless, NIH's agreement to fund research using these lines was premised on confirmation that all the cell lines in question were derived from blastocysts that were donated without payment, with voluntary, informed consent, and pursuant to an IRB-approved protocol. The precise details of the consent process for the NIH-approved cell lines may not have included each element called for in the National Academies' Guidelines. In particular, the Guidelines require informed consent from all embryo, gamete, and somatic cell donors, even anonymous gamete donors. For the

[4]"President Discusses Stem Cell Research," August 9, 2001. <http://www.whitehouse.gov/news/releases/2001/08/20010809-2.html>.

NIH-approved cell lines, the presence or absence of anonymously donated gametes cannot be confirmed, thus rendering impossible a determination of whether consent was obtained from all gamete donors. The NIH-approved cell lines were, however, derived from embryos that were donated under protocols that were substantially similar to those contemplated by the Guidelines.

Norms and procedures evolve, but it would be unnecessarily rigid to discourage institutions that follow the National Academies' Guidelines from working on the cell lines that are eligible for federal funding. The protocols under which the NIH-approved cell lines were derived were consistent with ethical norms then in place, were substantially similar to those now adopted in these Guidelines, and were adequately documented. The Committee considers the NIH-approved cell lines to be a special category because they are governed by a unique set of federal pronouncements (presidential statement and NIH rules). The intention of "grandfathering" the NIH-approved cell lines is to avoid precluding hES cell research that would otherwise be rendered difficult or impossible for investigators using NIH funding who wish to follow the National Academies' Guidelines. The clarification is not intended to "encourage" the use of these cell lines, either inside or outside the United States. For these reasons, retroactive application of the Guidelines is not warranted in this circumstance.

Therefore, the Guidelines are amended by adding a new Section 1.4:

1.4 Use of NIH-Approved hES Cell Lines

(a) It is acceptable to use hES cell lines that were approved in August 2001 for use in U.S. federally funded research.

(b) ESCRO committees should include on their registry a list of NIH-approved cell lines that have been used at their institution in accord with the requirement in Section 2.0 of the Guidelines.

(c) Presence on the list of NIH-approved cell lines constitutes adequate documentation of provenance, as per Section 6.1 of the Guidelines.

IMPORTATION OF hES CELL LINES INTO AN INSTITUTION OR JURISDICTION

Institutions following the National Academies' Guidelines may find themselves considering proposals for the importation of cell lines derived according to different rules, such as those from the United Kingdom, Canada, and the California Institute for Regenerative Medicine. These cell lines, while meeting all legal requirements of the respective jurisdictions for cell line derivation, may not have been derived in a manner that accords in every detail with the National Academies' Guidelines. For example, hES cell line derivations in the United Kingdom are managed through a licensing procedure that differs from the IRB and ESCRO committee review processes recommended in the Guidelines. Within the United States, state laws may vary from the Guidelines. California's laws, regulations, and guidelines, for example, though consistent with the Guidelines, apply some additional requirements concerning the details of the consent form, conflict-of-interest disclosures, and management of adverse medical events that may result from the donation of oocytes. As other states regulate such research, some state laws may differ from the Guidelines in some details but be sufficiently similar to be substantially equivalent.

Section 7.0 of the National Academies' Guidelines anticipates this problem in the international context. Section 7.0 specifically contemplates acceptance of cell lines derived under the extant legal and ethical regimes of another country provided that those regimes are substantially equivalent to the regime laid out in the Guidelines. This deference facilitates collaboration among institutions and shows proper respect for the diversity of authority in this area. This is analogous to the technique by which the U.S. federal government determines whether to accept the ethical and procedural norms of foreign research ethics review bodies as acceptable proxies for domestic IRB review.

Section 7.0 of the current National Academies' Guidelines reads: "If a U.S.-based investigator collaborates with an investigator in another country, the ESCRO committee may determine that the procedures prescribed by the foreign institution afford protections consistent with these guidelines, and the ESCRO committee may approve the substitution of some of or all of the foreign procedures for its own."

Therefore, without in any way suggesting that the addition of a new section should be construed by ESCRO committees to revoke any of the requirements of these Guidelines with respect to new donations or cell line derivations undertaken at their own institutions, the Guidelines are amended

by adding a new Section 1.5. This section applies to cell lines derived both before and after release of the Guidelines. ESCRO committees can review pre-2005 derivations and determine whether or not they are acceptable, following the guidance in new Section 1.5.

1.5 Acceptability of Research Using hES Cell Lines Imported from Other Institutions or Jurisdictions

(a) Before approving use of hES cell lines imported from other institutions or jurisdictions, ESCRO committees should consider whether such cell lines have been "acceptably derived."

(b) "Acceptably derived" means that the cell lines were derived from gametes or embryos for which

(1) the donation protocol was reviewed and approved by an IRB or, in the case of donations taking place outside the United States, a substantially equivalent oversight body;
(2) consent to donate was voluntary and informed;
(3) donation was made with reimbursement policies consistent with these Guidelines; and
(4) donation and derivation complied with the extant legal requirements of the relevant jurisdiction.

(c) ESCRO committees should include on their registry a list of cell lines that have been imported from other institutions or jurisdictions and information on the specific guidelines, regulations, or statutes under which the derivation of the imported cell lines was conducted. This is in accord with the requirement in Section 2.0 of the Guidelines that calls for ESCRO committees to maintain registries listing the cell lines in use at their institutions.

ESCRO COMMITTEES SERVING MULTIPLE INSTITUTIONS

The report *Guidelines for Human Embryonic Stem Cell Research* laid out a series of recommendations pertaining to the composition and role of ESCRO committees. Based on feedback from the community, it appears that

some of these recommendations need clarification. Although the text of Chapter 3 contains the statement that "In some cases, smaller institutions may wish to avail themselves of the services of larger facilities that have ESCRO committees," the idea that it is acceptable for institutions to use a nonlocal (external) ESCRO committee was unintentionally omitted from the wording of Section 2.0 of the Guidelines. Furthermore, since the Guidelines were issued in April 2005, it has become clear that there are other models for establishing ESCRO committees consistent with the principles of the Guidelines. New alternatives for the organization of IRB reviews are currently emerging that can serve as models for ESCRO review.

For example, the National Cancer Institute (NCI) has established a "Central IRB Initiative"[5] that is "designed to help reduce the administrative burden on local IRBs and investigators while continuing a high level of protection for human research participants." The NCI states that a local IRB's use of the Central IRB would facilitate the review of clinical trial protocols. The initiative is sponsored by NCI in consultation with the Department of Health and Human Services' Office for Human Research Protections (OHRP). OHRP's current guidance in the form of "Frequently Asked Questions" on its Web site[6] addresses institutions that do not have internal IRBs and provides options that include negotiating agreements with other institutions to have research reviewed as well as the use of commercial or independent IRBs. Finally, a November 2005 workshop summary report on "Alternative Models of IRB Review"[7] sponsored by the National Institutes of Health, OHRP, AAMC, and the American Society for Clinical Oncology explored the use of up to 10 alternative models, such as sharing materials among local IRBs, institutions relying on review by the IRB of another institution, and sites forming consortia to use a single IRB in a collaborative process. Although acceptance of the use of such alternative models for IRBs has not yet been indicated in updated guidance from OHRP or the Food and Drug Administration, the trend toward collaborative efforts is a topic that is actively under discussion and offers the possibility of more efficient and timely IRB (and, by analogy, ESCRO committee) review. The Tri-Institutional ESCRO Committee established by Rockefeller University, Memorial-Sloan Kettering Cancer Center, and Weill Medical College of Cornell University is an example of a single committee serving three research

[5]See <http://www.ncicirb.org/> for more information about the initiative.
[6]<http://www.hhs.gov/ohrp/faq.html>.
[7]<http://www.hhs.gov/ohrp/sachrp/documents/AltModIRB.pdf>.

institutions. Although the Committee on Guidelines for Human Embryonic Stem Cell Research quite clearly intended to allow for the use of shared or central ESCRO committees, it failed to state that explicitly. Therefore, Section 2.0 of the Guidelines is amended. (New wording is underlined.)

For projects involving more than one institution, there have also been concerns about the difficulty of multiple ESCRO committee reviews. Section 2.1 is added to explicitly allow—but not require—that multi-institution collaborations can be reviewed by a single ESCRO committee.

2.0 ESTABLISHMENT OF AN INSTITUTIONAL EMBRYONIC STEM CELL RESEARCH OVERSIGHT COMMITTEE

To provide oversight of all issues related to derivation and use of hES cell lines and to facilitate education of investigators involved in hES cell research, each institution should have activities involving hES cells overseen by an Embryonic Stem Cell Research Oversight (ESCRO) committee. This committee could be internal to a single institution or established jointly with one or more other institutions. Alternatively, an institution may have its proposals reviewed by an ESCRO committee of another institution, or by an independent ESCRO committee. An ESCRO committee should include independent representatives of the lay public as well as persons with expertise in developmental biology, stem cell research, molecular biology, assisted reproduction, and ethical and legal issues in hES cell research. It must have suitable scientific, medical, and ethical expertise to conduct its own review and should have the resources needed to coordinate the management of the various other reviews required for a particular protocol. A preexisting committee could serve the functions of the ESCRO committee provided that it has the recommended expertise and representation to perform the various roles described in this report. For example, an institution might elect to constitute an ESCRO committee from among some members of an IRB. But the ESCRO committee should not be a subcommittee of the IRB, as its responsibilities extend beyond human subject protections. Furthermore, much hES cell research does not require IRB review. The ESCRO committee should

(1) provide oversight over all issues related to derivation and use of hES cell lines,
(2) review and approve the scientific merit of research protocols,
(3) review compliance of all in-house hES cell research with all relevant regulations and these guidelines,
(4) maintain registries of hES cell research conducted at the institution and hES cell lines derived or imported by institutional investigators, and
(5) facilitate education of investigators involved in hES cell research.

An institution that uses an external ESCRO committee should nevertheless ensure that the registry and educational functions of an internal ESCRO committee are carried out by the external ESCRO committee on its behalf or internally by other administrative units.

2.1 For projects that involve more than one institution, review of the scientific merit, justification, and compliance status of the research may be carried out by a single ESCRO committee if all participating institutions agree to accept the results of the review.

FROZEN IVF BLASTOCYSTS DERIVED FROM ANONYMOUS SPERM DONORS: ABSENCE OF INFORMED CONSENT

Members of the scientific community raised concerns that the National Academies' Guidelines require that donors of all embryos, gametes, and somatic cells give informed consent for the use of their tissues for the derivation of human embryonic stem cell lines. Specifically, Section 3.3 of the Guidelines states that "When donor gametes have been used in the IVF process, resulting blastocysts may not be used for research without consent of all gamete donors." This requirement might preclude the use of frozen blastocysts from IVF clinics, which do not customarily request informed consent from sperm donors. The Committee, therefore, was asked to consider the effects this requirement might have on the available supply of

blastocysts for hES cell research and whether the population of frozen blastocysts now residing at IVF clinics needs to be "grandfathered" or exempt from the requirement for sperm donor consent.

To evaluate these effects, the Committee contacted the Society for Assisted Reproductive Technology (SART), which is actively involved in the collection of data on outcomes from its member IVF clinics. SART works closely with the Centers for Disease Control and Prevention in compliance with the Fertility Clinic Success Rate and Certification Act of 1992 (Wyden Act) to reflect accurately outcomes of the procedures commonly used in IVF practices.[8] The information returned in response to the Committee's request indicated that the number of blastocysts created with anonymous donor sperm in SART member practices is only about 3.5 percent.[9]

Given this small number, it is the Committee's view that maintaining the requirement for sperm donor consent in cases where human embryonic stem cell lines are to be derived from excess IVF clinic blastocysts should not significantly affect the availability of blastocysts for donation to research. The Committee, therefore, has concluded that it is not necessary to modify the Guidelines by "grandfathering" the frozen embryo population in IVF clinics and exempting them from the informed consent requirement for sperm donors. In light of the inability to determine whether any of these donors would have foregone sperm donation had they known of possible nonreproductive uses of the resulting blastocysts, the existing Guidelines reasonably balance respect for the gamete donors' expectations with the needs of the research community.

CONSIDERING THE SCIENCE IN hES CELL RESEARCH PROPOSALS: ADVICE FOR ESCRO COMMITTEES

It has been brought to the Committee's attention that some ESCRO committees would appreciate additional guidance on how to evaluate research proposals that are submitted for ESCRO committee review. In several places, the Guidelines emphasize the need to consider the scientific rationale for an experiment as part of the ethical analysis of the experiment. Although this section of this report does not amend the Guidelines, the

[8]See <http://www.sart.org/WhatIsSART.html> for more information about this data collection effort.

[9]2004 SART CORS© database.

Committee has compiled a list of questions that ESCRO committees may wish to consider when evaluating the scientific aspects of proposals for research involving hES cells. Many of these questions are contained in the 2005 report *Guidelines for Human Embryonic Stem Cell Research* but are distributed throughout the report. Not all of these questions will be applicable to every situation. Neither will answers to these questions necessarily be definitive with respect to the acceptability of the proposed research. Their goal is to ensure that the relevant scientific and ethical issues are considered.

Sample Questions for Reviewing hES Cell Research

- What is the scientific question being asked by the proposed research involving hES cells? Does the underlying hypothesis address an important scientific question? Could the question reasonably be addressed in any other way?
- Does the research team have the appropriate expertise and training in deriving or culturing either human or nonhuman stem cells? If training is the primary purpose of the proposal, is the training being conducted under the supervision of appropriate experts?
- Has the investigator articulated a compelling rationale for using human stem cells instead of nonhuman stem cells?
- Has the investigator articulated a compelling rationale for using hES cells instead of other types of stem cells?
- Has the investigator justified the selection of the stem cell line(s) to be used?
- Has the investigator articulated a rationale for creating a new stem cell line or could the proposed research be conducted with existing cell lines? If more than one cell line is to be derived, has the investigator justified the number he/she proposes to make?

Additional questions arise in considering protocols involving introduction of hES cells or cellular derivatives thereof into an animal host to form a chimera. Some of those questions were addressed in the 2005 *Guidelines for Human Embryonic Stem Cell Research*, and the committee intends to revisit these issues in future discussions.

Appendix A
National Academies' Guidelines for Human Embryonic Stem Cell Research, Amended as of February 2007[1]

1.0 Introduction
2.0 Establishment of an Institutional Embryonic Stem Cell Research Oversight Committee
3.0 Procurement of Gametes, Blastocysts, or Cells for hES Generation
4.0 Derivation of hES Cell Lines
5.0 Banking and Distribution of hES Cell Lines
6.0 Research Use of hES Cell Lines
7.0 International Collaboration
8.0 Conclusion

1.0 INTRODUCTION

In this chapter we collect all the recommendations made throughout the report and translate them into a series of formal guidelines. These guidelines focus on the derivation, procurement, banking, and use of human embryonic stem (hES) cell lines. They provide an oversight process that will help to ensure that research with hES cells is conducted in a responsible and ethically sensitive manner and in compliance with all regulatory requirements

[1]New or modified wording is indicated by underlining.

pertaining to biomedical research in general. The National Academies are issuing these guidelines for the use of the scientific community, including researchers in university, industry, or other private-sector research organizations.

1.1(a) What These Guidelines Cover

These guidelines cover all derivation of hES cell lines and all research that uses hES cells derived from

(1) blastocysts made for reproductive purposes and later obtained for research from in vitro fertilization (IVF) clinics,
(2) blastocysts made specifically for research using IVF,
(3) Somatic cell nuclear transfer (NT) into oocytes.

The guidelines do not cover research that uses nonhuman stem cells.

Many, but not all, of the guidelines and concerns addressed in this report are common to other areas of human stem cell research, such as

(1) research that uses human adult stem cells,
(2) research that uses fetal stem cells or embryonic germ cells derived from fetal tissue; such research is covered by federal statutory restrictions at 42 U.S.C. 289g-2(a) and federal regulations at 45 CFR 46.210.

Institutions and investigators conducting research using such materials should consider which individual provisions of these guidelines are relevant to their research.

1.1(b) Reproductive Uses of NT

These guidelines also do not apply to reproductive uses of nuclear transfer (NT), which are addressed in the 2002 report *Scientific and Medical Aspects of Human Reproductive Cloning*, in which the National Academies recommended that "Human reproductive cloning should not now be practiced. It is dangerous and likely to fail." Although these guidelines do not specifically address human reproductive cloning, it continues to be the view of the National Academies that research aimed at the reproductive cloning of a human being should not be conducted at this time.

1.2 Categories of hES Cell Research

These guidelines specify categories of research that

(a) Are permissible after currently mandated reviews and proper notification of the relevant research institution.
(b) Are permissible after additional review by an Embryonic Stem Cell Research Oversight (ESCRO) committee, as described in Section 2.0 of the guidelines.
(c) Should not be conducted at this time.

Because of the sensitive nature of some aspects of hES cell research, these guidelines in many instances set a higher standard than is required by laws or regulations with which institutions and individuals already must comply.

1.2(a) hES Cell Research Permissible After Currently Mandated Reviews

Purely in vitro hES cell research that uses previously derived hES cell lines is permissible provided that the ESCRO committee or equivalent body designated by the investigator's institution (see Section 2.0) receives documentation of the provenance of the cell lines including (i) documentation of the use of an acceptable informed consent process that was approved by an Institutional Review Board (IRB) or foreign equivalent for their derivation (consistent with Section 3.6); and (ii) documentation of compliance with any additional required review by an Institutional Animal Care and Use Committee (IACUC), Institutional Biosafety Committee (IBC), or other institutionally mandated review.

1.2(b) hES Cell Research Permissible Only After Additional Review and Approval

(1) Generation of new lines of hES cells by whatever means.
(2) Research involving the introduction of hES cells into nonhuman animals at any stage of embryonic, fetal, or postnatal development; particular attention should be paid to the probable pattern and effects of differentiation and integration of the human cells into the nonhuman animal tissues.
(3) Research in which the identity of the donors of blastocysts, gametes, or somatic cells from which the hES cells were derived is readily ascertainable or might become known to the investigator.

1.2(c) hES Cell Research That Should Not Be Permitted at This Time

The following types of research should not be conducted at this time:

(1) Research involving in vitro culture of any intact human embryo, regardless of derivation method, for longer than 14 days or until formation of the primitive streak begins, whichever occurs first.
(2) Research in which hES cells are introduced into nonhuman primate blastocysts or in which any embryonic stem cells are introduced into human blastocysts.

In addition:

(3) No animal into which hES cells have been introduced at any stage of development should be allowed to breed.

1.3 Obligations of Investigators and Institutions

All scientific investigators and their institutions, regardless of their field, bear the ultimate responsibility for ensuring that they conduct themselves in accordance with professional standards and with integrity. In particular, people whose research involves hES cells should work closely with oversight bodies, demonstrate respect for the autonomy and privacy of those who donate gametes, blastocysts, or somatic cells, and be sensitive to public concerns about research that involves human embryos.

1.4 Use of NIH-Approved hES Cell Lines

(a) It is acceptable to use hES cell lines that were approved in August 2001 for use in U.S. federally funded research.

(b) ESCRO committees should include on their registry a list of NIH-approved cell lines that have been used at their institution in accord with the requirement in Section 2.0 of the Guidelines.

(c) Presence on the list of NIH-approved cell lines constitutes adequate documentation of provenance, as per Section 6.1 of the Guidelines.

1.5 Acceptability of Research Using hES Cell Lines Imported from Other Institutions or Jurisdictions

(a) Before approving use of hES cell lines imported from other institutions or jurisdictions, ESCRO committees should consider whether such cell lines have been "acceptably derived."

(b) "Acceptably derived" means that the cell lines were derived from gametes or embryos for which

(1) The donation protocol was reviewed and approved by an IRB or, in the case of donations taking place outside the United States, a substantially equivalent oversight body;
(2) Consent to donate was voluntary and informed;
(3) Donation was made with reimbursement policies consistent with these Guidelines; and
(4) Donation and derivation complied with the extant legal requirements of the relevant jurisdiction.

(c) ESCRO committees should include on their registry a list of cell lines that have been imported from other institutions or jurisdictions and information on the specific guidelines, regulations, or statutes under which the derivation of the imported cell lines was conducted. This is in accord with the requirement in Section 2.0 of the Guidelines that calls for ESCRO committees to maintain registries listing the cell lines in use at their institutions.

2.0 ESTABLISHMENT OF AN INSTITUTIONAL EMBRYONIC STEM CELL RESEARCH OVERSIGHT COMMITTEE

To provide oversight of all issues related to derivation and use of hES cell lines and to facilitate education of investigators involved in hES cell research, each institution should have activities involving hES cells overseen by an Embryonic Stem Cell Research Oversight (ESCRO) committee. This committee could be internal to a single institution or established jointly with one or more other institutions. Alternatively, an institution may have its proposals reviewed by an ESCRO committee of another institution, or by an independent ESCRO committee. An ESCRO committee should include independent representatives of the lay public as well as persons with expertise in developmental biology, stem cell research, molecular biology, assisted reproduction, and ethical and legal issues in hES cell research. It must have suitable scientific, medical, and ethical expertise to conduct its own review

and should have the resources needed to coordinate the management of the various other reviews required for a particular protocol. A preexisting committee could serve the functions of the ESCRO committee provided that it has the recommended expertise and representation to perform the various roles described in this report. For example, an institution might elect to constitute an ESCRO committee from among some members of an IRB. But the ESCRO committee should not be a subcommittee of the IRB, as its responsibilities extend beyond human subject protections. Furthermore, much hES cell research does not require IRB review. The ESCRO committee should

(1) provide oversight over all issues related to derivation and use of hES cell lines,
(2) review and approve the scientific merit of research protocols,
(3) review compliance of all in-house hES cell research with all relevant regulations and these guidelines,
(4) maintain registries of hES cell research conducted at the institution and hES cell lines derived or imported by institutional investigators, and
(5) facilitate education of investigators involved in hES cell research.

An institution that uses an external ESCRO committee should nevertheless ensure that the registry and educational functions of an internal ESCRO committee are carried out by the external ESCRO committee on its behalf or internally by other administrative units.

2.1 For projects that involve more than one institution, review of the scientific merit, justification, and compliance status of the research may be carried out by a single ESCRO committee if all participating institutions agree to accept the results of the review.

3.0 PROCUREMENT OF GAMETES, BLASTOCYSTS, OR CELLS FOR hES GENERATION

3.1 An IRB, as described in federal regulations at 45 CFR 46.107, should review the procurement of all gametes, blastocysts, or somatic cells for the purpose of generating new hES cell lines, including the procurement of blastocysts in excess of clinical need from infertility clinics, blastocysts made through IVF specifically for research purposes, and oocytes, sperm, and

somatic cells donated for development of hES cell lines derived through NT or by parthenogenesis or androgenesis.

3.2 Consent for donation should be obtained from each donor at the time of donation. Even people who have given prior indication of their intent to donate to research any blastocysts that remain after clinical care should nonetheless give informed consent at the time of donation. Donors should be informed that they retain the right to withdraw consent until the blastocysts are actually used in cell-line derivation.

3.3 When donor gametes have been used in the IVF process, resulting blastocysts may not be used for research without consent of all gamete donors.

3.4a No payments, cash or in-kind, may be provided for donating blastocysts in excess of clinical need for research purposes. People who elect to donate stored blastocysts for research should not be reimbursed for the costs of storage prior to the decision to donate.

3.4b Women who undergo hormonal induction to generate oocytes specifically for research purposes (such as for NT) should be reimbursed only for direct expenses incurred as a result of the procedure, as determined by an IRB. No payments, cash or in-kind, should be provided for donating oocytes for research purposes. Similarly, no payments should be made for donations of sperm for research purposes or of somatic cells for use in NT.

3.5 To facilitate autonomous choice, decisions related to the creation of embryos for infertility treatment should be free of the influence of investigators who propose to derive or use hES cells in research. Whenever it is practicable, the attending physician responsible for the infertility treatment and the investigator deriving or proposing to use hES cells should not be the same person.

3.6 In the context of donation of gametes or blastocysts for hES cell research, the informed consent process, should, at a minimum, provide the following information:

(a) A statement that the blastocysts or gametes will be used to derive hES cells for research that may include research on human transplantation.

(b) A statement that the donation is made without any restriction or direction regarding who may be the recipient of transplants of the cells derived, except in the case of autologous donation.
(c) A statement as to whether the identities of the donors will be readily ascertainable to those who derive or work with the resulting hES cell lines.
(d) If the identities of the donors are retained (even if coded), a statement as to whether donors wish to be contacted in the future to receive information obtained through studies of the cell lines.
(e) An assurance that participants in research projects will follow applicable and appropriate best practices for donation, procurement, culture, and storage of cells and tissues to ensure, in particular, the traceability of stem cells. (Traceable information, however, must be secured to ensure confidentiality.)
(f) A statement that derived hES cells and/or cell lines might be kept for many years.
(g) A statement that the hES cells and/or cell lines might be used in research involving genetic manipulation of the cells or the mixing of human and nonhuman cells in animal models.
(h) Disclosure of the possibility that the results of study of the hES cells may have commercial potential and a statement that the donor will not receive financial or any other benefits from any future commercial development.
(i) A statement that the research is not intended to provide direct medical benefit to the donor(s) except in the case of autologous donation.
(j) A statement that embryos will be destroyed in the process of deriving hES cells.
(k) A statement that neither consenting nor refusing to donate embryos for research will affect the quality of any future care provided to potential donors.
(l) A statement of the risks involved to the donor.

In addition, donors could be offered the option of agreeing to some forms of hES cell research but not others. For example, donors might agree to have their materials used for deriving new hES cell lines but might not want their

materials used, for example, for NT. The consent process should fully explore whether donors have objections to any specific forms of research to ensure that their wishes are honored.

3.7 Clinical personnel who have a conscientious objection to hES cell research should not be required to participate in providing donor information or securing donor consent for research use of gametes or blastocysts. That privilege should not extend to the care of a donor or recipient.

3.8 Researchers may not ask members of the infertility treatment team to generate more oocytes than necessary for the optimal chance of reproductive success. An infertility clinic or other third party responsible for obtaining consent or collecting materials should not be able to pay for or be paid for the material obtained (except for specifically defined cost-based reimbursements and payments for professional services).

4.0 DERIVATION OF hES CELL LINES

4.1 Requests to the ESCRO committee for permission to attempt derivation of new hES cell lines from donated embryos or blastocysts must include evidence of IRB approval of the procurement process (see Section 3.0 above).

4.2 The scientific rationale for the need to generate new hES cell lines, by whatever means, must be clearly presented, and the basis for the numbers of embryos and blastocysts needed should be justified.

4.3 Research teams should demonstrate appropriate expertise or training in derivation or culture of either human or nonhuman ES cells before permission to derive new lines is given.

4.4 When NT experiments involving either human or nonhuman oocytes are proposed as a route to generation of ES cells, the protocol must have a strong scientific rationale. Proposals that include studies to find alternatives to donated oocytes in this research should be encouraged.

4.5 Neither blastocysts made using NT (whether produced with human or nonhuman oocytes) nor parthenogenetic or androgenetic human embryos may be transferred to a human or nonhuman uterus or cultured as intact embryos in vitro for longer than 14 days or until formation of the primitive streak, whichever occurs first.

4.6 Investigators must document how they will characterize, validate, store, and distribute any new hES cell lines and how they will maintain the confidentiality of any coded or identifiable information associated with the lines (see Section 5.0 below).

5.0 BANKING AND DISTRIBUTION OF hES CELL LINES

There are several models for the banking of human biological materials, including hES cells. The most relevant is the U.K. Stem Cell Bank. The guidelines developed by this and other groups generally adhere to key ethical principles that focus on the need for consent of donors and a system for monitoring adherence to ethical, legal, and scientific requirements. As hES cell research advances, it will be increasingly important for institutions that are obtaining, storing, and using cell lines to have confidence in the value of stored cells—that is, that they were obtained ethically and with the informed consent of donors, that they are well characterized and screened for safety, and that the conditions under which they are maintained and stored meet the highest scientific standards. Institutions engaged in hES research should seek mechanisms for establishing central repositories for hES cell lines—through partnerships or augmentation of existing quality research cell line repositories and should adhere to high ethical, legal, and scientific standards. At a minimum, an institutional registry of stem cell lines should be maintained.

5.1 Institutions that are banking or plan to bank hES cell lines should establish uniform guidelines to ensure that donors of material give informed consent through a process approved by an IRB and that meticulous records are maintained about all aspects of cell culture. Uniform tracking systems and common guidelines for distribution of cells should be established.

5.2 Any facility engaged in obtaining and storing hES cell lines should consider the following standards:

(a) Creation of a committee for policy and oversight purposes and creation of clear and standardized protocols for banking and withdrawals.

(b) Documentation requirements for investigators and sites that deposit cell lines, including

(i) A copy of the donor consent form.
(ii) Proof of Institutional Review Board approval of the procurement process.
(iii) Available medical information on the donors, including results of infectious-disease screening.
(iv) Available clinical, observational, or diagnostic information about the donor(s).
(v) Critical information about culture conditions (such as media, cell passage, and safety information).
(vi) Available cell line characterization (such as karyotype and genetic markers).

A repository has the right of refusal if prior culture conditions or other items do not meet its standards.

(c) A secure system for protecting the privacy of donors when materials retain codes or identifiable information, including but not limited to
(i) A schema for maintaining confidentiality (such as a coding system).
(ii) A system for a secure audit trail from primary cell lines to those submitted to the repository.
(iii) A policy governing whether and how to deliver clinically significant information back to donors.

(d) The following standard practices:
(i) Assignment of a unique identifier to each sample.
(ii) A process for characterizing cell lines.
(iii) A process for expanding, maintaining, and storing cell lines.
(iv) A system for quality assurance and control.
(v) A Web site that contains scientific descriptions and data related to the cell lines available.
(vi) A procedure for reviewing applications for cell lines.
(vii) A process for tracking disbursed cell lines and recording their status when shipped (such as number of passages).
(viii) A system for auditing compliance.
(ix) A schedule of charges.
(x) A statement of intellectual property policies.

(xi) When appropriate, creation of a clear Material Transfer Agreement or user agreement.
(xii) A liability statement.
(xiii) A system for disposal of material.

(e) Clear criteria for distribution of cell lines, including but not limited to evidence of approval of the research by an ESCRO committee or equivalent body at the recipient institution.

6.0 RESEARCH USE OF hES CELL LINES

Once hES cell lines have been derived, investigators and institutions, through ESCRO committees and other relevant committees (such as an IACUC, an IBC, or a radiation safety committee) should monitor their use in research.

6.1 Institutions should require documentation of the provenance of all hES cell lines, whether the cells were imported into the institution or generated locally. Notice to the institution should include evidence of IRB approval of the procurement process and of adherence to basic ethical and legal principles of procurement. In the case of lines imported from another institution, documentation that these criteria were met at the time of derivation will suffice.

6.2 In vitro experiments involving the use of already derived and coded hES cell lines will not need review beyond the notification required in Section 6.1.

6.3 Each institution should maintain a registry of its investigators who are conducting hES cell research and ensure that all registered users are kept up to date with changes in guidelines and regulations regarding the use of hES cells.

6.4 All protocols involving the combination of hES cells with nonhuman embryos, fetuses, or adult animals must be submitted to the local IACUC for review of animal welfare issues and to the ESCRO committee for consideration of the consequences of the human contributions to the resulting chimeras. (See also Section 1.2(c)(3) concerning breeding of chimeras.)

6.5 Transplantation of differentiated derivatives of hES cells or even hES cells themselves into adult animals will not require extensive ESCRO committee review. If there is a possibility that the human cells could contribute in a major organized way to the brain of the recipient animal, however, the scientific justification for the experiments must be strong, and proof of principle using nonhuman (preferably primate) cells, is desirable.

6.6 Experiments in which hES cells, their derivatives, or other pluripotent cells are introduced into nonhuman fetuses and allowed to develop into adult chimeras need more careful consideration because the extent of human contribution to the resulting animal may be higher. Consideration of any major functional contributions to the brain should be a main focus of review. (See also Section 1.2(c)(3) concerning breeding of chimeras.)

6.7 Introduction of hES cells into nonhuman mammalian blastocysts should be considered only under circumstances in which no other experiment can provide the information needed. (See also Sections 1.2(c)(2) and 1.2(c)(3) concerning restrictions on breeding of chimeras and production of chimeras with nonhuman primate blastocysts.)

6.8 Research use of existing hES cells does not require IRB review unless the research involves introduction of the hES cells or their derivatives into patients or the possibility that the identity of the donors of the blastocysts, gametes, or somatic cells is readily ascertainable or might become known to the investigator.

7.0 INTERNATIONAL COLLABORATION

If a U.S.-based investigator collaborates with an investigator in another country, the ESCRO committee may determine that the procedures prescribed by the foreign institution afford protections consistent with these guidelines, and the ESCRO committee may approve the substitution of some of or all of the foreign procedures for its own.

8.0 CONCLUSION

The substantial public support for hES cell research and the growing trend by many nonfederal funding agencies and state legislatures to support this field requires a set of guidelines to provide a framework for hES cell re-

search. In the absence of the oversight that would come with unrestricted federal funding of this research, these guidelines will offer reassurance to the public and to Congress that the scientific community is attentive to ethical concerns and is capable of self-regulation while moving forward with this important research.

To help ensure that these guidelines are taken seriously, stakeholders in hES cell research—sponsors, funding sources, research institutions, relevant oversight committees, professional societies, and scientific journals, as well as investigators—should develop policies and practices that are consistent with the principles inherent in these guidelines. Funding agencies, professional societies, journals, and institutional review panels can provide valuable community pressure and impose appropriate sanctions to ensure compliance. For example, ESCRO committees and IRBs should require evidence of compliance when protocols are reviewed for renewal, funding agencies should assess compliance when reviewing applications for support, and journals should require that evidence of compliance accompanies publication of results.

As individual states and private entities move into hES cell research, it will be important to initiate a national effort to provide a formal context in which the complex moral and oversight questions associated with this work can be addressed on a continuing basis. Both the state of hES cell research and clinical practice and public policy surrounding these topics are in a state of flux and are likely to be so for several years. Therefore, the committee believes that a national body should be established to assess periodically the adequacy of the policies and guidelines proposed in this document and to provide a forum for a continuing discussion of issues involved in hES cell research. New policies and standards may be appropriate for issues that cannot now be foreseen. The organization that sponsors this body should be politically independent and without conflicts of interest, should be respected in the lay and scientific communities, and able to call on suitable expertise to support this effort.

Appendix B

Committee Biographical Sketches

CO-CHAIRS

R. Alta Charo, J.D., is the Warren P. Knowles Professor of Law and Bioethics at the University of Wisconsin–Madison, on the faculties of both the Law School and Medical School, and, in 2006, was Visiting Professor of Law at the University of California, Berkeley, Boalt Hall School of Law. Professor Charo is the author of nearly 100 articles, book chapters, and government reports on topics including voting rights, environmental law, family planning and abortion law, medical genetics law, reproductive technology policy, science policy, and medical ethics. Professor Charo is a member of the boards of the Alan Guttmacher Institute and the Foundation for Genetic Medicine, a member of the National Medical Advisory Committee of the Planned Parenthood Federation of America, and a member of the ethics advisory boards of the International Society for Stem Cell Research, the Juvenile Diabetes Research Foundation and WiCell. In 2005, she was appointed to the ethics standards working group of the California Institute for Regenerative Medicine and was elected as a fellow of the Wisconsin Academy of Sciences, Arts, and Letters. In 1994, Professor Charo served on the NIH Human Embryo Research Panel, and from 1996 to 2001 she was a member of the presidential National Bioethics Advisory Commission where she participated in drafting its reports on *Cloning Human Beings* (1997); *Research Involving Persons with Mental Disorders That May Affect*

Decisionmaking Capacity (1998); *Research Involving Human Biological Materials: Ethical Issues and Policy Guidance* (1999); *Ethical Issues in Human Stem Cell Research* (1999); *Ethical and Policy Issues in International Research: Clinical Trials in Developing Countries*, and *Ethical and Policy Issues in Research Involving Human Participants* (2001). Since 2001, she has been a member of the National Academies' Board on Life Sciences and since 2006, she has been a member of the Institute of Medicine's Board on Population Health and Public Health Practices. Professor Charo was elected to the Institute of Medicine in 2006.

Richard O. Hynes, Ph.D., is the Daniel K. Ludwig Professor for Cancer Research at the MIT Center for Cancer Research and Department of Biology, and a Howard Hughes Medical Institute investigator. He was formerly head of the Biology Department and then director of the Center for Cancer Research at MIT. His research focuses on fibronectins and integrins and the molecular basis of cellular adhesion, both in normal development and in pathological situations, such as cancer, thrombosis, and inflammation. Dr. Hynes' current interests are cancer invasion and metastasis, angiogenesis, and animal models of human disease states. He is a member of the National Academy of Sciences and the Institute of Medicine and is a Fellow of the Royal Society of London and the American Academy of Arts and Sciences. In 1997, he received the Gairdner International Foundation Award. In 2000, he served as president of the American Society for Cell Biology and testified before Congress about the need for federal support and oversight of embryonic stem cell research. He co-chaired the 2005 National Academies' *Guidelines for Human Embryonic Stem Cell Research*.

MEMBERS

Eli Y. Adashi, M.D., M.S., FACOG, is currently the Dean of Medicine and Biological Sciences and the Frank L. Day Professor of Biology, the Warren Alpert Medical School of Brown University. Previously, Dr. Adashi served as the professor and chair of the Department of Obstetrics and Gynecology at the University of Utah Health Sciences Center. Dr. Adashi is a member of the Institute of Medicine of the National Academies, a member of the Association of American Physicians, and a fellow of the American Association for the Advancement of Science. Dr. Adashi is a former member of the Advisory Council of the National Institute of Child Health and Human Development and a former president of the Society for Reproductive Endo-

crinologists, the Society for Gynecologic Investigation, and the American Gynecological and Obstetrical Society. Dr. Adashi is also a former examiner and director of the Division of Reproductive Endocrinology of the American Board of Obstetrics and Gynecology. Finally, Dr. Adashi is a founding member and treasurer and, more recently, chair of the advisory committee of the Geneva-based Bertarelli Foundation, dedicated to promoting the welfare of the infertile couple and to addressing the current "epidemic" of high-order multiple gestations.

Brigid L.M. Hogan, Ph.D., is the George Barth Geller Professor and Chair of the Department of Cell Biology, Duke University Medical Center. Prior to joining Duke, Dr. Hogan was an Investigator of the Howard Hughes Medical Institute and Hortense B. Ingram Professor in the Department of Cell Biology at Vanderbilt University Medical Center. Dr. Hogan earned her Ph.D. in Biochemistry at the University of Cambridge. After completing her Ph.D. she was a postdoctoral fellow in the Department of Biology at MIT. Before moving to the United States in 1988, Dr. Hogan was head of the Molecular Embryology Laboratory at the National Institute for Medical Research in London. Her research focuses on the genetic control of embryonic development and morphogenesis, using the mouse as a model system. Her laboratory developed methods for deriving mouse pluripotential embryonic germ (EG) cell lines. She was Co-Chair for Science of the 1994 NIH Human Embryo Research Panel and a member of the 2001/2002 National Academies' Panel on Scientific and Medical Aspects of Human Cloning. Within the past few years, Dr. Hogan has been elected to the Royal Society of London, the American Academy of Arts and Sciences, the Institute of Medicine, and the National Academy of Sciences.

Marcia Imbrescia is the current owner of Peartree Design, a landscape design firm, and was previously the media director for Drumbeater, a high-technology advertising agency. She holds B.A. degrees in marketing and journalism, and a graduate certificate in landscape design. Ms. Imbrescia has a passion for health advocacy and helping people with illness and disability. She is a member of the Board of Trustees of the Arthritis Foundation (AF), for which she has participated as a volunteer at the chapter and national levels. She served as member (1996–1998, 2001) and chairperson (2002–2003) of AF's American Juvenile Arthritis Organization. In 1992, she received the Volunteer of the Year Award from the Massachusetts Chapter of AF. Her volunteer efforts include program development, conference planning, public speaking, fundraising, and advocacy. She served on the

National Academies' Committee on Guidelines for Human Embryonic Stem Cell Research in 2004–2005.

Terry Magnuson, Ph.D., is Sarah Graham Kenan Professor and chair of the Department of Genetics at the University of North Carolina. He also directs the Carolina Center for Genome Sciences, and is the program director of cancer genetics at the Lineberger Comprehensive Cancer Center. Dr. Magnuson's research interests include mammalian genetics, genomics, and development. His laboratory has developed a high-throughput system to study the effects of mutations on mouse development with mouse embryonic stem cells. He is particularly interested in the role of chromatin remodeling complexes in processes such as autosomal imprinting, X-inactivation, and anterior-posterior patterning of axial structures in mammals. He is a member of the Board of Directors of the Genetics Society of America and of the Society for Developmental Biology.

Linda B. Miller, OTR, M.S. in Hospital Administration, is President of the Washington, D.C.-based Volunteer Trustees Foundation, a consortium of not-for-profit hospital governing boards. She has extensive experience in trustee education, advocacy, and the legal, ethical, and policy issues facing voluntary health care institutions. Recently, she has worked closely with the states' attorneys general in developing guidelines for protecting the community interest in the sale and conversion of nonprofit hospitals, as well as in designing models for practice and legal oversight. She was elected to membership in the Institute of Medicine in 1997.

Ms. Miller has been a frequent speaker on health policy issues and has been published extensively in both the medical and popular press, including the *New England Journal of Medicine*, *Health Affairs*, *USA Today*, the *Washington Post,* and *New York Times,* among others. She served as a Special Assistant to the Secretary of Health, Education and Welfare (now HHS) and on numerous health-related policy councils and advisory committees, including the National Institutes of Health's Consensus Panel on Liver Transplantation and, most recently, the Institute of Medicine's Committee on Spinal Cord Injury. Ms. Miller currently serves on the Advisory Board of the University of Louisville-based Institute for Cellular Therapeutics, headed by Dr. Suzanne Ildstad, which does research in adult bone marrow transplant, and has been a member of several academic and health care institutions' board of governors, including Blythedale Childrens Hospital in New York, Capital Hospice in the national capital region, and Cornell University's Alumni Council, among others.

Jonathan D. Moreno, Ph.D., is the David and Lyn Silfen University Professor at the University of Pennsylvania. Until 2007, he was the Emily Davie and Joseph S. Kornfeld Professor of Biomedical Ethics at the University of Virginia where he also directed the Center for Biomedical Ethics. Dr. Moreno is a member of the Institute of Medicine of the National Academies. He is also a bioethics advisor for the Howard Hughes Medical Institute, a faculty affiliate of the Kennedy Institute of Ethics at Georgetown University, and a Fellow of the Hastings Center. During 1995–1996 he was Senior Policy and Research Analyst for the President's Advisory Committee on Human Radiation Experiments and during 1998–2000 was a senior consultant for the National Bioethics Advisory Commission. He co-chaired the 2005 National Academies' Committee on Guidelines for Human Embryonic Stem Cell Research and is a consultant to the Ethical, Social, and Cultural Program of the Bill & Melinda Gates Foundation Grand Challenges in Global Health initiative, for ethical and regulatory issues regarding stem cell research in China.

Stuart H. Orkin,[1] M.D., is the David G. Nathan Professor of Pediatrics at Harvard Medical School, Chair of the Department of Pediatric Onocology at the Dana-Farber Cancer Institute, and an Investigator with the Howard Hughes Medical Institute. His laboratory utilizes multidisciplinary approaches to understand how mammalian cells choose specific fates and how mutations in important transcriptional regulators lead to developmental defects or malignancy. Recent and ongoing work falls into several overlapping areas, including study of essential hematopoietic transcription factors, the genetic pathogenesis of two forms of leukemia, and whether some of the lessons of hematopoiesis may be applied to consideration of the pathogenesis of solid tumors. Finally, the fundamental properties of stem cells—pluripotency and self-renewal—are being addressed from a biochemical perspective in mouse embryonic stem (ES) cells. In the future, his laboratory will pursue the functions of the associated proteins in order to unravel the biochemistry of ES fate specification. This strategy may ultimately suggest how directed manipulation of somatic cells to an ES cell fate might be achieved.

Pilar N. Ossorio, Ph.D., J.D., is Associate Professor of Law and Bioethics at the University of Wisconsin–Madison, and Program Faculty in the Graduate

[1]Resigned from committee effective December 18, 2006.

Program in Population Health at UW. Prior to taking her position at UW, she was Director of the Genetics Section at the Institute for Ethics at the American Medical Association, and taught as an adjunct faculty member at the University of Chicago Law School. For the 2006 calendar year, Professor Ossorio was a visiting professor of law at the University of California, Berkeley, Boalt Hall School of Law.

Dr. Ossorio received her Ph.D. in Microbiology and Immunology in 1990 from Stanford University. She went on to complete a postdoctoral fellowship in cell biology at Yale University School of Medicine. Throughout the early 1990s, Dr. Ossorio also worked as a consultant for the federal program on the Ethical, Legal, and Social Implications (ELSI) of the Human Genome Project, and in 1994, she took a full-time position with the Department of Energy's ELSI program. In 1993, she served on the Ethics Working Group for President Clinton's Health Care Reform Task Force. Dr. Ossorio received her J.D. from the University of California at Berkeley School of Law (Boalt Hall) in 1997. While at Boalt she was elected to the legal honor society Order of the Coif and received several awards for outstanding legal scholarship.

Dr. Ossorio is a fellow of the American Association for the Advancement of Science (AAAS), on the editorial board of the *American Journal of Bioethics*, an advisor to NHGRI on ethical issues in large-scale sequencing, and a member of UW's institutional review board for health sciences research. She is a past member of AAAS's Committee on Scientific Freedom and Responsibility, a past member of the National Cancer Policy Board (Institute of Medicine), and has been a member or chair of several working groups on genetics and ethics. She has published scholarly articles in bioethics, law, and molecular biology.

E. Albert Reece, M.D., Ph.D., is currently Dean of the University of Maryland School of Medicine and Vice President for Medical Affairs at the University of Maryland, Baltimore. Previously, he was Vice Chancellor and Dean of the University of Arkansas College of Medicine. Dr. Reece received his undergraduate degree from Long Island University, his M.D. (Magna Cum Laude) from New York University, his Ph.D. degree in biochemistry from the University of the West Indies, and his M.B.A. degree from the Fox School of Business and Management of Temple University. He completed a residency in OB/GYN at Columbia University–Presbyterian Hospital, and a fellowship in maternal-fetal medicine at Yale University School of Medicine. He served on the faculty at Yale for 10 years, and was the Chairman of the Department of Obstetrics, Gynecology, and Reproductive Sciences at

Temple University. Dr. Reece has published over 400 journal articles, book chapters, and abstracts, and 9 textbooks including *Diabetes in Pregnancy*; *Medicine of the Fetus & Mother*; and *Fundamentals of Ultrasound in Obstetrics & Gynecology*. He is an editor for the *Journal of Maternal-Fetal Medicine* and a reviewer for several other scientific journals. His research focuses on diabetes in pregnancy, birth defects, and prenatal diagnosis. Dr. Reece is a member of the Institute of Medicine.

Joshua R. Sanes, Ph.D., is Professor of Molecular and Cellular Biology and the Paul J. Finnegan Family Director of the Center for Brain Science at Harvard University. He was previously Alumni Endowed Professor of Neurobiology at the Washington University School of Medicine. Dr. Sanes earned a B.A. in biochemistry and psychology at Yale and a Ph.D. in Neurobiology at Harvard. He studies the formation of the synapses that interconnect nerve cells, including pioneering work on the signals exchanged between nerve cells and their target muscles as new connections are made. He is also using the vertebrate visual system to examine how nerve cells develop and migrate to the right location in the body. He was elected a Fellow of the American Association for the Advancement of Science in 1992 and a member of the National Academy of Sciences in 2002.

Harold T. Shapiro, Ph.D., is President Emeritus of both Princeton University and the University of Michigan and is currently Professor of Economics and Public Affairs at Princeton University. His research interests include bioethics, the social role of higher education, hospital/medical center administration, university administration, econometrics, statistics, and economics. Dr. Shapiro currently chairs the Board of Trustees of the Alfred P. Sloan Foundation, is presiding director for the Dow Chemical Company, and is a member of numerous boards including the Robert Wood Johnson Medical School, HCA, the Merck Vaccine Advisory Board, the Knight Foundation Commission on Intercollegiate Athletics, U.S. Olympic Committee, and the Stem Cell Institute of New Jersey. He is a former Chair of the Association of American Universities and the National Bioethics Advisory Committee and Vice Chair of the President's Council of Advisors on Science and Technology. He has also served on the Board of Directors of the National Bureau of Economic Research, Inc. and the Board of Trustees of the Universities Research Association, Inc. He has chaired and served on numerous National Academies committees including the Committee on the Organizational Structure of the National Institutes of Health and the Committee on Particle Physics. Dr. Shapiro was awarded the 2006 American Association for the

Advancement of Science's William D. Carey Lecture for his leadership in science policy. He earned a Ph.D. in economics from Princeton University and holds 14 honorary doctorates.

John E. Wagner, Jr., M.D., is a professor of pediatrics at the University of Minnesota Medical School. He is the first recipient of the Children's Cancer Research Fund/Hageboeck Family Chair in Pediatric Oncology and also holds the Variety Club Endowed Chair in Molecular and Cellular Therapy. He is the director of the division of Pediatric Hematology/Oncology and Bone Marrow Transplantation and Scientific Director of Clinical Research of the Stem Cell Institute. Dr. Wagner is a member of numerous societies, including the American Society of Hematology, the International Society of Experimental Hematology, and the American Society of Blood and Marrow Transplantation. He is a member of several honorary societies including Alpha Omega Alpha (1980), the American Society of Clinical Investigation (2000), and the Association of American Physicians (2006). Dr. Wagner holds a patent on the isolation of the pluripotential quiescent stem cell population. Dr. Wagner holds a B.A. in Biological Sciences and a B.A. in Psychology from the University of Delaware and an M.D. from Jefferson Medical College. Dr. Wagner's research has focused on the development of novel cellular therapies for tissue repair and suppression of the immune response using subpopulations of neonatal umbilical cord blood and adult bone marrow and peripheral blood. Projects are funded by both NIH (P01 CA65493, Biology and Transplantation of the Human Stem Cell; and N01-HB-37164, Somatic Cell Therapeutics) and industry (ViaCell, Inc., on the transplantation of expanded umbilical cord blood hematopoietic stem cells; and Athersys, Inc., on the large-scale development of multipotent adult progenitor cells). In addition, Dr. Wagner pioneered the use of embryo selection to "create" a perfectly tissue matched stem cell donor in the treatment of genetic disease. Dr. Wagner has authored more than 180 articles and book chapters on the subject of hematopoietic stem cell transplantation. He currently co-chairs the Graft Sources and Manipulation Working Committee of the Center of the International Blood and Marrow Transplant Research, serves on the Scientific Board of Directors of the National Marrow Donor Program, and is a member of the Scientific and Medical Accountability Standards Working Group of the California Institute of Regenerative Medicine. Dr. Wagner has previously served as a member of the Institute of Medicine's Committee on Establishing a National Cord Blood Stem Cell Banking Program.

2008 Amendments to the National Academies'

Guidelines for Human Embryonic Stem Cell Research,

NAT'L ACADEMY OF SCIENCES (2008)

2008 AMENDMENTS

THE NATIONAL ACADEMIES' GUIDELINES FOR HUMAN EMBRYONIC STEM CELL RESEARCH

Human Embryonic Stem Cell Research Advisory Committee

Board on Life Sciences
Division on Earth and Life Studies

Board on Health Sciences Policy
Institute of Medicine

NATIONAL RESEARCH COUNCIL *AND*
INSTITUTE OF MEDICINE
OF THE NATIONAL ACADEMIES

THE NATIONAL ACADEMIES PRESS
Washington, D.C.
www.nap.edu

THE NATIONAL ACADEMIES PRESS 500 Fifth Street, NW Washington, DC 20001

NOTICE: The project that is the subject of this report was approved by the Governing Board of the National Research Council, whose members are drawn from the councils of the National Academy of Sciences, the National Academy of Engineering, and the Institute of Medicine. The members of the committee responsible for the report were chosen for their special competences and with regard for appropriate balance.

This study was supported by The Ellison Medical Foundation, The Greenwall Foundation, and the Howard Hughes Medical Institute. Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the authors and do not necessarily reflect the views of the organizations or agencies that provided support for the project.

International Standard Book Number-13: 978-0-309-12220-7
International Standard Book Number-10: 0-309-12220-1

Suggested Citation: National Research Council and Institute of Medicine. 2008. *2008 Amendments to the National Academies' Guidelines for Human Embryonic Stem Cell Research*. Washington, DC: The National Academies Press.

Cover: A cluster of motor neurons and neural fibers derived from human embryonic stem cells in the lab of University of Wisconsin–Madison stem cell researcher and neurodevelopmental biologist Su-Chan Zhang. The motor neurons are shown in red, neural fibers appear green, and the blue specks indicate DNA in cell nuclei. These motor neurons were developed from one of James Thomson's original human embryonic stem cell lines. Copyright for the photograph is held by the University of Wisconsin's Board of Regents.

Additional copies of this report are available from the National Academies Press, 500 Fifth Street, NW, Lockbox 285, Washington, DC 20055; (800) 624-6242 or (202) 334-3313 (in the Washington metropolitan area); Internet, http://www.nap.edu.

Printed in the United States of America

THE NATIONAL ACADEMIES

Advisers to the Nation on Science, Engineering, and Medicine

The **National Academy of Sciences** is a private, nonprofit, self-perpetuating society of distinguished scholars engaged in scientific and engineering research, dedicated to the furtherance of science and technology and to their use for the general welfare. Upon the authority of the charter granted to it by the Congress in 1863, the Academy has a mandate that requires it to advise the federal government on scientific and technical matters. Dr. Ralph J. Cicerone is president of the National Academy of Sciences.

The **National Academy of Engineering** was established in 1964, under the charter of the National Academy of Sciences, as a parallel organization of outstanding engineers. It is autonomous in its administration and in the selection of its members, sharing with the National Academy of Sciences the responsibility for advising the federal government. The National Academy of Engineering also sponsors engineering programs aimed at meeting national needs, encourages education and research, and recognizes the superior achievements of engineers. Dr. Charles Vest is president of the National Academy of Engineering.

The **Institute of Medicine** was established in 1970 by the National Academy of Sciences to secure the services of eminent members of appropriate professions in the examination of policy matters pertaining to the health of the public. The Institute acts under the responsibility given to the National Academy of Sciences by its congressional charter to be an adviser to the federal government and, upon its own initiative, to identify issues of medical care, research, and education. Dr. Harvey V. Fineberg is president of the Institute of Medicine.

The **National Research Council** was organized by the National Academy of Sciences in 1916 to associate the broad community of science and technology with the Academy's purposes of furthering knowledge and advising the federal government. Functioning in accordance with general policies determined by the Academy, the Council has become the principal operating agency of both the National Academy of Sciences and the National Academy of Engineering in providing services to the government, the public, and the scientific and engineering communities. The Council is administered jointly by both Academies and the Institute of Medicine. Dr. Ralph J. Cicerone and Dr. Charles Vest are chair and vice chair, respectively, of the National Research Council.

www.national-academies.org

HUMAN EMBRYONIC STEM CELL RESEARCH ADVISORY COMMITTEE

R. ALTA CHARO (*Cochair*), University of Wisconsin, Madison, WI
RICHARD O. HYNES (*Cochair*), Massachusetts Institute of Technology, Cambridge, MA
ELI Y. ADASHI, Brown University, Providence, RI
BRIGID L.M. HOGAN, Duke University Medical Center, Durham, NC
MARCIA IMBRESCIA, Arthritis Foundation, Lynnfield, MA
TERRY MAGNUSON, University of North Carolina, Chapel Hill, NC
LINDA B. MILLER, Volunteer Trustees Foundation, Washington, DC
JONATHAN D. MORENO, University of Pennsylvania, Philadelphia, PA
PILAR N. OSSORIO, University of Wisconsin, Madison, WI
E. ALBERT REECE, University of Maryland, Baltimore, MD
JOSHUA R. SANES, Harvard University, Cambridge, MA
HAROLD T. SHAPIRO, Princeton University, Princeton, NJ
JOHN E. WAGNER, Jr., University of Minnesota, Minneapolis, MN

Staff

ADAM P. FAGEN, Study Co-Director, Board on Life Sciences
BRUCE M. ALTEVOGT, Study Co-Director, Board on Health Sciences Policy
FRANCES E. SHARPLES, Director, Board on Life Sciences
ANDREW M. POPE, Director, Board on Health Sciences Policy
AMANDA P. CLINE, Senior Program Assistant, Board on Life Sciences
NORMAN GROSSBLATT, Senior Editor, Division on Earth and Life Studies

BOARD ON LIFE SCIENCES

Staff

FRANCES E. SHARPLES, Director
JO L. HUSBANDS, Scholar/Senior Project Director
ADAM P. FAGEN, Senior Program Officer
ANN H. REID, Senior Program Officer
MARILEE K. SHELTON-DAVENPORT, Senior Program Officer
ANNA FARRAR, Financial Associate
AMANDA P. CLINE, Senior Program Assistant
REBECCA L. WALTER, Senior Program Assistant

Acknowledgments

The Committee acknowledges the input received from members of the stem cell research and oversight communities and the speakers and participants in its meetings.

This report has been reviewed in draft form by individuals chosen for their diverse perspectives and technical expertise, in accordance with procedures approved by the National Research Council's Report Review Committee. The purpose of this independent review is to provide candid and critical comments that will assist the institution in making its published report as sound as possible and to ensure that the report meets institutional standards for objectivity, evidence, and responsiveness to the study charge. The review comments and draft manuscript remain confidential to protect the integrity of the deliberative process. We wish to thank the following individuals for their review of this report:

George Q. Daley, Children's Hospital Boston and Harvard Medical School
Norman Fost, University of Wisconsin–Madison
Henry T. Greely, Stanford Law School
Geoffrey Lomax, California Institute for Regenerative Medicine
Gail R. Martin, University of California, San Francisco
P. Pearl O'Rourke, Partners HealthCare System
James Thomson, University of Wisconsin–Madison
Laurie Zoloth, Northwestern University

Although the reviewers listed above have provided many constructive comments and suggestions, they were not asked to endorse the conclusions

or recommendations, nor did they see the final draft of the report before its release. The review of this report was overseen by **Janet Rowley**, University of Chicago Medical Center, and **Floyd Bloom**, Scripps Research Institute (retired). Appointed by the National Research Council, they were responsible for making certain that an independent examination of this report was carried out in accordance with institutional procedures and that all review comments were carefully considered. Responsibility for the final content of this report rests entirely with the authoring committee and the institution.

Contents

Introduction 1

Applicability of the Guidelines to Non-Embryonic Human Pluripotent Stem Cells 4
- From Section 1 6
- From Section 3 7
- From Section 4 9
- From Section 5 10
- Section 7 10

Clarification of the Meaning of "Proper Notification" 13
- 1.3(a) hES Cell Research Permissible after Currently Mandated Reviews 14
- Sections 6.1 and 6.2 15

Public Openness and ESCRO Committee Audits 15
- 2.0 Establishment of an Institutional Embryonic Stem Cell Research Oversight Committee 16

Clarification of Policy Regarding Reimbursement for Oocyte Donors 17
- Section 3.4(b) 19

References 20

Appendixes

A National Academies' Guidelines for Human Embryonic Stem Cell Research, Amended as of September 2008 21

B Glossary 39

C Committee Biographical Sketches 47

2008 Amendments to the National Academies' Guidelines for Human Embryonic Stem Cell Research

INTRODUCTION

The National Academies' report *Guidelines for Human Embryonic Stem Cell Research* (NRC and IOM, 2005) was developed by the Committee on Guidelines for Human Embryonic Stem Cell Research and released in April 2005. The body of the report provided the background and rationale for the choices involved in formulating the Guidelines, which were compiled in its final chapter. Because human embryonic stem (hES) cell research touches on many ethical, legal, scientific, and policy issues, the Guidelines are intended to make explicit how research with hES cells can be pursued most responsibly. The Guidelines are intended to address researchers primarily in the United States, but they may be applicable internationally as well.

The 2005 publication of the Guidelines offered a common set of ethical standards for a field that, because of the absence of comprehensive federal funding, was lacking national standards for research. Although the Guidelines have proved useful since 2005, it was recognized soon after their initial issuance that some aspects of them needed clarification in light of experience and that they must be kept up to date given the rapid pace of scientific developments in the field of stem cell research. The National Academies established the Human Embryonic Stem Cell Research Advisory Committee for that purpose in 2006 with support from the Ellison Medical Foundation, the Greenwall Foundation, and the Howard Hughes Medical Institute. It issued its first set of amendments to the Guidelines in 2007 (NRC and IOM, 2007).

Statement of Task of the Human Embryonic Stem Cell Research Advisory Committee

The Advisory Committee will meet 2 to 3 times per year over a period of 36 months to (1) monitor and review scientific developments and changing ethical, legal, and policy issues related to human embryonic stem cell research, (2) discuss the need for revisions to the Guidelines for Human Embryonic Stem Cell Research, and (3) prepare periodic reports to update the Guidelines as needed. Minimal but necessary changes may be issued as letter reports, but more extensive modifications may necessitate the preparation of traditional reports to fully provide the rationale for the changes.

Sources of information that will be considered by the Advisory Committee will include public symposia organized by the Committee to review developments in stem cell science and how these impact the ethical and policy issues surrounding hES cell research.

The Human Embryonic Stem Cell Research Advisory Committee continues to engage in a number of efforts to gather information about the need, if any, for revision of the Guidelines. For example, the Committee conducted three regional meetings (in southern California, Chicago, and the Boston area) in the first half of 2007 for those involved in institutional Embryonic Stem Cell Research Oversight (ESCRO) committees to hear from people in the field about their experiences in implementing the Guidelines and any problems they have encountered. In addition, the Committee participated in a day-long session on ESCRO committees at the annual meeting of Public Responsibility in Medicine and Research (PRIM&R) in December 2007 to gather more feedback from the community.

The Committee also met in March and August 2007 and in February 2008 to hear from invited speakers who addressed issues that the Committee has taken under consideration for potential further amendments to the Guidelines. Finally, the Committee is planning a second symposium (its first was held in November 2006) for November 2008 to hear invited speakers review the latest scientific developments, describe how the developments might affect analyses of associated ethical issues, and identify possible effects on the workability or justifiability of the current Guidelines. The meeting will

focus in part on recent developments in moving toward clinical translation of stem cell therapeutics. The Committee has also established an electronic mailing list for ESCRO committee members and staff to communicate and share questions and answers, and members of the Committee have been actively soliciting input from their colleagues and receiving comments via a Web site[1] established for the purpose.

As it did in 2007, the Committee identified issues that appeared to warrant consideration of revisions of the Guidelines. The present report addresses those issues in a second brief set of amendments. Most important, the Committee is issuing this second set of amendments to address new scientific developments in reprogramming of somatic cells to pluripotency by adding a new section (Section 7) and revising other relevant sections of the Guidelines. It is also issuing several other minor amendments to

- Clarify the obligations of investigators to notify and obtain approval from their institutions' ESCRO committees before initiating any hES cell experiments and to provide for the possibility of "expedited review" of some hES cell experimental protocols—Section 1.3(a)[2], Section 6.1, and Section 6.2.

- Clarify what is included in "direct expenses" for allowable reimbursements to women donating oocytes—Section 3.4(b).

- Further enumerate the registration and auditing responsibilities of institutions conducting hES cell research to improve public access to information and ensure that ESCRO committees are carrying out their responsibilities appropriately—Section 2.0.

In addition, inconsistencies in the original numbering of the Guidelines have led to some confusion. Various sections of the Guidelines, particularly within Section 1, have been renumbered in these amendments for greater clarity.

Future deliberations of the Committee will address items for which additional information-gathering and more extensive debate and discussion may be necessary. For example, based on the National Institutes of Health (NIH) determination that the pre-2001 "presidential" lines were derived from embryos donated with informed consent and without financial induce-

[1]http://www.nationalacademies.org/stemcells

[2]Formerly Section 1.2(a). As explained below, several sections of the Guidelines, particularly within Section 1, are being renumbered in these amendments for greater clarity.

ment (NIH, 2001), the 2007 Guidelines deemed those lines to have been acceptably derived (see Sections 1.4 and 1.5 and associated discussion in NRC and IOM, 2007). In light of questions raised when the present report was already near completion about the derivation or use of some of those lines (Streiffer, 2008), and as per its charge, the Committee will monitor developments as to the ethics and policy regarding the lines in question in order to consider whether any future changes in the Guidelines are warranted. Stem cell research oversight committees are, of course, free to set their own policies about the use of these lines according to the principles outlined in Section 1.6 of the Guidelines (as renumbered in this document). The Committee is also aware that the scientific and oversight communities desire additional guidance on how to evaluate research that requires the development of chimeras. In response, the Committee has added some text in the new Section 7.3(c) [as well as 1.3(b)] and also plans to address research involving chimeras at the meeting it is organizing for November 2008.

These amended Guidelines supersede those issued in 2005 and 2007 by the Committee on Guidelines for Human Embryonic Stem Cell Research and the Human Embryonic Stem Cell Research Advisory Committee, respectively. It is important that the clarifications and amendments presented here be interpreted in the context of the complete set of amended Guidelines, which is included at the end of this report (Appendix A). In addition, the glossary included in the 2005 *Guidelines for Human Embryonic Stem Cell Research* (NRC and IOM, 2005) has been amended by adding definitions for the terms *hPS cells* and *multipotent*, and the entire glossary is reprinted as Appendix B.

APPLICABILITY OF THE GUIDELINES TO NON-EMBRYONIC HUMAN PLURIPOTENT STEM CELLS

The original Guidelines released in 2005 were addressed specifically to research with hES cell lines, although institutions and investigators conducting research on human adult stem cells or fetal stem cells were encouraged to "consider which individual provisions of these guidelines are relevant to their research." Because the Guidelines were developed primarily for research with hES cells, however, it was not made explicit which provisions of the Guidelines might apply to other types of stem cells.

There have been several recent reports on reprogramming of somatic cells to pluripotency (for definitions see glossary, Appendix B). In light of the production of so-called induced pluripotent stem (iPS) cell lines derived

by introducing sets of genes into, first, murine somatic cells (Takahashi and Yamanaka, 2006) and, later, human somatic cells (Takahashi et al., 2007; Yu et al., 2007; Park et al., 2008), it seems prudent to consider more explicitly which provisions of the Guidelines should apply also to stem cells of types other than hES cells. This is not to suggest that the need for research with hES cells is supplanted by the availability of other pluripotent stem cells. It is far from clear at this point which cell types will prove to be the most useful for regenerative medicine, and it is likely that each will have some utility. Such iPS cells are currently derived by introduction of retroviruses that carry the inducing genes. This derivation procedure raises serious issues about their potential for use in therapy, inasmuch as it is known that inserted retroviruses can cause cancer, and research will be necessary to develop alternative means to derive iPS cells or to circumvent the potential tumorigenicity. Furthermore, the demonstration that iPS cells are indeed pluripotent relies on careful comparisons with hES cells; for either cell type to be used therapeutically in regenerative medicine, methods need to be developed to promote their differentiation into specialized cell types and to evaluate the safety of introducing cell populations that may contain some pluripotent cells into patients. Much further research will be required on both hES and iPS cells to develop the required procedures, including drawing appropriate comparisons between them. Understanding of the potential for differentiation of hES cells, iPS cells, or, indeed, adult multipotent (capable of differentiation into a limited spectrum of differentiated cell types)[3] stem cells will require testing in animals and screening for potential tumorigenicity. Therefore, issues arising from such human-animal chimera experiments pertain to all these cell types.

For those reasons and in response to inquiries from the scientific community, the Human Embryonic Stem Cell Research Advisory Committee has consulted with experts and carefully considered potential modifications of the Guidelines to cover other pluripotent and multipotent stem cells, which the Committee presents herein. The intention is not to extend unnecessarily the oversight of stem cell research where it is already adequately monitored under existing regulations and guidelines. For example, derivation of human pluripotent stem cell lines from sources other than embryos does not involve ethical or policy issues beyond those normally encountered in sampling any tissue from human subjects, although *use* of such cells may raise issues similar to those for embryonically derived cells. Derivation of iPS cells and

[3]A multipotent stem cell can give rise to other types of cells but it is limited in its ability to differentiate. An example is found in the multipotent stem cells in bone marrow that give rise to all blood cells but not other cell types.

of other non-embryonic human pluripotent stem cells (hereafter referred to as hPS cells) does not require special stem cell expertise and is adequately covered by current Institutional Review Board (IRB) regulations. It does not require additional review by an ESCRO committee. The Committee notes in particular that under federal regulations, even IRBs would not be required to review the generation of hPS cells from existing anonymized somatic cells from surgical waste, tissue banks, or commercial entities that provide tissue for research, nor would they be required to review the generation of hPS cells from cadaveric tissue, whether or not it is anonymized. Similarly, with few exceptions, purely *in vitro* experiments with hPS cells do not raise ethical concerns beyond those encountered with any human cell line and also do not require ESCRO committee review.

However, as mentioned above, introduction of any hPS cells and introduction of some multipotent stem cells (such as neural stem cells) into animals raises issues similar to those pertaining to hES cells. The earlier versions of the Guidelines placed responsibility for review of such experiments with hES cells in the hands of ESCRO committees and Institutional Animal Care and Use Committees (IACUCs), and it is logical to do the same for hPS cells and for stem cells with more limited potential for differentiation. The revisions presented in this document provide guidance on the levels of review for various categories of experiments with iPS and other hPS cells and on categories of research for which such review is not necessary. Most of the changes appear in a new Section 7, "Recommendations for Research on Non-Embryo-Derived Human Pluripotent Stem Cells (hPS Cells)", although some provisions of Sections 1, 3, 4, and 5 are also affected, as follows (new or revised wording is underlined, and deleted text appears in ~~strikeout~~ form):

From Section 1

1.1 What These Guidelines Cover

1.1(a) These guidelines cover all derivation of hES cell lines and all research that uses hES cells derived from

(i) blastocysts made for reproductive purposes and later obtained for research from *in vitro* fertilization (IVF) clinics,
(ii) blastocysts made specifically for research using IVF,
(iii) somatic cell nuclear transfer (NT) into oocytes.

1.1(b) ~~Many, but not all,~~ Some of the ~~guidelines and~~ concerns addressed in this report are common to other ~~areas~~ types of human stem cell research; as such, certain of these Guidelines should also apply to those other types of research. For example, ~~such as~~

(i) research that uses human adult stem cells.

(ii) research that uses fetal stem cells or embryonic germ cells derived from fetal tissue; such research is covered by federal statutory restrictions at 42 USC 289g-2(a) and federal regulations at 45 CFR 46.210.

(iii) research that uses human pluripotent stem (hPS) cells derived from non-embryonic sources, such as spermatogonial stem cells and "induced pluripotent" stem cells derived from somatic cells by introduction of genes or otherwise (so-called iPS cells), and other pluripotent cells yet to be developed.

Recommendations as to which guidelines apply to other hPS cells are collected in Section 7 below. Institutions and investigators conducting research ~~using such materials~~ with adult and fetal stem cells should also consider which individual provisions of these guidelines are relevant to their research.

1.1(c) The guidelines do not cover research that uses nonhuman stem cells.

From Section 3

3.1 An IRB, as described in federal regulations at 45 CFR 46.107, should review all new procurements of all gametes, blastocysts, or somatic cells for the purpose of generating new hES or hPS cell lines. This includes the procurement of blastocysts in excess of clinical need from infertility clinics; blastocysts made through IVF specifically for research purposes; ~~and~~ oocytes, sperm, and somatic cells donated for development of hES cell lines derived

through NT or by parthenogenesis or androgenesis; and hPS cells derived by any means and that require human subjects review.

3.6 In the context of donation of gametes, blastocysts, or somatic cells for hES cell research, or for hPS cell research that requires human subjects review, the informed-consent process should, at a minimum, provide the following information:

(a) A statement that the blastocysts, gametes, or somatic cells will be used to derive hES or hPS cells for research that may include research on human transplantation.
(b) A statement that the donation is made without any restriction or direction regarding who may be the recipient of transplants of the cells derived, except in the case of autologous donation.
(c) A statement as to whether the identities of the donors will be readily ascertainable to those who derive or work with the resulting hES or hPS cell lines.
(d) If the identities of the donors are retained (even if coded), a statement as to whether donors wish to be contacted in the future to receive information obtained through studies of the cell lines.
(e) An assurance that participants in research projects will follow applicable and appropriate best practices for donation, procurement, culture, and storage of cells and tissues to ensure, in particular, the traceability of stem cells. (Traceable information, however, must be secured to ensure confidentiality.)
(f) A statement that derived hES or hPS cells and/or cell lines might be kept for many years.
(g) A statement that the hES or hPS cells and/or cell lines might be used in research involving genetic manipulation of the cells or mixing of human and nonhuman cells in animal models.
(h) Disclosure of the possibility that the results of study of the hES or hPS cells may have commercial potential and a statement that the donor will not receive financial or any other benefits from any future commercial development.
(i) A statement that the research is not intended to provide direct medical benefit to the donor(s) except in the case of autologous donation.

(j) A statement that embryos will be destroyed in the process of deriving hES cells.
(k) A statement that neither consenting nor refusing to donate embryos for research will affect the quality of any future care provided to potential donors.
(l) A statement of the risks involved to donors.

In addition, donors could be offered the option of agreeing to some forms of hES cell research but not others. For example, donors might agree to have their materials used for deriving new hES cell lines but not want their materials used, for example, for NT. The consent process should fully explore whether donors have objections to any specific forms of research to ensure that their wishes are honored. Investigators and stem cell banks are, of course, free to choose which cell lines to accept, and are not obligated to accept cell lines for which maintaining information about specific research use prohibitions would be unduly burdensome.

New derivations of stem cell lines from banked tissues obtained prior to the adoption of these guidelines are permissible provided that the original donations were made in accordance with the legal requirements in force at the place and time of donation. This includes gametes, blastocysts, adult stem cells, somatic cells, or other tissue. In the event that these banked tissues retain identifiers linked to living individuals, human subjects protections may apply.

From Section 4

4.6 Investigators must document how they will characterize, validate, store, and distribute any new hES cell lines and how they will maintain the confidentiality of any coded or identifiable information associated with the lines (see Section 5.0 below). Investigators are encouraged to apply the same procedures and standards for characterization, validation, storage, and distribution to hPS cell lines.

From Section 5

5.0 BANKING AND DISTRIBUTION OF hES CELL LINES

There are several models for the banking of human biological materials, including hES cells. The most relevant is the U.K. Stem Cell Bank. The guidelines developed by this and other groups generally adhere to key ethical principles that focus on the need for consent of donors and a system for monitoring adherence to ethical, legal, and scientific requirements. As hES cell research advances, it will be increasingly important for institutions that are obtaining, storing, and using cell lines to have confidence in the value of stored cells—that is, that they were obtained ethically and with the informed consent of donors, that they are well characterized and screened for safety, and that the conditions under which they are maintained and stored meet the highest scientific standards. Institutions engaged in hES research should seek mechanisms for establishing central repositories for hES cell lines—through partnerships or augmentation of existing quality research cell line repositories—and should adhere to high ethical, legal, and scientific standards. At a minimum, an institutional registry of stem cell lines should be maintained. Institutions are encouraged to consider the use of the same procedures for banking and distribution of hPS cell lines.

Section 7

7.0 RECOMMENDATIONS FOR RESEARCH ON NON-EMBRYO-DERIVED HUMAN PLURIPOTENT STEM CELLS (hPS CELLS)

7.1 Derivation

Because non-embryo-derived hPS cells are derived from human material, their derivation is covered by existing IRB regulations concerning review and informed consent. No ESCRO committee review is necessary, although the IRB may always seek the advice of an ESCRO committee if it seems desirable. The IRB review

should consider proper consent for use of the derived hPS cells. Some of the recommendations for informed consent that apply to hES cells also apply to hPS cells (see Section 3.6), including informed consent to genetic manipulation of resulting pluripotent stem cells and their use for transplantation into animals and humans and, potentially, in future commercial development.

7.2 Use in *in Vitro* Experiments

Use of hPS cells in purely *in vitro* experiments need not be subject to any review beyond that necessary for any human cell line except that any experiments designed or expected to yield gametes (oocytes or sperm) should be subject to ESCRO committee review.

7.3 Use in Experiments Involving Transplantation of hPS Cells into Animals at Any Stage of Development or Maturity

7.3(a) Research involving transplantation of pluripotent human cells derived from non-embryonic sources into nonhuman animals at any stage of embryonic, fetal, or postnatal development should be reviewed by ESCRO committees and IACUCs, as are similar experiments that use hES cells.

7.3(b) ESCRO committees should review the provenance of hPS cells as they review the provenance of hES cells (see Section 1.6) to ensure that the cell lines were derived according to ethical procedures of informed consent as monitored by an IRB or equivalent oversight body.

7.3(c) Proposals for the use of hPS cells in animals should be considered in one of the following categories:

(i) Permissible after currently mandated reviews and proper documentation [see Section 1.3(a)]: experiments that are exempt from full ESCRO committee review but not IACUC review (experiments that involve only transplantation into postnatal animals with no likelihood of contributing to the central nervous system or germ line).

(ii) Permissible after additional review by an ESCRO committee, as described in Section 2.0 of the Guidelines [see Section 1.3(b)]: experiments in which there is a significant possibility that the implanted hPS cells could give rise to neural or gametic cells and tissues. Such experiments need full ESCRO committee and IACUC review and would include generation of all preimplantation chimeras and neural transplantation into embryos or perinatal animals. Particular attention should be paid to at least three factors: the extent to which the implanted cells colonize and integrate into the animal tissue; the degree of differentiation of the implanted cells; and the possible effects of the implanted cells on the function of the animal tissue.

(iii) Should not be conducted at this time [see Section 1.3(c)]:

(1) Experiments that involve transplantation of hPS cells into human blastocysts.

(2) Research in which hPS cells are introduced into nonhuman primate embryos, pending further research that will clarify the potential of such introduced cells to contribute to neural tissue or to the germ line.

7.4 Multipotent Neural Stem Cells

It is also relevant to note that neural[4] stem cells, although not pluripotent, are multipotent and may have the potential to contribute to neural tissue in chimeric animals. ESCRO committees should decide whether they wish to review and monitor such experiments with neural stem cells in a similar fashion.

7.5 Prohibition on Breeding

No animal into which hPS cells have been introduced such that they could contribute to the germ line should be allowed to breed.

[4]Referring to cells of the nervous system that give rise to both neurons and glia.

7.6 Guidance for Banking and Distribution

Institutions should consider the value of banking and distributing hPS cells using the guidance and rules that are already in place for hES cells and the value of including hPS cell lines in their registries.

CLARIFICATION OF THE MEANING OF "PROPER NOTIFICATION"

Section 1.3 (formerly Section 1.2) of the Guidelines specifies research that is "permissible after currently mandated review and *proper notification* of the relevant research institution" (emphasis added). Section 1.3(a) clarifies which documentation is required for determining the provenance of the cell lines, but it does not address what "proper notification" entails. Similarly, Sections 6.1 and 6.2 concerning research use of hES cell lines refer to "notification" and "notice" but do not specify what notification entails.

Use of the word "notification" has led some ESCRO committee representatives to ask whether the Guidelines intend that investigators fulfill this requirement by merely informing ESCRO committees that the research would be occurring (that is, the investigator would determine and inform, but the ESCRO committee would have no role). That is not what was intended. The discussion in the 2005 report states that the "ESCRO committee should ensure that the procurement process has been appropriate by requiring documentation that it was approved by an IRB and adhered to basic principles of ethically responsible procurement" (NRC and IOM, 2005, pp. 54-55). Thus, the ESCRO committee—not the investigator—must decide whether the proposed research is purely *in vitro* research with existing hES cell lines that meet appropriate standards for procurement.

The original Guidelines Committee intended that notification involve the ESCRO committee but allow expedited review procedures, such as those used in the context of IRBs. The federal regulations for IRBs outline the procedure as follows (45 CFR 46.110[5]):

> Under an expedited review procedure, the review may be carried out by the IRB chairperson or by one or more experienced reviewers designated by the chairperson from among members of the IRB. In reviewing the research, the reviewers may ex-

[5]http://www.hhs.gov/ohrp/humansubjects/guidance/45cfr46.htm#46.110.

> ercise all of the authorities of the IRB except that the reviewers may not disapprove the research. . . .
>
> (c) Each IRB which uses an expedited review procedure shall adopt a method for keeping all members advised of research proposals which have been approved under the procedure.

ESCRO committees are therefore called on to establish procedures for reviewing purely *in vitro* research that uses previously and appropriately derived hES cell lines; these reviews may be expedited at the discretion of an ESCRO committee. The former Section 1.2(a) [renumbered as 1.3(a)] of the Guidelines is therefore revised to clarify this point.

1.3(a) hES cell research permissible after currently mandated reviews

Purely *in vitro* hES cell research that uses previously derived hES cell lines is permissible provided that the ESCRO committee or equivalent body designated by the investigator's institution (see Section 2.0) receives documentation of the provenance of the cell lines, including (i) documentation of the use of an acceptable informed-consent process that was approved by an Institutional Review Board (IRB) or foreign equivalent for their derivation (consistent with Section 3.6) and (ii) documentation of compliance with any additional required review by an Institutional Animal Care and Use Committee (IACUC), Institutional Biosafety Committee (IBC), or other institutionally mandated review. To determine whether the proposed research meets the requirements of this section, the ESCRO committee may choose to conduct an *expedited review* of such research proposals. In this context, "expedited review" means that the ESCRO committee chair or others designated by the committee chair can act on behalf of the committee to determine that the hES cells have been acceptably derived (see Section 1.6) and report to the entire committee.

In addition, Sections 6.1 and 6.2 are revised to be consistent with the changes in the newly revised and renumbered 1.3(a):

6.1 Institutions should require documentation of the provenance of all hES cell lines, whether the cells were imported into the institution or generated locally. ~~Notice to~~ The institution should obtain ~~include~~ evidence of IRB approval of the procurement process and of adherence to basic ethical and legal principles of procurement as described in Sections 1.3(a) and 1.6. In the case of lines imported from another institution, documentation that these criteria were met at the time of derivation will suffice.

6.2 *In vitro* experiments involving the use of already derived and coded hES cell lines will not need review beyond the ~~notification required~~ review described in Sections 1.3(a) and ~~in Section~~ 6.1.

PUBLIC OPENNESS AND ESCRO COMMITTEE AUDITS

Research that uses hES cells remains controversial in the United States and is still subject to intense political scrutiny. Therefore, it is important to sustain public confidence in the integrity of the institutions and researchers conducting hES cell research; this is one of the reasons that the Guidelines were developed. The Human Embryonic Stem Cell Research Advisory Committee continues to believe that it is in the interests of researchers and their institutions to ensure that the Guidelines of the National Academies or other relevant bodies (such as state regulations and guidelines of the International Society for Stem Cell Research) are being appropriately implemented to ensure that both the public and policy-makers may have a high level of confidence that institutions and their researchers are conducting the research responsibly. As part of this assurance, the public should have reasonable access to information on the types of hES cell research being conducted at an institution and evidence that the research conforms to the requirements of the guidelines being followed by that institution.

For those reasons, the committee is amending the Guidelines in two ways. First, Section 2.0 calls for registries of hES cell research to be maintained by institutional ESCRO committees. Although the original intent was that the information in a registry be available to the public, this intent was not explicit in the Guidelines. The committee is therefore amending the wording of Section 2.0 to make that clear. Second, although the committee cannot impose legally enforceable requirements, it is adding a strong suggestion that institutions at which hES cell research is being conducted carry out pe-

riodic audits (for example, every 3-5 years) of their ESCRO committees to ensure that these groups are performing their duties as intended as a good management practice. The emphasis of the audits should be on documenting decisions regarding the acceptability of research proposals and on verifying that cell lines in use at the institution were acceptably derived. Institutions should also make at least the general findings and preferably the details of the audits available to the public. The amended wording (underlined) of Section 2.0 is as follows:

2.0 ESTABLISHMENT OF AN INSTITUTIONAL EMBRYONIC STEM CELL RESEARCH OVERSIGHT COMMITTEE

To provide oversight of all issues related to derivation and use of hES cell lines and to facilitate education of investigators involved in hES cell research, each institution should have activities involving hES cells overseen by an Embryonic Stem Cell Research Oversight (ESCRO) committee. This committee could be internal to a single institution or established jointly with one or more other institutions. Alternatively, an institution may have its proposals reviewed by an ESCRO committee of another institution, or by an independent ESCRO committee. An ESCRO committee should include independent representatives of the lay public as well as persons with expertise in developmental biology, stem cell research, molecular biology, assisted reproduction, and ethical and legal issues in hES cell research. It must have suitable scientific, medical, and ethical expertise to conduct its own review and should have the resources needed to coordinate the management of the various other reviews required for a particular protocol. A pre-existing committee could serve the functions of the ESCRO committee provided that it has the ~~recommended~~ expertise recommended here and representation to perform the various roles described in this report. For example, an institution might elect to constitute an ESCRO committee from among some members of an IRB. But the ESCRO committee should not be a subcommittee of the IRB, as its responsibilities extend beyond human subject protections. Furthermore, much hES cell research does not require IRB review. The ESCRO committee should

(a) Provide oversight over all issues related to derivation and use of hES cell lines.
(b) Review and approve the scientific merit of research protocols.
(c) Review compliance of all in-house hES cell research with all relevant regulations and these guidelines.
(d) Maintain registries of hES cell research conducted at the institution and hES cell lines derived or imported by institutional investigators. An institution conducting stem cell research should make information from the registries (including, but not necessarily limited to, project abstracts and sources of funding) available to the public and the media through the institution's Web site.
(e) Facilitate education of investigators involved in hES cell research.

An institution that maintains its own ESCRO committee should conduct periodic audits of the committee to verify that it is carrying out its responsibilities appropriately. Auditable records include documentation of decisions regarding the acceptability of research proposals and verification that cell lines in use at the institution were acceptably derived (see Section 1.6). Institutions should make the results of the audits available to the public.

An institution that uses an external ESCRO committee should nevertheless ensure that the registry and educational functions of an internal ESCRO committee are carried out by the external ESCRO committee on its behalf or internally by other administrative units. Those institutions that use external ESCRO committees are also responsible for ensuring that these committees are likewise carrying out their responsibilities appropriately.

CLARIFICATION OF POLICY REGARDING REIMBURSEMENT OF OOCYTE DONORS

It was pointed out in the report *Guidelines for Human Embryonic Stem Cell Research* (NRC and IOM, 2005) that although there is widespread consensus that donors should not be paid for blastocysts donated for research,

there is less of a consensus about inducements for women to donate oocytes or for men to donate sperm for research purposes. Oocyte donation solely for research purposes is the issue of most concern because of its invasiveness, its inconvenience, and the risks posed by the procedure (reviewed in IOM and NRC, 2007). If the need for oocytes in hES cell research increases, however, it is possible that donations from clinical procedures or for nonfinancial motives may prove insufficient to meet the demand. In such cases, investigators might want to recruit oocyte donors, and it is from this circumstance that the issue of whether such donors should be paid arises.

Guidelines for Human Embryonic Stem Cell Research contained a long discussion (Chapter 5) of the arguments for and against payment of oocyte donors, which will not be repeated here. In short, one side argues for fair and just remuneration of participants in research, in which inducements are commonly provided for competent adult research subjects provided that the research risks are reasonable in relation to the potential research benefits. Furthermore, because payment is legal and widely practiced for egg donation for reproductive purposes, many find the forbidding of payment in the research context difficult to justify. Others, however, oppose any payment, whether for research or reproduction. Typically, they caution against any form of payment that may create an "undue inducement" that could compromise a prospective donor's evaluation of the risks posed by donation or the voluntariness of her choices. Furthermore, opponents of payment often embed their objections in a larger set of concerns about the "commodification of life," which also apply to payment for human tissue of any sort and to the patenting of genes and other issues. Complicating these principled debates are more pragmatic concerns: whether (and how much) payment is needed to ensure a sufficient supply of oocytes for nuclear transfer and other forms of specialized stem cell research, and the interchangeability of cell lines, material transfers, and the future of collaborative stem cell research if various state and national jurisdictions have different rules regarding reimbursement and compensation for oocyte donors.

The recommendation made by the Committee on Guidelines for Human Embryonic Stem Cell Research in 2005 was that women who undergo hormonal induction to generate oocytes specifically for research purposes should be reimbursed only for direct expenses incurred as a result of the procedure, as determined by an Institutional Review Board. Thus, the National Academies' Guidelines prohibit cash or in-kind payments for donating oocytes for research purposes. As pointed out in the earlier report (NRC and IOM,

2005) that position was based in part on the recognition that payments to oocyte donors raise concerns that might undermine public confidence in the responsible management of hES cell research. The report also noted that the recommendation was intended to ensure consistency between procurement practices in the United States and in other countries that have major hES cell research programs and with the limitations enacted in specific states, facilitating collaboration among investigators in the United States and abroad. Since that time, however, California has provided a useful model in its finalized regulations (Title 17 CA Code of Regulations, Section 100020) that allows reimbursement of oocyte donors for "permissible expenses," which are clearly defined to include "actual lost wages." The state of Massachusetts has a similar policy. Although the original National Academies' Guidelines did not specifically mention lost wages as a reimbursable category of direct expenses, institutions and states that perform or support hES cell research should view the National Academies' Guidelines as open to the interpretation that "lost wages" is a legitimate category of reimbursable expenses. To make that explicit, the wording of Section 3.4(b) is modified as follows (new wording underlined):

> 3.4(b) Women who undergo hormonal induction to generate oocytes specifically for research purposes (such as for NT) should be reimbursed only for direct expenses incurred as a result of the procedure, as determined by an IRB. Direct expenses may include costs associated with travel, housing, child care, medical care, health insurance, and actual lost wages. No payments beyond reimbursements, cash or in-kind, should be provided for donating oocytes for research purposes. Similarly, no payments beyond reimbursements should be made for donations of sperm for research purposes or of somatic cells for use in NT.

The committee does not find persuasive the argument that this change has the effect of assigning differing values to the oocytes of different women based on their relative salaries. Reimbursement for lost wages is not a "price" being paid for oocytes. The intent is to leave all donors no better off, but also no worse off.

REFERENCES

IOM and NRC (Institute of Medicine and National Research Council). 2007. *Assessing the Medical Risks of Human Oocyte Donation for Stem Cell Research: Workshop Report*. Washington, D.C.: The National Academies Press.

NIH (National Institutes of Health). 2001. Funding of Research Using Specified Existing Human Embryonic Stem Cells. Notice NOT-OD-01-059, National Institutes of Health, release date August 27, 2001.

NRC and IOM. 2005. *Guidelines for Human Embryonic Stem Cell Research*. Washington, D.C.: The National Academies Press.

NRC and IOM. 2007. *2007 Amendments to the National Academies' Guidelines for Human Embryonic Stem Cell Research*. Washington, D.C.: The National Academies Press.

Park, In-Hyun, Rui Zhao, Jason A. West, Akiko Yabuuchi, Hongguang Hu, Tan A. Ince, Paul H. Lerou, M. William Lensch, and George Q. Daley. 2008. Reprogramming of human somatic cells to pluripotency with defined factors. *Nature* 451: 141-146.

Streiffer, Robert. 2008. Informed Consent and Federal Funding for Stem Cell Research, *Hastings Center Report* 38(3): 40-47.

Takahashi, Kazutoshi, and Shinya Yamanaka. 2006. Induction of Pluripotent Stem Cells from Mouse Embryonic and Adult Fibroblast Cultures by Defined Factors. *Cell* 126: 663-676.

Takahashi, Kazutoshi, Koji Tanabe, Mari Ohnuki, Megumi Narita, Tomoko Ichisaka, Kiichiro Tomoda, and Shinya Yamanaka. 2007. Induction of pluripotent stem cells from adult fibroblasts by defined factors. *Cell* 131: 1-12.

Yu, Junying, Maxim A. Vodyanik, Kim Smuga-Otto, Jessica Antosiewicz-Bourget, Jennifer L. Frane, Shulan Tian, Jeff Nie, Gudrun A. Jonsdottir, Victor Ruotti, Ron Stewart, Igor I. Sluvkin, and James A. Thomson. 2007. Induced pluripotent stem cell lines derived from human somatic cells. *Science* 318: 1917-1920.

Appendix A

National Academies' Guidelines for Human Embryonic Stem Cell Research Amended as of September 2008[1]

1.0 Introduction
2.0 Establishment of an Institutional Embryonic Stem Cell Research Oversight Committee
3.0 Procurement of Gametes, Blastocysts, or Cells for hES Generation
4.0 Derivation of hES Cell Lines
5.0 Banking and Distribution of hES Cell Lines
6.0 Research Use of hES Cell Lines
7.0 Recommendations for Research on Non-Embryo-Derived Human Pluripotent Stem Cells (hPS Cells)
8.0 International Collaboration
9.0 Conclusion

1.0 INTRODUCTION

In this chapter we collect all the recommendations made throughout the report and translate them into a series of formal guidelines. These guidelines focus on the derivation, procurement, banking, and use of human embryonic stem (hES) cell lines. They provide an oversight process that will help to ensure that research with hES cells is conducted in a responsible and ethically sensitive manner and in compliance with all regulatory requirements pertaining to biomedical research in general. The National Academies are issuing

[1]New or modified wording is indicated by underlining, deleted text by ~~strikeout~~.

these guidelines for the use of the scientific community, including researchers in university, industry, or other private-sector research organizations.

1.1 What These Guidelines Cover

1.1(a) These guidelines cover all derivation of hES cell lines and all research that uses hES cells derived from

(i) blastocysts made for reproductive purposes and later obtained for research from *in vitro* fertilization (IVF) clinics,
(ii) blastocysts made specifically for research using IVF,
(iii) somatic cell nuclear transfer (NT) into oocytes.

1.1(b) Some of the ~~guidelines and~~ concerns addressed in this report are common to other ~~areas~~ types of human stem cell research; as such, certain of these Guidelines should also apply to those other types of research. For example, ~~such as~~

(i) research that uses human adult stem cells,

(ii) research that uses fetal stem cells or embryonic germ cells derived from fetal tissue; such research is covered by federal statutory restrictions at 42 U.S.C. 289g-2(a) and federal regulations at 45 CFR 46.210,

(iii) research using human pluripotent stem (hPS) cells derived from non-embryonic sources, such as spermatogonial stem cells and "induced pluripotent" stem cells derived from somatic cells by introduction of genes or otherwise (so-called iPS cells), as well as other pluripotent cells yet to be developed.

Recommendations as to which guidelines apply to other hPS cells are collected in Section 7 below. Institutions and investigators conducting research ~~using such materials~~ with adult and fetal stem cells should also consider which individual provisions of these guidelines are relevant to their research.

1.1(c) The guidelines do not cover research that uses nonhuman stem cells.

1.2 Reproductive Uses of NT

These guidelines also do not apply to reproductive uses of nuclear transfer (NT), which are addressed in the 2002 report *Scientific and Medical Aspects of Human Reproductive Cloning*, in which the National Academies recommended that "Human reproductive cloning should not now be practiced. It is dangerous and likely to fail." Although these guidelines do not specifically address human reproductive cloning, it continues to be the view of the National Academies that research aimed at the reproductive cloning of a human being should not be conducted at this time.

1.3 Categories of hES Cell Research

These guidelines specify categories of research that:

- Are permissible after currently mandated reviews and proper notification of the relevant research institution.
- Are permissible after additional review by an Embryonic Stem Cell Research Oversight (ESCRO) committee, as described in Section 2.0 of the guidelines.
- Should not be conducted at this time.

Because of the sensitive nature of some aspects of hES cell research, these guidelines in many instances set a higher standard than is required by laws or regulations with which institutions and individuals already must comply.

1.3(a) hES cell research permissible after currently mandated reviews

Purely *in vitro* hES cell research that uses previously derived hES cell lines is permissible provided that the ESCRO committee or equivalent body designated by the investigator's institution (see Section 2.0) receives documentation of the provenance of the cell lines including (i) documentation of the use of an acceptable informed consent process that was approved by an Institutional Review Board (IRB) or foreign equivalent for their derivation (consistent with Section 3.6) and (ii) documentation of compliance with any additional required review by an Institutional Animal Care and Use Committee (IACUC), Institutional Biosafety Committee (IBC), or other institutionally mandated review. To determine whether the proposed research meets the requirements of this section, the ESCRO committee may choose to conduct an *expedited review* of such research proposals. In this context, "expedited review" means that the ESCRO committee chair or others des-

ignated by the committee chair act on behalf of the committee to determine that the hES cells have been acceptably derived (see Section 1.6) and report to the entire committee.

1.3(b) hES cell research permissible only after additional review and approval

(i) Generation of new lines of hES cells by whatever means.

(ii) Research involving the introduction of hES cells into nonhuman animals at any stage of embryonic, fetal, or postnatal development. Particular attention should be paid to at least three factors: the extent to which the implanted cells colonize and integrate into the animal tissue; the degree of differentiation of the implanted cells; and the possible effects of the implanted cells on the function of the animal tissue.

(iii) Research in which the identity of the donors of blastocysts, gametes, or somatic cells from which the hES cells were derived is readily ascertainable or might become known to the investigator.

1.3(c) hES cell research that should not be permitted at this time

The following types of research should not be conducted at this time:

(i) Research involving *in vitro* culture of any intact human embryo, regardless of derivation method, for longer than 14 days or until formation of the primitive streak begins, whichever occurs first.

(ii) Research in which hES cells are introduced into nonhuman primate blastocysts or in which any embryonic stem cells are introduced into human blastocysts.

In addition:

(iii) No animal into which hES cells have been introduced such that they could contribute to the germ line should be allowed to breed.

1.4 Obligations of Investigators and Institutions

All scientific investigators and their institutions, regardless of their field, bear the ultimate responsibility for ensuring that they conduct themselves in accordance with professional standards and with integrity. In particular, people whose research involves hES cells should work closely with oversight bodies, demonstrate respect for the autonomy and privacy of those who donate gametes, blastocysts, or somatic cells and be sensitive to public concerns about research that involves human embryos.

1.5 Use of NIH-Approved hES Cell Lines

1.5(a) It is acceptable to use hES cell lines that were approved in August 2001 for use in U.S. federally funded research.

1.5(b) ESCRO committees should include on their registry a list of NIH-approved cell lines that have been used at their institution in accord with the requirement in Section 2.0 of the Guidelines.

1.5(c) Presence on the list of NIH-approved cell lines constitutes adequate documentation of provenance, as per Section 6.1 of the Guidelines.

1.6 Acceptability of Research Using hES Cell Lines Imported from Other Institutions or Jurisdictions

1.6(a) Before approving use of hES and hPS cell lines imported from other institutions or jurisdictions, ESCRO committees should consider whether such cell lines have been "acceptably derived."

1.6(b) "Acceptably derived" means that the cell lines were derived from gametes or embryos for which

(i) the donation protocol was reviewed and approved by an IRB or, in the case of donations taking place outside the United States, a substantially equivalent oversight body;
(ii) consent to donate was voluntary and informed;
(iii) donation was made with reimbursement policies consistent with these Guidelines; and
(iv) donation and derivation complied with the extant legal requirements of the relevant jurisdiction.

1.6(c) ESCRO committees should include on their registry a list of cell lines that have been imported from other institutions or jurisdictions and information on the specific guidelines, regulations, or statutes under which the derivation of the imported cell lines was conducted. This is in accord with the requirement in Section 2.0 of the Guidelines that calls for ESCRO committees to maintain registries listing the cell lines in use at their institutions.

2.0 ESTABLISHMENT OF AN INSTITUTIONAL EMBRYONIC STEM CELL RESEARCH OVERSIGHT COMMITTEE

To provide oversight of all issues related to derivation and use of hES cell lines and to facilitate education of investigators involved in hES cell research, each institution should have activities involving hES cells overseen by an Embryonic Stem Cell Research Oversight (ESCRO) committee. This committee could be internal to a single institution or established jointly with one or more other institutions. Alternatively, an institution may have its proposals reviewed by an ESCRO committee of another institution, or by an independent ESCRO committee. An ESCRO committee should include independent representatives of the lay public as well as persons with expertise in developmental biology, stem cell research, molecular biology, assisted reproduction, and ethical and legal issues in hES cell research. It must have suitable scientific, medical, and ethical expertise to conduct its own review and should have the resources needed to coordinate the management of the various other reviews required for a particular protocol. A pre-existing committee could serve the functions of the ESCRO committee provided that it has the ~~recommended~~ expertise recommended here and representation to perform the various roles described in this report. For example, an institution might elect to constitute an ESCRO committee from among some members of an IRB. But the ESCRO committee should not be a subcommittee of the IRB, as its responsibilities extend beyond human subject protections. Furthermore, much hES cell research does not require IRB review. The ESCRO committee should:

(a) Provide oversight over all issues related to derivation and use of hES cell lines.
(b) Review and approve the scientific merit of research protocols.
(c) Review compliance of all in-house hES cell research with all relevant regulations and these guidelines.
(d) Maintain registries of hES cell research conducted at the institution and hES cell lines derived or imported by institutional investigators.

An institution conducting stem cell research should make information from the registries (including, but not necessarily limited to, project abstracts and source of funding) available to the public and the media through the institution's Web site.

(e) Facilitate education of investigators involved in hES cell research.

An institution that maintains its own ESCRO committee should also conduct periodic audits of the committee to verify that it is carrying out its responsibilities appropriately. Auditable records include documentation of decisions regarding the acceptability of research proposals and verification that cell lines in use at the institution were acceptably derived (see Section 1.6). Institutions should make the results of these audits available to the public.

An institution that uses an external ESCRO committee should nevertheless ensure that the registry and educational functions of an internal ESCRO committee are carried out by the external ESCRO committee on its behalf or internally by other administrative units. Those institutions that use external ESCRO committees are also responsible for ensuring that these committees are likewise carrying out their responsibilities appropriately.

2.1 For projects that involve more than one institution, review of the scientific merit, justification, and compliance status of the research may be carried out by a single ESCRO committee if all participating institutions agree to accept the results of the review.

3.0 PROCUREMENT OF GAMETES, BLASTOCYSTS, OR CELLS FOR hES GENERATION

3.1 An IRB, as described in federal regulations at 45 CFR 46.107, should review all new procurements of all gametes, blastocysts, or somatic cells for the purpose of generating new hES or hPS cell lines. This includes the procurement of blastocysts in excess of clinical need from infertility clinics; blastocysts made through IVF specifically for research purposes; ~~and~~ oocytes, sperm, and somatic cells donated for development of hES cell lines derived through NT or by parthenogenesis or androgenesis; and hPS cells derived by any means that require human subjects review.

3.2 Consent for donation should be obtained from each donor at the time of donation. Even people who have given prior indication of their intent to donate to research any blastocysts that remain after clinical care should

nonetheless give informed consent at the time of donation. Donors should be informed that they retain the right to withdraw consent until the blastocysts are actually used in cell line derivation.

3.3 When donor gametes have been used in the IVF process, resulting blastocysts may not be used for research without consent of all gamete donors.

3.4 Payment and Reimbursement

3.4(a) No payments, cash or in-kind, may be provided for donating blastocysts in excess of clinical need for research purposes. People who elect to donate stored blastocysts for research should not be reimbursed for the costs of storage prior to the decision to donate.

3.4(b) Women who undergo hormonal induction to generate oocytes specifically for research purposes (such as for NT) should be reimbursed only for direct expenses incurred as a result of the procedure, as determined by an IRB. Direct expenses may include costs associated with travel, housing, child care, medical care, health insurance, and actual lost wages. No payments beyond reimbursements, cash or in-kind, should be provided for donating oocytes for research purposes. Similarly, no payments beyond reimbursements should be made for donations of sperm for research purposes or of somatic cells for use in NT.

3.5 To facilitate autonomous choice, decisions related to the creation of embryos for infertility treatment should be free of the influence of investigators who propose to derive or use hES cells in research. Whenever it is practicable, the attending physician responsible for the infertility treatment and the investigator deriving or proposing to use hES cells should not be the same person.

3.6 In the context of donation of gametes, blastocysts, or somatic cells for hES cell research or for hPS cell research that requires human subjects review, the informed-consent process, should, at a minimum, provide the following information.

(a) A statement that the blastocysts, gametes, or somatic cells will be used to derive hES or hPS cells for research that may include research on human transplantation.
(b) A statement that the donation is made without any restriction or direction regarding who may be the recipient of transplants of the cells derived, except in the case of autologous donation.

(c) A statement as to whether the identities of the donors will be readily ascertainable to those who derive or work with the resulting hES or hPS cell lines.
(d) If the identities of the donors are retained (even if coded), a statement as to whether donors wish to be contacted in the future to receive information obtained through studies of the cell lines.
(e) An assurance that participants in research projects will follow applicable and appropriate best practices for donation, procurement, culture, and storage of cells and tissues to ensure, in particular, the traceability of stem cells. (Traceable information, however, must be secured to ensure confidentiality.)
(f) A statement that derived hES or hPS cells and/or cell lines might be kept for many years.
(g) A statement that the hES or hPS cells and/or cell lines might be used in research involving genetic manipulation of the cells or the mixing of human and nonhuman cells in animal models.
(h) Disclosure of the possibility that the results of study of the hES or hPS cells may have commercial potential and a statement that the donor will not receive financial or any other benefits from any future commercial development.
(i) A statement that the research is not intended to provide direct medical benefit to the donor(s) except in the case of autologous donation.
(j) A statement that embryos will be destroyed in the process of deriving hES cells.
(k) A statement that neither consenting nor refusing to donate embryos for research will affect the quality of any future care provided to potential donors.
(l) A statement of the risks involved to the donor.

In addition, donors could be offered the option of agreeing to some forms of hES cell research but not others. For example, donors might agree to have their materials used for deriving new hES cell lines but might not want their materials used, for example, for NT. The consent process should fully explore whether donors have objections to any specific forms of research to ensure that their wishes are honored. Investigators and stem cell banks are, of course, free to choose which cell lines to accept, and are not obligated to accept cell lines for which maintaining information about specific research use prohibitions would be unduly burdensome.

New derivations of stem cell lines from banked tissues obtained prior to the adoption of these guidelines are permissible provided that the original dona-

tions were made in accordance with the legal requirements in force at the place and time of donation. This includes gametes, blastocysts, adult stem cells, somatic cells, or other tissue. In the event that these banked tissues retain identifiers linked to living individuals, human subjects protections may apply.

3.7 Clinical personnel who have a conscientious objection to hES cell research should not be required to participate in providing donor information or securing donor consent for research use of gametes or blastocysts. That privilege should not extend to the care of a donor or recipient.

3.8 Researchers may not ask members of the infertility treatment team to generate more oocytes than necessary for the optimal chance of reproductive success. An infertility clinic or other third party responsible for obtaining consent or collecting materials should not be able to pay for or be paid for the material obtained (except for specifically defined cost-based reimbursements and payments for professional services).

4.0 DERIVATION OF hES CELL LINES

4.1 Requests to the ESCRO committee for permission to attempt derivation of new hES cell lines from donated embryos or blastocysts must include evidence of IRB approval of the procurement process (see Section 3.0 above).

4.2 The scientific rationale for the need to generate new hES cell lines, by whatever means, must be clearly presented, and the basis for the numbers of embryos and blastocysts needed should be justified.

4.3 Research teams should demonstrate appropriate expertise or training in derivation or culture of either human or nonhuman ES cells before permission to derive new lines is given.

4.4 When NT experiments involving either human or nonhuman oocytes are proposed as a route to generation of ES cells, the protocol must have a strong scientific rationale. Proposals that include studies to find alternatives to donated oocytes in this research should be encouraged.

4.5 Neither blastocysts made using NT (whether produced with human or nonhuman oocytes) nor parthenogenetic or androgenetic human embryos

may be transferred to a human or nonhuman uterus or cultured as intact embryos *in vitro* for longer than 14 days or until formation of the primitive streak, whichever occurs first.

4.6 Investigators must document how they will characterize, validate, store, and distribute any new hES cell lines and how they will maintain the confidentiality of any coded or identifiable information associated with the lines (see Section 5.0 below). Investigators are encouraged to apply the same procedures and standards for characterization, validation, storage, and distribution to hPS cell lines.

5.0 BANKING AND DISTRIBUTION OF hES CELL LINES

There are several models for the banking of human biological materials, including hES cells. The most relevant is the U.K. Stem Cell Bank. The guidelines developed by this and other groups generally adhere to key ethical principles that focus on the need for consent of donors and a system for monitoring adherence to ethical, legal, and scientific requirements. As hES cell research advances, it will be increasingly important for institutions that are obtaining, storing, and using cell lines to have confidence in the value of stored cells—that is, that they were obtained ethically and with the informed consent of donors, that they are well characterized and screened for safety, and that the conditions under which they are maintained and stored meet the highest scientific standards. Institutions engaged in hES research should seek mechanisms for establishing central repositories for hES cell lines—through partnerships or augmentation of existing quality research cell line repositories and should adhere to high ethical, legal, and scientific standards. At a minimum, an institutional registry of stem cell lines should be maintained. Institutions are encouraged to consider the use of the same procedures for banking and distribution of hPS cell lines.

5.1 Institutions that are banking or plan to bank hES cell lines should establish uniform guidelines to ensure that donors of material give informed consent through a process approved by an IRB and that meticulous records are maintained about all aspects of cell culture. Uniform tracking systems and common guidelines for distribution of cells should be established.

5.2 Any facility engaged in obtaining and storing hES cell lines should consider the following standards:

(a) Creation of a committee for policy and oversight purposes and creation of clear and standardized protocols for banking and withdrawals.

(b) Documentation requirements for investigators and sites that deposit cell lines, including
  (i) A copy of the donor consent form.
  (ii) Proof of Institutional Review Board approval of the procurement process.
  (iii) Available medical information on the donors, including results of infectious-disease screening.
  (iv) Available clinical, observational, or diagnostic information about the donor(s).
  (v) Critical information about culture conditions (such as media, cell passage, and safety information).
  (vi) Available cell line characterization (such as karyotype and genetic markers).

A repository has the right of refusal if prior culture conditions or other items do not meet its standards.

(c) A secure system for protecting the privacy of donors when materials retain codes or identifiable information, including but not limited to
  (i) A schema for maintaining confidentiality (such as a coding system).
  (ii) A system for a secure audit trail from primary cell lines to those submitted to the repository.
  (iii) A policy governing whether and how to deliver clinically significant information back to donors.

(d) The following standard practices:
  (i) Assignment of a unique identifier to each sample.
  (ii) A process for characterizing cell lines.
  (iii) A process for expanding, maintaining, and storing cell lines.
  (iv) A system for quality assurance and control.
  (v) A Web site that contains scientific descriptions and data related to the cell lines available.
  (vi) A procedure for reviewing applications for cell lines.
  (vii) A process for tracking disbursed cell lines and recording their status when shipped (such as number of passages).
  (viii) A system for auditing compliance.

(ix) A schedule of charges.
(x) A statement of intellectual property policies.
(xi) When appropriate, creation of a clear Material Transfer Agreement or user agreement.
(xii) A liability statement.
(xiii) A system for disposal of material.

(e) Clear criteria for distribution of cell lines, including but not limited to evidence of approval of the research by an embryonic stem cell research oversight committee or equivalent body at the recipient institution.

6.0 RESEARCH USE OF hES CELL LINES

Once hES cell lines have been derived, investigators and institutions, through ESCRO committees and other relevant committees (such as an IACUC, an IBC, or a radiation safety committee) should monitor their use in research.

6.1 Institutions should require documentation of the provenance of all hES cell lines, whether the cells were imported into the institution or generated locally. ~~Notice to~~ The institution should obtain ~~include~~ evidence of IRB approval of the procurement process and of adherence to basic ethical and legal principles of procurement as described in Sections 1.3(a) and 1.6. In the case of lines imported from another institution, documentation that these criteria were met at the time of derivation will suffice.

6.2 *In vitro* experiments involving the use of already derived and coded hES cell lines will not need review beyond the ~~notification required~~ review described in Sections 1.3(a) and ~~in Section~~ 6.1.

6.3 Each institution should maintain a registry of its investigators who are conducting hES cell research and ensure that all registered users are kept up to date with changes in guidelines and regulations regarding the use of hES cells.

6.4 All protocols involving the combination of hES cells with nonhuman embryos, fetuses, or adult animals must be submitted to the local IACUC for review of animal welfare issues and to the ESCRO committee for consideration of the consequences of the human contributions to the resulting chimeras. (See also Section 1.3(c)(iii) concerning breeding of chimeras.)

6.5 Transplantation of differentiated derivatives of hES cells or even hES cells themselves into adult animals will not require extensive ESCRO committee review. If there is a possibility that the human cells could contribute in a major organized way to the brain of the recipient animal, however, the scientific justification for the experiments must be strong, and proof of principle using nonhuman (preferably primate) cells, is desirable.

6.6 Experiments in which hES cells, their derivatives, or other pluripotent cells are introduced into nonhuman fetuses and allowed to develop into adult chimeras need more careful consideration because the extent of human contribution to the resulting animal may be higher. Consideration of any major functional contributions to the brain should be a main focus of review. (See also Section 1.3(c)(iii) concerning breeding of chimeras.)

6.7 Introduction of hES cells into nonhuman mammalian blastocysts should be considered only under circumstances in which no other experiment can provide the information needed. (See also Sections 1.3(c)(ii) and 1.3(c)(iii) concerning restrictions on breeding of chimeras and production of chimeras with nonhuman primate blastocysts.)

6.8 Research use of existing hES cells does not require IRB review unless the research involves introduction of the hES cells or their derivatives into patients or the possibility that the identity of the donors of the blastocysts, gametes, or somatic cells is readily ascertainable or might become known to the investigator.

7.0 RECOMMENDATIONS FOR RESEARCH ON NON-EMBRYO-DERIVED HUMAN PLURIPOTENT STEM CELLS (hPS CELLS)

7.1 Derivation

Because non-embryo-derived hPS cells are derived from human material, their derivation is covered by existing IRB regulations concerning review and informed consent. No ESCRO committee review is necessary, although the IRB may always seek the advice of an ESCRO committee if it seems desirable. The IRB review should consider proper consent for use of the derived hPS cells. Some of the recommendations for informed consent that apply to hES cells also apply to hPS cells (see Section 3.6), including informed consent to genetic manipulation of resulting pluripotent stem cells and their

use for transplantation into animals and humans and, potentially, in future commercial development.

7.2 Use in *in Vitro* Experiments

Use of hPS cells in purely *in vitro* experiments need not be subject to any review beyond that necessary for any human cell line except that any experiments designed or expected to yield gametes (oocytes or sperm) should be subject to ESCRO committee review.

7.3 Use in Experiments Involving Transplantation of hPS Cells into Animals at Any Stage of Development or Maturity

7.3(a) Research involving transplantation of pluripotent human cells derived from non-embryonic sources into nonhuman animals at any stage of embryonic, fetal, or postnatal development should be reviewed by ESCRO committees and IACUCs, as are similar experiments that use hES cells.

7.3(b) ESCRO committees should review the provenance of hPS cells as they review the provenance of hES cells (see Section 1.6) to ensure that the cell lines were derived according to ethical procedures of informed consent as monitored by an IRB or equivalent oversight body.

7.3(c) Proposals for use of hPS cells in animals should be considered in one of the following categories:

> (i) Permissible after currently mandated reviews and proper documentation [see Section 1.3(a)]: experiments that are exempt from full ESCRO committee review but not IACUC review (experiments that involve only transplantation into postnatal animals with no likelihood of contributing to the central nervous system or germ line).
>
> (ii) Permissible after additional review by an ESCRO committee, as described in Section 2.0 of the Guidelines [see Section 1.3(b)]: experiments in which there is a significant possibility that the implanted hPS cells could give rise to neural or gametic cells and tissues. Such experiments need full ESCRO committee and IACUC review and would include generation of all preimplantation chimeras and neural transplantation into embryos or perinatal animals. Particular attention should be paid to at least three factors: the extent to which the

implanted cells colonize and integrate into the animal tissue; the degree of differentiation of the implanted cells; and the possible effects of the implanted cells on the function of the animal tissue.

(iii) Should not be conducted at this time [see Section 1.3(c)]:

(1) Experiments that involve transplantation of hPS cells into human blastocysts.
(2) Research in which hPS cells are introduced into nonhuman primate embryos, pending further research that will clarify the potential of such introduced cells to contribute to neural tissue or to the germ line.

7.4 Multipotent Neural Stem Cells

It is also relevant to note that neural stem cells, although not pluripotent, are multipotent and may have the potential to contribute to neural tissue in chimeric animals. ESCRO committees should decide whether they wish to review and monitor such experiments with neural stem cells in a similar fashion.

7.5 Prohibition on Breeding

No animal into which hPS cells have been introduced such that they could contribute to the germ line should be allowed to breed.

7.6 Guidance for Banking and Distribution

Institutions should consider the value of banking and distributing hPS cells using the guidance and rules that are already in place for hES cells and the value of including hPS cell lines in their registries.

8.0 INTERNATIONAL COLLABORATION

If a U.S.-based investigator collaborates with an investigator in another country, the ESCRO committee may determine that the procedures prescribed by the foreign institution afford protections consistent with these guidelines, and the ESCRO committee may approve the substitution of some of or all of the foreign procedures for its own.

9.0 CONCLUSION

The substantial public support for hES cell research and the growing trend by many nonfederal funding agencies and state legislatures to support this field requires a set of guidelines to provide a framework for hES cell research. In the absence of the oversight that would come with unrestricted federal funding of this research, these guidelines will offer reassurance to the public and to Congress that the scientific community is attentive to ethical concerns and is capable of self-regulation while moving forward with this important research.

To help ensure that these guidelines are taken seriously, stakeholders in hES cell research—sponsors, funding sources, research institutions, relevant oversight committees, professional societies, and scientific journals, as well as investigators—should develop policies and practices that are consistent with the principles inherent in these guidelines. Funding agencies, professional societies, journals, and institutional review panels can provide valuable community pressure and impose appropriate sanctions to ensure compliance. For example, ESCROs and IRBs should require evidence of compliance when protocols are reviewed for renewal, funding agencies should assess compliance when reviewing applications for support, and journals should require that evidence of compliance accompanies publication of results.

As individual states and private entities move into hES cell research, it will be important to initiate a national effort to provide a formal context in which the complex moral and oversight questions associated with this work can be addressed on a continuing basis. Both the state of hES cell research and clinical practice and public policy surrounding these topics are in a state of flux and are likely to be so for several years. Therefore, the committee believes that a national body should be established to assess periodically the adequacy of the policies and guidelines proposed in this document and to provide a forum for a continuing discussion of issues involved in hES cell research. New policies and standards may be appropriate for issues that cannot now be foreseen. The organization that sponsors this body should be politically independent and without conflicts of interest, should be respected in the lay and scientific communities, and able to call on suitable expertise to support this effort.

Appendix B

Glossary[1]

Adult stem cell—An undifferentiated cell found in a differentiated tissue that can renew itself and (with limitations) differentiate to yield the specialized cell types of the tissue from which it originated.

Androgenesis—Development in which the embryo contains only paternal chromosomes.

Autologous transplant—Transplanted tissue derived from the intended recipient of the transplant. Such a transplant helps to avoid complications of immune rejection.

Blastocoel—The cavity in the center of a blastocyst.

Blastocyst—A preimplantation embryo of 50–250 cells depending on age. The blastocyst consists of a sphere made up of an outer layer of cells (the trophectoderm), a fluid-filled cavity (the blastocoel), and a cluster of cells on the interior (the inner cell mass).

Blastomere—A single cell from a morula or early blastocyst, before the differentiation into trophectoderm and inner cell mass.

Bone marrow—The soft, living tissue that fills most bone cavities and contains hematopoietic stem cells, from which all red and white blood cells evolve. The bone marrow also contains mesenchymal stem cells from which a number of cell types arise, including chondrocytes, which produce cartilage, and fibroblasts, which produce connective tissue.

[1]New or modified wording is indicated by underlining, deleted text by ~~strikeout~~.

Chimera—An organism composed of cells derived from at least two genetically different cell types. The cells could be from the same or separate species.

Differentiation—The process whereby an unspecialized early embryonic cell acquires the features of a specialized cell, such as a heart, liver, or muscle cell.

DNA—Deoxyribonucleic acid, a chemical found primarily in the nucleus of cells. DNA carries the instructions for making all the structures and materials the body needs to function.

Ectoderm—The outermost of the three primitive germ layers of the embryo; it gives rise to skin, nerves, and brain.

Egg cylinder—An asymmetric embryonic structure that helps to determine the body plan of the mouse.

Electroporation—Method of introducing DNA into a cell.

Embryo—An animal in the early stages of growth and differentiation that are characterized by cleavage, laying down of fundamental tissues, and the formation of primitive organs and organ systems; especially the developing human individual from the time of implantation to the end of the eighth week after conception, after which stage it becomes known as a fetus.[2]

Embryoid bodies (EBs)—Clumps of cellular structures that arise when embryonic stem cells are cultured. Embryoid bodies contain tissue from all three germ layers: endoderm, mesoderm, and ectoderm. Embryoid bodies are not part of normal development and occur only in vitro.

Embryonic disk—A group of cells derived from the inner cell mass of the blastocyst, which later develops into an embryo. The disk consists of three germ layers known as the endoderm, mesoderm, and ectoderm.

Embryonic germ (EG) cells—Cells found in a specific part of the embryo or fetus called the gonadal ridge that normally develop into mature gametes. The germ cells differentiate into the gametes (oocytes or sperm).

---

[2]http://www.nlm.nih.gov/medlineplus/mplusdictionary.html. In common parlance, "embryo" is used more loosely and variably to refer to all stages of development from fertilization until some ill-defined stage when it is called a fetus. There are strictly defined scientific terms such as "zygote," "morula," and "blastocyst" that refer to specific stages of preimplantation development (see Chapter 2 of NRC and IOM, 2005). In this report, we have used the more precise scientific terms where relevant but have used the term "embryo" where more precision seemed likely to confuse rather than clarify.

Embryonic stem (ES) cells—Primitive (undifferentiated) cells derived from the early embryo that have the potential to become a wide variety of specialized cell types.

Endoderm—Innermost of the three primitive germ layers of the embryo; it later gives rise to the lungs, liver, and digestive organs.

Enucleated cell—A cell whose nucleus has been removed.

Epidermis—The outer cell layers of the skin.

Epigenetic—Refers to modifications in gene expression that are controlled by heritable but potentially reversible changes in DNA methylation or chromatin structure without involving alteration of the DNA sequence.

Epithelium—Layers of cells in various organs, such as the epidermis of the skin or the lining of the gut. These cells serve the general functions of protection, absorption, and secretion, and play a specialized role in moving substances through tissue layers. Their ability to regenerate is excellent; the cells of an epithelium may replace themselves as frequently as every 24 hours from the pools of specialized stem cells.

Feeder cell layer—Cells that are used in culture to maintain pluripotent stem cells. Feeder cells usually consist of mouse embryonic fibroblasts.

Fertilization—The process whereby male and female gametes unite to form a zygote (fertilized egg).

Fibroblasts—Cells from many organs that give rise to connective tissue.

Gamete—A mature male or female germ cell, that is, sperm or oocyte, respectively.

Gastrulation—The procedure by which an animal embryo at an early stage of development produces the three primary germ layers: ectoderm, mesoderm, and endoderm.

Gene—A functional unit of heredity that is a segment of DNA located in a specific site on a chromosome. A gene usually directs the formation of an enzyme or other protein.

Gene targeting—A procedure used to produce a mutation in a specific gene.

Genital ridge—Anatomic site in the early fetus where primordial germ cells are formed.

Genome—The complete genetic material of an organism.

Genotype—Genetic constitution of an individual.

Germ cell—A sperm or egg or a cell that can become a sperm or egg. All other body cells are called somatic cells.

Germ layer—In early development, the embryo differentiates into three distinct germ layers (ectoderm, endoderm, and mesoderm), each of which gives rise to different parts of the developing organism.

Germ line—The cell lineage from which the oocyte and sperm are derived.

Gonadal ridge—Anatomic site in the early fetus where primordial germ cells (PGCs) are formed.

Gonads—The sex glands—testis and ovary.

Hematopoietic—Blood-forming.

Hematopoietic stem cell (HSC)—A stem cell from which all red and white blood cells evolve and that may be isolated from bone marrow or umbilical cord blood for use in transplants.

Hepatocyte—Liver cell.

Heterologous—From genetically different individuals.

hES cell—Human embryonic stem cell; a type of pluripotent stem cell.

Histocompatibility antigens—Glycoproteins on the surface membranes of cells that enable the body's immune system to recognize a cell as native or foreign and that are determined by the major histocompatibility complex.

Homologous recombination—Recombining of two like DNA molecules, a process by which gene targeting produces a mutation in a specific gene.

hPS cells—Human pluripotent stem cells derived from non-embryonic sources.

Hybrid—An organism that results from a cross between gametes of two different genotypes.

Immune system cells—White blood cells, or leukocytes, that originate in the bone marrow. They include antigen-presenting cells, such as dendritic cells, T and B lymphocytes, macrophages, and neutrophils, among many others.

Immunodeficient mice—Genetically altered mice used in transplantation experiments because they usually do not reject transplanted tissue.

Immunogenic—Related to or producing an immune response.

Immunosuppressive—Suppressing a natural immune response.

Implantation—The process in which a blastocyst implants into the uterine wall, where a placenta forms to nurture the growing fetus.

Inner cell mass—The cluster of cells inside the blastocyst that give rise to the embryonic disk of the later embryo and, ultimately, the fetus.

Interspecific—Between species.

In utero—In the uterus.

In vitro—Literally, "in glass," in a laboratory dish or test tube; in an artificial environment.

***In vitro* fertilization (IVF)**—An assisted reproductive technique in which fertilization is accomplished outside the body.

In vivo—In the living subject; in a natural environment.

Karyotype—The full set of chromosomes of a cell arranged with respect to size, shape, and number.

Leukemia inhibitory factor (LIF)—A growth factor necessary for maintaining mouse embryonic stem cells in a proliferative, undifferentiated state.

Mesenchymal stem cells—Stem cells found in bone marrow and elsewhere from which a number of cell types can arise, including chondrocytes, which produce cartilage, and fibroblasts, which produce connective tissue.

Mesoderm—The middle layer of the embryonic disk, which consists of a group of cells derived from the inner cell mass of the blastocyst; it is formed at gastrulation and is the precursor to bone, muscle, and connective tissue.

Morula—A solid mass of 16–32 cells that resembles a mulberry and results from the cleavage (cell division without growth) of a zygote (fertilized egg).

Mouse embryonic fibroblast (MEF)—Cells used as feeder cells in culturing pluripotent stem cells.

Multipotent—Capable of differentiation into a limited spectrum of differentiated cell types.

Neural stem cell (NSC)—A stem cell found in adult neural tissue that can give rise to neurons, astrocytes, and oligodendrocytes.

Nuclear transfer (NT)—Replacing the nucleus of one cell with the nucleus of another cell.

Oocyte—Developing egg; usually a large and immobile cell.

Ovariectomy—Surgical removal of an ovary.

Parthenogenesis—Development in which the embryo contains only maternal chromosomes.

Passage—A round of cell growth and proliferation in culture.

Phenotype—Visible properties of an organism produced by interaction of genotype and environment.

Placenta—The oval or discoid spongy structure in the uterus from which the fetus derives its nourishment and oxygen.

Pluripotent cell—A cell that has the capability of developing into cells of all germ layers (endoderm, ectoderm, and mesoderm).

Precursor cells—In fetal or adult tissues, partly differentiated cells that divide and give rise to differentiated cells. Also known as progenitor cells.

Preimplantation genetic diagnosis (PGD)—A procedure applied to IVF embryos to determine which ones carry deleterious mutations predisposing to hereditary diseases.

Primary germ layers—The three initial embryonic germ layers—endoderm, mesoderm, and ectoderm—from which all other somatic tissue types develop.

Primordial germ cell—A cell appearing during early development that is a precursor to a germ cell.

Primitive streak—The initial band of cells from which the embryo begins to develop. The primitive streak establishes and reveals the embryo's head-tail and left-right orientations.

Pseudopregnant—Refers to a female primed with hormones to accept a blastocyst for implantation.

Somatic cell—Any cell of a plant or animal other than a germ cell or germ cell precursor.

Somatic cell nuclear transfer (SCNT)—The transfer of a cell nucleus from a somatic cell into an egg (oocyte) whose nucleus has been removed.

Stem cell—A cell that has the ability to divide for indefinite periods *in vivo* or in culture and to give rise to specialized cells.

Teratoma—A tumor composed of tissues from the three embryonic germ layers. Usually found in ovary or testis. Produced experimentally in animals by injecting pluripotent stem cells to determine the stem cells' abilities to differentiate into various types of tissues.

Tissue culture—Growth of tissue *in vitro* on an artificial medium for experimental research.

Transfection—A method by which experimental DNA may be put into a cultured cell.

Transgene—A gene that has been incorporated into a cell or organism and passed on to successive generations.

Transplantation—Removal of tissue from one part of the body or from one individual and its implantation or insertion into another, especially by surgery.

Trophectoderm—The outer layer of the developing blastocyst that will ultimately form the embryonic side of the placenta.

Trophoblast—The extraembryonic tissue responsible for negotiating implantation, developing into the placenta, and controlling the exchange of oxygen and metabolites between mother and embryo.

Undifferentiated—Not having changed to become a specialized cell type.

Xenograft or xenotransplant—A graft or transplant of cells, tissues, or organs taken from a donor of one species and grafted into a recipient of another species.

Zygote—A cell formed by the union of male and female germ cells (sperm and egg, respectively).

Appendix C

Committee Biographical Sketches

COCHAIRS

R. Alta Charo, JD, is the Warren P. Knowles Professor of Law and Bioethics at the University of Wisconsin–Madison, on the faculties of both the Law School and the Medical School. In 2006, she was Visiting Professor of Law the University of California, Berkeley Boalt Hall School of Law. Professor Charo is the author of nearly 100 articles, book chapters, and government reports on such topics as voting rights, environmental law, family planning and abortion law, medical genetics law, reproductive technology policy, science policy, and medical ethics. Professor Charo is a member of the boards of the Alan Guttmacher Institute and the Foundation for Genetic Medicine, a member of the National Medical Advisory Committee of the Planned Parenthood Federation of America, and a member of the ethics advisory boards of the International Society for Stem Cell Research, the Juvenile Diabetes Research Foundation, and WiCell. In 2005, she was appointed to the ethics standards working group of the California Institute for Regenerative Medicine and was elected a fellow of the Wisconsin Academy of Sciences, Arts and Letters. In 1994, Professor Charo served on the National Institutes of Health Human Embryo Research Panel; and from 1996 to 2001, she was a member of the presidential National Bioethics Advisory Commission and participated in drafting its reports *Cloning Human Beings* (1997), *Research Involving Persons with Mental Disorders That May Affect Decisionmaking Capacity* (1998), *Research Involving Human Biological Materials: Ethical Issues and Policy Guidance* (1999), *Ethical Issues in Human Stem Cell Research* (1999), *Ethical and Policy Issues in International Research: Clinical Trials in Developing Countries* (2001), and *Ethical and Policy Issues in Research Involving Human Participants* (2001). She was a member of the National Academies' Board on Life Sciences from 2001 until 2007 and since 2006 has been a member of the Institute of Medicine (IOM) Board on

Population Health and Public Health Practices. Professor Charo was elected to IOM in 2006.

Richard O. Hynes, PhD, is the Daniel K. Ludwig Professor for Cancer Research at the David H. Koch Institute for Integrative Cancer Research and Department of Biology at MIT and a Howard Hughes Medical Institute Investigator. He was formerly head of the Biology Department and then director of the Center for Cancer Research at the Massachusetts Institute of Technology. His research focuses on fibronectins and integrins and the molecular basis of cellular adhesion, both in normal development and in pathological situations, such as cancer, thrombosis, and inflammation. Dr. Hynes's current interests are cancer invasion and metastasis, angiogenesis, and animal models of human disease states. He is a member of the National Academy of Sciences and the Institute of Medicine and is a fellow of the Royal Society of London and the American Academy of Arts and Sciences. In 1997, he received the Gairdner International Foundation Award. In 2000, he served as president of the American Society for Cell Biology and testified before Congress about the need for federal support and oversight of embryonic stem cell research. He cochaired the 2005 National Academies *Guidelines for Human Embryonic Stem Cell Research* and is a governor of the Wellcome Trust, UK.

MEMBERS

Eli Y. Adashi, MD, MS, FACOG, is professor of medical science and the former dean of medicine and biological sciences and the Frank L. Day Professor of Biology at the Warren Alpert Medical School of Brown University. Previously, Dr. Adashi served as the professor and chair of the Department of Obstetrics and Gynecology at the University of Utah Health Sciences Center. Dr. Adashi is a member of the Institute of Medicine, a member of the Association of American Physicians, and a fellow of the American Association for the Advancement of Science. Dr. Adashi is a former member of the Advisory Council of the National Institute of Child Health and Human Development and a former president of the Society for Reproductive Endocrinologists, the Society for Gynecologic Investigation, and the American Gynecological and Obstetrical Society. Dr. Adashi is also a former examiner and director of the Division of Reproductive Endocrinology of the American Board of Obstetrics and Gynecology. He is a founding member and treasurer and more recently chair of the advisory committee of the Geneva-based Bertarelli Foundation,

dedicated to promoting the welfare of the infertile couple and to addressing the current "epidemic" of high-order multiple gestations.

Brigid L.M. Hogan, PhD, is the George Barth Geller Professor and chair of the Department of Cell Biology, Duke University Medical Center. Before joining Duke, Dr. Hogan was an investigator of the Howard Hughes Medical Institute and Hortense B. Ingram Professor in the Department of Cell Biology at Vanderbilt University Medical Center. Dr. Hogan earned her PhD in biochemistry at the University of Cambridge. She was then a postdoctoral fellow in the Department of Biology at the Massachusetts Institute of Technology. Before moving to the United States in 1988, Dr. Hogan was head of the Molecular Embryology Laboratory at the National Institute for Medical Research in London. Her research focuses on the genetic control of embryonic development and morphogenesis, using the mouse as a model system. Her laboratory developed methods for deriving mouse pluripotential embryonic germ cell lines. She was cochair for science of the 1994 National Institutes of Health Human Embryo Research Panel and a member of the 2001-2002 National Academies Panel on Scientific and Medical Aspects of Human Cloning. Within the last few years, Dr. Hogan has been elected to the Royal Society of London, the American Academy of Arts and Sciences, the Institute of Medicine, and the National Academy of Sciences.

Marcia Imbrescia is the owner of Peartree Design, a landscape design firm, and was previously the media director for Drumbeater, a high-technology advertising agency. She holds BA degrees in marketing and journalism and a graduate certificate in landscape design. Ms. Imbrescia has a passion for health advocacy and helping people with illness and disability. She is a member of the Board of Trustees of the Arthritis Foundation (AF), for which she has participated as a volunteer at the chapter and national levels. She served as a member (1996-1998 and 2001) and chairperson (2002-2003) of AF's American Juvenile Arthritis Organization. In 1992, she received the Volunteer of the Year Award from the Massachusetts Chapter of AF. Her volunteer efforts include program development, conference planning, public speaking, fundraising, and advocacy. She served on the National Academies Committee on Guidelines for Human Embryonic Stem Cell Research in 2004-2005.

Terry Magnuson, PhD, is Sarah Graham Kenan Professor and chair of the Department of Genetics at the University of North Carolina. He also directs the Carolina Center for Genome Sciences and is the program director of cancer genetics at the Lineberger Comprehensive Cancer Center. Dr. Magnuson's

research interests include mammalian genetics, genomics, and development. His laboratory has developed a high-throughput system to study the effects of mutations on mouse development with mouse embryonic stem cells. He is particularly interested in the role of chromatin remodeling complexes in such processes as autosomal imprinting, X-inactivation, and anterior-posterior patterning of axial structures in mammals. He is an elected member of the American Academy of Arts and Sciences and was a member of the Board of Directors of the Genetics Society of America and of the Society for Developmental Biology.

Linda B. Miller, OTR, MS in hospital administration, is president of the Washington, DC–based Volunteer Trustees Foundation, a consortium of not-for-profit hospital governing boards. She has extensive experience in trustee education, advocacy, and the legal, ethical, and policy issues facing voluntary health care institutions. Recently, she has worked closely with the states' attorneys general in developing guidelines for protecting the community interest in the sale and conversion of nonprofit hospitals and in designing models for practice and legal oversight. She was elected to membership in the Institute of Medicine (IOM) in 1997.

Ms. Miller has been a frequent speaker on health-policy issues and has been published extensively in both the medical and popular press, including the *New England Journal of Medicine*, *Health Affairs*, *USA Today*, the *Washington Post,* and the *New York Times*. She served as a special assistant to the secretary of health, education, and welfare (now the Department of Health and Human Services) and on numerous health-related policy councils and advisory committees, including the National Institutes of Health's Consensus Panel on Liver Transplantation and, most recently, IOM's Committee on Spinal Cord Injury. Ms. Miller serves on the Advisory Board of the University of Louisville–based Institute for Cellular Therapeutics, headed by Suzanne Ildstad, which does research in adult bone marrow transplantation, and has been a member of several academic and health-care institutions' boards of governors, including those of Blythedale Children's Hospital in New York, Capital Hospice in the national capital region, and Cornell University's Alumni Council.

Jonathan D. Moreno, PhD, is the David and Lyn Silfen University Professor and professor of medical ethics and of the history and sociology of science at the University of Pennsylvania. He is also a senior fellow at the Center for American Progress. Until 2007, he was the Emily Davie and Joseph S. Kornfeld Professor of Biomedical Ethics at the University of Virginia, where

he also directed the Center for Biomedical Ethics. Dr. Moreno is a member of the Institute of Medicine. He is also a bioethics adviser for the Howard Hughes Medical Institute, a faculty affiliate of the Kennedy Institute of Ethics at Georgetown University, and a fellow of the Hastings Center. During 1995-1996, he was senior policy and research analyst for the President's Advisory Committee on Human Radiation Experiments; and during 1998-2000, he was a senior consultant for the National Bioethics Advisory Commission. He cochaired the 2005 National Academies Committee on Guidelines for Human Embryonic Stem Cell Research and is a consultant to the Ethical, Social and Cultural Program of the Bill & Melinda Gates Foundation Grand Challenges in Global Health initiative for ethical and regulatory issues related to stem cell research in China.

Pilar N. Ossorio, PhD, JD, is associate professor of law and bioethics at the University of Wisconsin–Madison and program faculty in the Graduate Program in Population Health at the university. Before taking her position there, she was director of the Genetics Section of the Institute for Ethics at the American Medical Association and taught as an adjunct faculty member at the University of Chicago Law School. For the 2006 calendar year, Professor Ossorio was a visiting professor of law at the University of California, Berkeley Boalt Hall School of Law.

Dr. Ossorio received her PhD in microbiology and immunology in 1990 from Stanford University. She went on to complete a postdoctoral fellowship in cell biology at Yale University School of Medicine. Throughout the early 1990s, Dr. Ossorio worked as a consultant for the federal program on the Ethical, Legal, and Social Implications (ELSI) of the Human Genome Project; in 1994, she took a full-time position with the Department of Energy's ELSI program. In 1993, she served on the Ethics Working Group for President Clinton's Health Care Reform Task Force. Dr. Ossorio received her JD from the Boalt Hall School of Law in 1997. While there, she was elected to the legal honor society Order of the Coif and received several awards for outstanding legal scholarship.

Dr. Ossorio is a fellow of the American Association for the Advancement of Science (AAAS), on the Editorial Board of the *American Journal of Bioethics*, an adviser to the National Human Genome Research Institute on ethical issues in large-scale sequencing, and a member of the University of Wisconsin's institutional review board for health-sciences research. She is a past member of AAAS's Committee on Scientific Freedom and Responsibility, a past member of the National Cancer Policy Board in the Institute of Medicine, and a past member or chair of several working groups on genet-

ics and ethics. She has published scholarly articles in bioethics, law, and molecular biology.

E. Albert Reece, MD, PhD, MBA, is dean of the University of Maryland School of Medicine and vice president for medical affairs at the University of Maryland, Baltimore. Previously, he was vice chancellor and dean of the University of Arkansas College of Medicine. Dr. Reece received his undergraduate degree from Long Island University, his MD (Magna Cum Laude) from New York University, his PhD in biochemistry from the University of the West Indies, and his MBA from the Fox School of Business and Management of Temple University. He completed a residency in obstetrics and gynecology at Columbia University–Presbyterian Hospital and a fellowship in maternal-fetal medicine at Yale University School of Medicine. He served on the faculty at Yale for 10 years and was the chairman of the Department of Obstetrics, Gynecology and Reproductive Sciences at Temple University. Dr. Reece has published over 400 journal articles, book chapters, and abstracts and nine textbooks, including *Diabetes in Pregnancy*, *Medicine of the Fetus & Mother*, and *Fundamentals of Obstetric & Gynecologic Ultrasound*. He is an editor for the *Journal of Maternal-Fetal Medicine* and a reviewer for several other scientific journals. His research focuses on diabetes in pregnancy, birth defects, and prenatal diagnosis. Dr. Reece is a member of the Institute of Medicine.

Joshua R. Sanes, PhD, is professor of molecular and cellular biology and the Paul J. Finnegan Family Director of the Center for Brain Science at Harvard University. He was previously Alumni Endowed Professor of Neurobiology at the Washington University School of Medicine. Dr. Sanes earned a BA in biochemistry and psychology at Yale and a PhD in Neurobiology at Harvard. He studies the formation of the synapses that interconnect nerve cells, including pioneering work on the signals exchanged between nerve cells and their target muscles as new connections are made. He is also using the vertebrate visual system to examine how nerve cells develop and migrate to the right location in the body. He was elected a fellow of the American Association for the Advancement of Science in 1992 and a member of the National Academy of Sciences in 2002.

Harold T. Shapiro, PhD, is president emeritus of both Princeton University and the University of Michigan and is currently professor of economics and public affairs at Princeton University. His research interests include bioethics, the social role of higher education, hospital and medical-center administra-

tion, university administration, econometrics, statistics, and economics. Dr. Shapiro chairs the Board of Trustees of the Alfred P. Sloan Foundation, is presiding director for the Dow Chemical Company, and is a member of numerous boards, including the Robert Wood Johnson Medical School, HCA, the Merck Vaccine Advisory Board, the Knight Foundation Commission on Intercollegiate Athletics, the U.S. Olympic Committee, and the Stem Cell Institute of New Jersey. He is a former chair of the Association of American Universities and the National Bioethics Advisory Committee and vice chair of the President's Council of Advisors on Science and Technology. He has also served on the Board of Directors of the National Bureau of Economic Research, Inc. and the Board of Trustees of the Universities Research Association, Inc. He has chaired and served on numerous National Academies committees, including the Committee on the Organizational Structure of the National Institutes of Health and the Committee on Particle Physics. Dr. Shapiro was named the 2006 American Association for the Advancement of Science William D. Carey Lecturer for his leadership in science policy. He earned a PhD in economics from Princeton University and holds 14 honorary doctorates.

John E. Wagner, Jr., MD, is a professor of pediatrics at the University of Minnesota Medical School. He is the first recipient of the Children's Cancer Research Fund/Hageboeck Family Chair in Pediatric Oncology and also holds the Variety Club Endowed Chair in Molecular and Cellular Therapy. He is the director of the Division of Pediatric Hematology/Oncology and Bone Marrow Transplantation and scientific director of clinical research of the Stem Cell Institute. Dr. Wagner is a member of numerous societies, including the American Society of Hematology, the International Society of Experimental Hematology, and the American Society of Blood and Marrow Transplantation. He is a member of several honorary societies, including Alpha Omega Alpha (1980), the American Society of Clinical Investigation (2000), and the Association of American Physicians (2006). Dr. Wagner holds a patent on the isolation of the pluripotential quiescent stem cell population. Dr. Wagner holds a BA in biological sciences and a BA in psychology from the University of Delaware and an MD from Jefferson Medical College. Dr. Wagner's research has focused on the development of novel cellular therapies for tissue repair and suppression of the immune response using subpopulations of neonatal umbilical cord blood and adult bone marrow and peripheral blood. His projects are funded by the National Institutes of Health and industry. In addition, Dr. Wagner pioneered the use of embryo selection to "create" a perfectly tissue-matched stem cell donor for the treat-

ment of genetic disease. Dr. Wagner has written more than 180 articles and book chapters on hematopoietic stem cell transplantation. He cochairs the Graft Sources and Manipulation Working Committee of the Center for International Blood and Marrow Transplant Research (CIBMTR), serves on the Scientific Board of Directors of the National Marrow Donor Program, and is a member of the Scientific and Medical Accountability Standards Working Group of the California Institute of Regenerative Medicine. Dr. Wagner has previously served as a member of the Institute of Medicine's Committee on Establishing a National Cord Blood Stem Cell Banking Program.

Final Report of the National Academies' Human Embryonic Stem Cell Research Advisory Committee and 2010 Amendments to The National Academies' Guidelines for Human Embryonic Stem Cell Research,

NAT'L ACADEMY OF SCIENCES (2010)

FINAL REPORT OF THE NATIONAL ACADEMIES' HUMAN EMBRYONIC STEM CELL RESEARCH ADVISORY COMMITTEE AND 2010 AMENDMENTS TO THE NATIONAL ACADEMIES' GUIDELINES FOR HUMAN EMBRYONIC STEM CELL RESEARCH

Human Embryonic Stem Cell Research Advisory Committee

Board on Life Sciences
Division on Earth and Life Studies

Board on Health Sciences Policy
Institute of Medicine

NATIONAL RESEARCH COUNCIL *AND*
INSTITUTE OF MEDICINE
OF THE NATIONAL ACADEMIES

THE NATIONAL ACADEMIES PRESS
Washington, D.C.
www.nap.edu

THE NATIONAL ACADEMIES PRESS 500 Fifth Street, NW Washington, DC 20001

NOTICE: The project that is the subject of this report was approved by the Governing Board of the National Research Council, whose members are drawn from the councils of the National Academy of Sciences, the National Academy of Engineering, and the Institute of Medicine. The members of the committee responsible for the report were chosen for their special competences and with regard for appropriate balance.

This study was supported by The Ellison Medical Foundation. Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the authors and do not necessarily reflect the views of the organizations or agencies that provided support for the project.

International Standard Book Number-13: 978-0-309-15600-4
International Standard Book Number-10: 0-309-15600-9

Additional copies of this report are available from the National Academies Press, 500 Fifth Street, NW, Lockbox 285, Washington, DC 20055; (800) 624-6242 or (202) 334-3313 (in the Washington metropolitan area); Internet, http://www.nap.edu.

Suggested citation: National Research Council and Institute of Medicine. 2010. *Final Report of the National Academies' Human Embryonic Stem Cell Research Advisory Committee and 2010 Amendments to the National Academies' Guidelines for Human Embryonic Stem Cell Research*. Washington, DC: The National Academies Press.

Cover: A cluster of motor neurons and neural fibers derived from human embryonic stem cells in the lab of University of Wisconsin-Madison stem cell researcher and neurodevelopmental biologist Su-Chan Zhang. These motor neurons were developed from one of James Thomson's original human embryonic stem cell lines. Copyright for the photograph is held by the University of Wisconsin's Board of Regents.

Additional copies of this report are available from the National Academies Press, 500 Fifth Street, NW, Lockbox 285, Washington, DC 20055; (800) 624-6242 or (202) 334-3313 (in the Washington metropolitan area); Internet, http://www.nap.edu.

Printed in the United States of America

THE NATIONAL ACADEMIES

Advisers to the Nation on Science, Engineering, and Medicine

The **National Academy of Sciences** is a private, nonprofit, self-perpetuating society of distinguished scholars engaged in scientific and engineering research, dedicated to the furtherance of science and technology and to their use for the general welfare. Upon the authority of the charter granted to it by the Congress in 1863, the Academy has a mandate that requires it to advise the federal government on scientific and technical matters. Dr. Ralph J. Cicerone is president of the National Academy of Sciences.

The **National Academy of Engineering** was established in 1964, under the charter of the National Academy of Sciences, as a parallel organization of outstanding engineers. It is autonomous in its administration and in the selection of its members, sharing with the National Academy of Sciences the responsibility for advising the federal government. The National Academy of Engineering also sponsors engineering programs aimed at meeting national needs, encourages education and research, and recognizes the superior achievements of engineers. Dr. Charles Vest is president of the National Academy of Engineering.

The **Institute of Medicine** was established in 1970 by the National Academy of Sciences to secure the services of eminent members of appropriate professions in the examination of policy matters pertaining to the health of the public. The Institute acts under the responsibility given to the National Academy of Sciences by its congressional charter to be an adviser to the federal government and, upon its own initiative, to identify issues of medical care, research, and education. Dr. Harvey V. Fineberg is president of the Institute of Medicine.

The **National Research Council** was organized by the National Academy of Sciences in 1916 to associate the broad community of science and technology with the Academy's purposes of furthering knowledge and advising the federal government. Functioning in accordance with general policies determined by the Academy, the Council has become the principal operating agency of both the National Academy of Sciences and the National Academy of Engineering in providing services to the government, the public, and the scientific and engineering communities. The Council is administered jointly by both Academies and the Institute of Medicine. Dr. Ralph J. Cicerone and Dr. Charles Vest are chair and vice chair, respectively, of the National Research Council.

www.national-academies.org

HUMAN EMBRYONIC STEM CELL RESEARCH ADVISORY COMMITTEE

R. ALTA CHARO (*Co-chair*), University of Wisconsin, Madison, WI[1]
RICHARD O. HYNES (*Co-chair*), Howard Hughes Medical Institute and Massachusetts Institute of Technology, Cambridge, MA
ELI Y. ADASHI, Brown University, Providence, RI
BRIGID L.M. HOGAN, Duke University Medical Center, Durham, NC
MARCIA IMBRESCIA, Peartree Design, Lynnfield, MA
TERRY MAGNUSON, University of North Carolina, Chapel Hill, NC
LINDA B. MILLER, Volunteer Trustees Foundation, Washington, DC
JONATHAN D. MORENO, University of Pennsylvania, Philadelphia, PA
PILAR N. OSSORIO, University of Wisconsin, Madison, WI
E. ALBERT REECE, University of Maryland, Baltimore, MD
JOSHUA R. SANES, Harvard University, Cambridge, MA
HAROLD T. SHAPIRO, Princeton University, Princeton, NJ
JOHN E. WAGNER, Jr., University of Minnesota, Minneapolis, MN

Staff

ADAM P. FAGEN, Senior Program Officer, Board on Life Sciences
BRUCE M. ALTEVOGT, Senior Program Officer, Board on Health Sciences Policy
FRANCES E. SHARPLES, Director, Board on Life Sciences
ANDREW M. POPE, Director, Board on Health Sciences Policy
AMANDA P. CLINE, Senior Program Assistant, Board on Life Sciences

[1] Professor Charo was appointed as a senior policy advisor in the Office of the Commissioner of the U.S. Food and Drug Administration (FDA) on August 31, 2009. None of her assigned tasks at FDA are related to the topics discussed in this report.

BOARD ON LIFE SCIENCES

Staff

FRANCES E. SHARPLES, Director
JO L. HUSBANDS, Scholar/Senior Project Director
KATHERINE BOWMAN, Senior Program Officer
ADAM P. FAGEN, Senior Program Officer
MARILEE K. SHELTON-DAVENPORT, Senior Program Officer
INDIA HOOK-BARNARD, Program Officer
ANNA FARRAR, Financial Associate
CARL-GUSTAV ANDERSON, Senior Program Assistant
AMANDA P. CLINE, Senior Program Assistant
AMANDA MAZZAWI, Program Assistant

BOARD ON HEALTH SCIENCES POLICY

Staff

ANDREW M. POPE, Director
AMY K.H. PACKMAN, Administrative Assistant
DONNA RANDALL, Financial Officer

Acknowledgments

The Committee acknowledges the input received from members of the stem cell research and oversight communities and the speakers and participants in its meetings.

This report has been reviewed in draft form by individuals chosen for their diverse perspectives and technical expertise, in accordance with procedures approved by the National Research Council's Report Review Committee. The purpose of this independent review is to provide candid and critical comments that will assist the institution in making its published report as sound as possible and to ensure that the report meets institutional standards for objectivity, evidence, and responsiveness to the study charge. The review comments and draft manuscript remain confidential to protect the integrity of the deliberative process. We wish to thank the following individuals for their review of this report:

George Q. Daley, Howard Hughes Medical Institute, Children's Hospital Boston, and Harvard Medical School
Fred H. Gage, Salk Institute for Biological Studies
Lawrence S.B. Goldstein, Howard Hughes Medical Institute and University of California, San Diego
Bernard Lo, University of California, San Francisco
Geoffrey Lomax, California Institute for Regenerative Medicine
Gail R. Martin, University of California, San Francisco
P. Pearl O'Rourke, Partners HealthCare System, Inc., and Harvard Medical School
Steven Peckman, University of California, Los Angeles

Janet D. Rowley, University of Chicago
Huda Y. Zoghbi, Howard Hughes Medical Institute and Baylor College of Medicine

Although the reviewers listed above have provided many constructive comments and suggestions, they were not asked to endorse the conclusions or recommendations, nor did they see the final draft of the report before its release. The review of this report was overseen by **Floyd E. Bloom**, The Scripps Research Institute. Appointed by the National Research Council, he was responsible for making certain that an independent examination of this report was carried out in accordance with institutional procedures and that all review comments were carefully considered. Responsibility for the final content of this report rests entirely with the authoring committee and the institution.

Contents

Introduction 1

The 2010 National Academies' Guidelines 4

The Future Role of the National Academies in Stem Cell Research Oversight 5

2010 Amendments to the National Academies' Guidelines for Human Embryonic Stem Cell Research 7

APPENDIXES

A National Institutes of Health Guidelines for Research Using Human Stem Cells 9

B Invited Participations at the August 7, 2009, Meeting of the Human Embryonic Stem Cell Research Advisory Committee 17

C National Academies' Guidelines for Human Embryonic Stem Cell Research, Amended as of May 2010 19

D Committee Member and Staff Biographies 37

FINAL REPORT OF THE NATIONAL ACADEMIES' HUMAN EMBRYONIC STEM CELL RESEARCH ADVISORY COMMITTEE AND 2010 AMENDMENTS TO THE NATIONAL ACADEMIES' GUIDELINES FOR HUMAN EMBRYONIC STEM CELL RESEARCH

INTRODUCTION

The 2005 National Academies' *Guidelines for Human Embryonic Stem Cell Research* laid out standards for responsible and ethical conduct in a controversial field of research that largely lacked federal funding or oversight. Those guidelines helped this important field of research to develop within a framework of defensible, self-imposed rules. The result was greater public confidence in the quality of the work. As certain states (California, Connecticut, Massachusetts, New York, Maryland, and others) have moved to regulate or fund this research, they have used the National Academies' Guidelines as a template on which to build their own state regulations. The international voluntary standards written by the International Society for Stem Cell Research (ISSCR) also tracked closely the National Academies' Guidelines.

Since their release, the National Academies' Guidelines have been adopted wholly or in large part by most major research institutions in the United States. This response included the creation of new Embryonic Stem Cell Research Oversight (ESCRO) committees, use of detailed guidance on informing gamete and embryo donors, and substantive limitations on the range of materials that would be used and how those experiments would be conducted. To assist the research community, the National Academies' Human Embryonic Stem Cell Research Advisory Committee has conducted regional and other outreach meetings to help investigators and ESCRO committee members to interpret and implement the Guidelines. The Advi-

sory Committee also updated the Guidelines in 2007 and 2008 to reflect the lessons learned by scientists and administrators around the country and to reflect changes in the science of stem cell research. Finally, the Advisory Committee organized or participated in several public workshops on key areas of concern, such as the medical risks of oocyte donation and the next steps toward translating bench science to clinical trials.

The inauguration of President Barack Obama in January 2009 led to a marked shift in federal policies on stem cell research. On March 9, President Obama issued Executive Order (EO) 13505, "Removing Barriers to Responsible Scientific Research Involving Human Stem Cells." (Federal Register Volume 74, Number 46, pp. 10667-10668). President Obama's EO stated that the "Secretary of Health and Human Services, through the Director of NIH [National Institutes of Health], may support and conduct responsible, scientifically worthy human stem cell research, including human embryonic stem cell research, to the extent permitted by law." While leaving untouched the "Dickey-Wicker" amendment,[1] which can only be changed by Congress and which effectively prohibits the use of federal funds to derive new human embryonic stem (hES) cell lines, the EO did rescind prior Executive branch policy. Specifically, the EO rescinded the previous policy that had restricted federal funding for hES cell research to *in vitro* work on lines derived before an earlier EO issued by President George W. Bush, by stating "The Presidential statement of August 9, 2001, limiting Federal funding for research involving human embryonic stem cells, shall have no further effect as a statement of governmental policy."

The EO issued by President Obama also called upon NIH to review its own existing guidance as well as other widely recognized guidelines on human stem cell research, including provisions establishing appropriate safeguards, and to develop and issue new NIH guidance for such research that is consistent with the EO's call to support "responsible, scientifically worthy" stem cell research. Without the restrictions placed upon it by the previous administration, the NIH announced that it would begin a broader

[1] The so-called "Dickey Wicker" amendment has been included in the annual federal appropriation for government-funded activities and has been interpreted to prevent the creation of new human embryonic stem cell lines using federal funds. For example, Section 509 of the Omnibus Appropriations Act 2009, enacted as Public Law 111-8) says:

> None of the funds made available in this Act may be used for—(1) the creation of a human embryo or embryos for research purposes; or (2) research in which a human embryo or embryos are destroyed, discarded, or knowingly subjected to risk of injury or death greater than that allowed for research on fetuses in utero under 45 CFE 46.204(b) and section 498(b) of the Public Health Service Act (42 U.S.C. 289g(b)).

program of funding extramural hES cell research according to its own new guidelines on eligibility for funding.

The NIH Guidelines on Human Stem Cell Research were issued on July 7, 2009 (Appendix A). They establish mechanisms to determine the eligibility of hES cell lines for federal research funding based on the principles that (1) responsible research with hES cells has the potential to improve our understanding of human health and illness and discover new ways to prevent and/or treat illness; and (2) individuals donating embryos for research purposes should do so freely, with voluntary and informed consent. Many of the provisions defining informed consent in the NIH guidelines closely resemble those of the National Academies, ISSCR, and others that predate the new NIH requirements. Thus, the NIH guidelines address both the evaluation of lines already in existence, derived under a variety of rules and guidelines, as well as lines yet to be derived. NIH has established a Working Group of the Advisory Committee to the Director of NIH to determine which hES cell lines were derived under conditions that meet the requirements of the NIH guidelines.[2]

It should be noted that the NIH guidelines prohibit the use of federal funding for research using hES cell lines derived from any source other than excess *in vitro* fertilization (IVF) embryos created for reproductive purposes. Thus research on lines that may, in the future, be derived by somatic cell nuclear transfer (SCNT), parthenogenesis, or from IVF embryos created specifically for research purposes is not currently eligible for federal funding. As a consequence, they would not be subject to the NIH guidelines, including its standards for ensuring voluntary, informed consent for donated materials.

The NIH has also established a new Registry of hES cell lines eligible for NIH funding, containing those lines that its Working Group deems to conform with the requirements of the guidelines.[3] The NIH approved the first list of hES cell lines for NIH funding on December 2, 2009, a second set on December 14, 2009, and additional lines in the first half of 2010 and indicated that it anticipated a continuing flow of approved hES cell lines to be listed on the NIH Registry. Use of those lines with federal funding will henceforth be governed by the NIH guidelines.

This letter report sets out an updated version of the National Academies' Guidelines, one that takes into account the new, expanded role of the NIH in overseeing hES cell research. It also identifies those avenues of continuing National Academies' involvement deemed most valuable by the research community and other significant stakeholders.

---

[2] See <http://www.nih.gov/news/health/sep2009/od-21.htm> for information about the Working Group.

[3] The Registry is available at <http://grants.nih.gov/stem_cells/registry/current.htm>.

THE 2010 NATIONAL ACADEMIES' GUIDELINES

Overall, there are three areas in which non-NIH guidelines will continue to be the source of guidance for hES cell research.

- First, because the continuing effect of the Dickey-Wicker amendment means that derivation of hES cell lines cannot be supported by federal funds, such derivations will need continuing oversight outside the NIH guidelines. And since the acceptability of the cell lines for use in NIH-funded research hinges on the underlying conditions of non-federally funded derivation, the NIH guidelines implicitly overlap many of the National Academies' Guidelines on derivation.

- Second, only hES cell lines derived from excess IVF embryos initially produced for reproductive purposes are currently eligible for NIH funding. Therefore, hES cell lines derived from other sources (e.g., from embryos produced by IVF for research purposes or by nuclear transfer or other methods) will not be eligible for NIH funding and not subject to the NIH guidelines; this work will continue to need oversight under other guidelines.

- Third, because the NIH guidelines only briefly address limits on the research uses to which embryonic stem cell lines may be put, other guidelines will continue to be useful for a wider range of experiments with chimeras than those currently identified by NIH.

To avoid complications, contradictions, and confusion, this Advisory Committee has developed an updated version of the National Academies' Guidelines that recognizes the new and increased influence of the NIH guidelines, and which incorporates references to the NIH guidelines as appropriate in the text of the National Academies' Guidelines. Where there is complete overlap, the Advisory Committee recommends that the NIH guidelines supersede its own. Where there are gaps or limitations in the NIH guidelines, the Advisory Committee recommends continued adoption of its own Guidelines.

The Advisory Committee also notes some areas in which there is tension between NIH, National Academies, and other guidelines or state funding rules, and identifies those for which some variation from National Academies' Guidelines is to be expected.

The first concerns the issue of egg donation. Since the issuance of the 2008 Amendments to the National Academies' Guidelines, the Ethics Com-

mittee of the State of New York's Empire State Stem Cell Board adopted a resolution allowing New York State-funded stem cell researchers to compensate women who donate their oocytes directly and solely to research for the time, risk and burden involved in donating.[4] Amounts of compensation are to be comparable to those received by women in New York State for similar donations for reproductive purposes. Compensation may not be based upon number or quality of eggs, but should cover only time and burden. While this Advisory Committee acknowledges that the circumstances surrounding the issue of compensation to oocyte donors continues to evolve, it chose not to change the National Academies' Guidelines. Therefore, the Advisory Committee leaves intact the wording of Section 3.4(b), recognizing that states and other entities may choose to set their own policies, as New York has done.

Second, the Advisory Committee notes that the requirement in the National Academies' Guidelines for consent of *all* gamete donors (see Section 3.3) is not reflected in the new NIH guidelines. Further, a number of states and research institutions have declined to adopt this rule, given the lack of clear legal need for such consent from anonymous donors. The Advisory Committee also notes that the Food and Drug Administration's (FDA's) recent tissue transplant rules require screening of gamete donors except in cases involving sexually intimate partners. This suggests that stem cell lines made with donor (i.e., screened) gametes may be marginally safer for tissue transplants and may be more useable for FDA-regulated trials and therapies. The Advisory Committee recognizes that this requirement may be widely overlooked, and that the issue will be relevant only for a small percentage of derivations. Nonetheless, the Advisory Committee still believes that the practice of obtaining informed consent from all gamete donors, as well as other relevant parties (e.g., intended parents), should continue to be followed because it is the most cautious and respectful standard for donation.

The combination of the new NIH guidelines and those National Academies' Guidelines remaining in effect will continue to represent a comprehensive and responsible approach as this research advances into the future.

THE FUTURE ROLE OF THE NATIONAL ACADEMIES IN STEM CELL RESEARCH OVERSIGHT

In addition to reviewing the National Academies' Guidelines, the Advisory Committee also considered the future role of the National Academies

[4] The resolution is available at <http://stemcell.ny.gov/docs/Compensation_of_Gamete_Donors_resolution_of_Funding_Comm.pdf>

in helping to guide responsible conduct in this field. The Advisory Committee dedicated most of its August 7, 2009, meeting to hear input from stakeholders from the stem cell research community and from those who have experience with the implementation of the National Academies' Guidelines; a list of these individuals participating in the meeting may be found in Appendix B.

One area of considerable discussion was the future of ESCRO committees, as most institutions that have been following the National Academies' or other non-federal guidelines since 2005 have established such committees. Most participants in the August 7 meeting thought that ESCRO/SCRO committees[5] play valuable roles and function in such a way that their elimination could leave gaps not filled by other oversight bodies (e.g., Institutional Review Boards, Institutional Animal Care and Use Committees, Institutional Biosafety Committees). It was stated that ESCRO committees could continue to be useful in maintaining deeper expertise on stem cell research than is necessarily provided by these other oversight bodies. ESCRO committees could also be helpful in assisting research institutions in monitoring developments in the field of stem cell research. In light of these comments, the Advisory Committee agrees that the continued use of ESCRO committees is useful, especially in circumstances where new hES cells are being derived. Even for research with existing cell lines funded by NIH—and therefore subject to NIH guidelines and the NIH hES cell registry—ESCRO committees could also help institutions by providing needed expertise and training for the members of their other committees.

The stakeholders at the August 2009 meeting also discussed whether the National Academies should continue to play a role by maintaining an activity, such as a roundtable, that would allow periodic meetings to discuss knowledge and policy gaps, new problems, and contentious issues. It was suggested that, in the future, the *uses* of stem cells, as opposed to derivation of new lines, are likely to provide a larger share of any controversy or concern surrounding stem cell research. Stakeholders at the meeting suggested that the National Academies are viewed as providing a neutral setting for discussions that can help guide research institutions to make appropriate decisions about research, particularly in areas that are outside the bounds of NIH funding. Several guests stated that research using chimeras represents one such area of potential concern, but that other issues (e.g., stem cell-derived gametes) are also likely to emerge that may provoke controversy. Other topics identified as being potentially important in the future for stem

[5] Other guidelines called for the establishment of Stem Cell Research Oversight (SCRO) committees whose mandate was not limited to *embryonic* stem cell research.

cell research guidance included the relative merits of hES cells vs. induced pluripotent stem cells and clinical trials and translational research.

Some of these topics may have little to do with the Guidelines themselves, but might make excellent topics for future workshops or studies. In light of these discussions, the Advisory Committee decided that:

- The Human Embryonic Stem Cell Research Advisory Committee should prepare this brief final report communicating to the stem cell research community those elements of the National Academies' Guidelines that should remain in effect and under what conditions.

- Following the completion of this task, the Advisory Committee should disband.

The Advisory Committee also discussed the feedback from stakeholders on future mechanisms for discussion of stem cell issues. Although government agencies such as the NIH, professional societies such as the ISSCR, consortia such as the Interstate Alliance on Stem Cell Research,[6] and meetings organized by many different organizations and institutions provide opportunities for discussion, there does not seem to be an ongoing neutral forum for productive discussion of stem cell issues. Participants at the committee's August 2009 meeting mentioned that the National Academies and the Advisory Committee had served this important convening function over the last several years, and there was a need for a similar continuing activity. Perhaps most needed is a forum that could bring together key stakeholders—including federal, state, academic, patient, and industry organizations and institutions— for periodic meetings that would address topics of shared interest and concern to the broader stem cell research, regenerative medicine, and policy communities.

2010 AMENDMENTS TO THE NATIONAL ACADEMIES' GUIDELINES FOR HUMAN EMBRYONIC STEM CELL RESEARCH

Finally, the Advisory Committee presents here an amended version of the National Academies' Guidelines (Appendix C) delineating those sections of the Guidelines that are superseded by the NIH rules for federally funded research.

[6] The Interstate Alliance (IASCR) is a voluntary body of states and affiliate countries and organizations interested in increasing opportunities for interstate collaboration on stem cell research. See <http://www.iascr.org/> for more information.

Appendix A

National Institutes of Health Guidelines for Research Using Human Stem Cells[1]

I. Scope of Guidelines

These Guidelines apply to the expenditure of National Institutes of Health (NIH) funds for research using human embryonic stem cells (hESCs) and certain uses of induced pluripotent stem cells (See Section IV). The Guidelines implement Executive Order 13505.

Long-standing HHS [Department of Health and Human Services] regulations for Protection of Human Subjects, 45 C.F.R. 46, Subpart A establish safeguards for individuals who are the sources of many human tissues used in research, including non-embryonic human adult stem cells and human induced pluripotent stem cells. When research involving human adult stem cells or induced pluripotent stem cells constitutes human subject research, Institutional Review Board review may be required and informed consent may need to be obtained per the requirements detailed in 45 C.F.R. 46, Subpart A. Applicants should consult *http://www.hhs.gov/ohrp/humansubjects/guidance/45cfr46.htm*.

It is also important to note that the HHS regulation, Protection of Human Subjects, 45 C.F.R. Part 46, Subpart A, may apply to certain research using hESCs. This regulation applies, among other things, to research involving individually identifiable private information about a living

[1] Available at <http://stemcells.nih.gov/policy/2009guidelines.htm>.

individual, 45 C.F.R. § 46.102(f). The HHS Office for Human Research Protections (OHRP) considers biological material, such as cells derived from human embryos, to be individually identifiable when they can be linked to specific living individuals by the investigators either directly or indirectly through coding systems. Thus, in certain circumstances, IRB review may be required, in addition to compliance with these Guidelines. Applicant institutions are urged to consult OHRP guidances at *http://www.hhs.gov/ohrp/policy/index.html#topics*.

To ensure that the greatest number of responsibly derived hESCs are eligible for research using NIH funding, these Guidelines are divided into several sections, which apply specifically to embryos donated in the U.S. and foreign countries, both before and on or after the effective date of these Guidelines. Section II (A) and (B) describe the conditions and review processes for determining hESC eligibility for NIH funds. Further information on these review processes may be found at *www.NIH.gov*. Sections IV and V describe research that is not eligible for NIH funding.

These guidelines are based on the following principles:

1. Responsible research with hESCs has the potential to improve our understanding of human health and illness and discover new ways to prevent and/or treat illness.
2. Individuals donating embryos for research purposes should do so freely, with voluntary and informed consent.

As directed by Executive Order 13505, the NIH shall review and update these Guidelines periodically, as appropriate.

II. Eligibility of Human Embryonic Stem Cells for Research with NIH Funding

For the purpose of these Guidelines, "human embryonic stem cells (hESCs)" are cells that are derived from the inner cell mass of blastocyst stage human embryos, are capable of dividing without differentiating for a prolonged period in culture, and are known to develop into cells and

tissues of the three primary germ layers.[2] Although hESCs are derived from embryos, such stem cells are not themselves human embryos. All of the processes and procedures for review of the eligibility of hESCs will be centralized at the NIH as follows:

A. Applicant institutions proposing research using hESCs derived from embryos donated in the U.S. on or after the effective date of these Guidelines may use hESCs that are posted on the new NIH Registry or they may establish eligibility for NIH funding by submitting an assurance of compliance with Section II (A) of the Guidelines, along with supporting information demonstrating compliance for administrative review by the NIH. For the purposes of this Section II (A), hESCs should have been derived from human embryos:
    1. that were created using in vitro fertilization for reproductive purposes and were no longer needed for this purpose;
    2. that were donated by individuals who sought reproductive treatment (hereafter referred to as "donor(s)") and who gave voluntary written consent for the human embryos to be used for research purposes; and
    3. for which all of the following can be assured and documentation provided, such as consent forms, written policies, or other documentation, provided:
        a. All options available in the health care facility where treatment was sought pertaining to the embryos no longer needed for reproductive purposes were explained to the individual(s) who sought reproductive treatment.
        b. No payments, cash or in kind, were offered for the donated embryos.
        c. Policies and/or procedures were in place at the health care facility where the embryos were donated that neither consenting nor refusing to donate embryos for research would affect the quality of care provided to potential donor(s).

[2] On February 23, 2010, NIH issued a request for public comment in the Federal Register on changing this definition to the following:

> For the Purpose of the Guidelines, 'human embryonic stem cells (hESCs)' are pluripotent cells that are derived from early stage human embryos, up to and including the blastocyst stage, are capable of dividing without differentiating for a prolonged period in culture, and are known to develop into cells and tissues of the three primary germ layers.

As of the publication of this report, no revisions have been formally issued. Readers are encouraged to consult <http://stemcells.nih.gov/> for the NIH current guidelines.

d. There was a clear separation between the prospective donor(s)'s decision to create human embryos for reproductive purposes and the prospective donor(s)'s decision to donate human embryos for research purposes. Specifically:
   i. Decisions related to the creation of human embryos for reproductive purposes should have been made free from the influence of researchers proposing to derive or utilize hESCs in research. The attending physician responsible for reproductive clinical care and the researcher deriving and/or proposing to utilize hESCs should not have been the same person unless separation was not practicable.
   ii. At the time of donation, consent for that donation should have been obtained from the individual(s) who had sought reproductive treatment. That is, even if potential donor(s) had given prior indication of their intent to donate to research any embryos that remained after reproductive treatment, consent for the donation for research purposes should have been given at the time of the donation.
   iii. Donor(s) should have been informed that they retained the right to withdraw consent for the donation of the embryo until the embryos were actually used to derive embryonic stem cells or until information which could link the identity of the donor(s) with the embryo was no longer retained, if applicable.

e. During the consent process, the donor(s) were informed of the following:
   i. that the embryos would be used to derive hESCs for research;
   ii. what would happen to the embryos in the derivation of hESCs for research;
   iii. that hESCs derived from the embryos might be kept for many years;
   iv. that the donation was made without any restriction or direction regarding the individual(s) who may receive medical benefit from the use of the hESCs, such as who may be the recipients of cell transplants.;
   v. that the research was not intended to provide direct medical benefit to the donor(s);

vi. that the results of research using the hESCs may have commercial potential, and that the donor(s) would not receive financial or any other benefits from any such commercial development;
vii. whether information that could identify the donor(s) would be available to researchers.

B. Applicant institutions proposing research using hESCs derived from embryos donated in the U.S. before the effective date of these Guidelines may use hESCs that are posted on the new NIH Registry or they may establish eligibility for NIH funding in one of two ways:
   1. By complying with Section II (A) of the Guidelines; or
   2. By submitting materials to a Working Group of the Advisory Committee to the Director (ACD), which will make recommendations regarding eligibility for NIH funding to its parent group, the ACD. The ACD will make recommendations to the NIH Director, who will make final decisions about eligibility for NIH funding.

      The materials submitted must demonstrate that the hESCs were derived from human embryos: 1) that were created using in vitro fertilization for reproductive purposes and were no longer needed for this purpose; and 2) that were donated by donor(s) who gave voluntary written consent for the human embryos to be used for research purposes.

      The Working Group will review submitted materials, e.g., consent forms, written policies or other documentation, taking into account the principles articulated in Section II (A), 45 C.F.R. Part 46, Subpart A, and the following additional points to consider. That is, during the informed consent process, including written or oral communications, whether the donor(s) were: (1) informed of other available options pertaining to the use of the embryos; (2) offered any inducements for the donation of the embryos; and (3) informed about what would happen to the embryos after the donation for research.

C. For embryos donated outside the United States before the effective date of these Guidelines, applicants may comply with either Section II (A) or (B). For embryos donated outside of the United States on or after the effective date of the Guidelines, applicants seeking to

determine eligibility for NIH research funding may submit an assurance that the hESCs fully comply with Section II (A) or submit an assurance along with supporting information, that the alternative procedural standards of the foreign country where the embryo was donated provide protections at least equivalent to those provided by Section II (A) of these Guidelines. These materials will be reviewed by the NIH ACD Working Group, which will recommend to the ACD whether such equivalence exists. Final decisions will be made by the NIH Director.

D. NIH will establish a new Registry listing hESCs eligible for use in NIH funded research. All hESCs that have been reviewed and deemed eligible by the NIH in accordance with these Guidelines will be posted on the new NIH Registry.

III. Use of NIH Funds

Prior to the use of NIH funds, funding recipients should provide assurances, when endorsing applications and progress reports submitted to NIH for projects using hESCs, that the hESCs are listed on the NIH registry.

IV. Research Using hESCs and/or Human Induced Pluripotent Stem Cells That, Although the Cells May Come from Eligible Sources, Is Nevertheless Ineligible for NIH Funding

This section governs research using hESCs and human induced pluripotent stem cells, i.e., human cells that are capable of dividing without differentiating for a prolonged period in culture, and are known to develop into cells and tissues of the three primary germ layers. Although the cells may come from eligible sources, the following uses of these cells are nevertheless ineligible for NIH funding, as follows:

A. Research in which hESCs (even if derived from embryos donated in accordance with these Guidelines) or human induced pluripotent stem cells are introduced into non-human primate blastocysts.
B. Research involving the breeding of animals where the introduction of hESCs (even if derived from embryos donated in accordance with these Guidelines) or human induced pluripotent stem cells may contribute to the germ line.

V. Other Research Not Eligible for NIH Funding

A. NIH funding of the derivation of stem cells from human embryos is prohibited by the annual appropriations ban on funding of human embryo research (Section 509, Omnibus Appropriations Act, 2009, Pub. L. 111-8, 3/11/09), otherwise known as the Dickey Amendment.

B. Research using hESCs derived from other sources, including somatic cell nuclear transfer, parthenogenesis, and/or IVF embryos created for research purposes, is not eligible for NIH funding.

Appendix B

Invited Participants at the August 7, 2009, Meeting of the Human Embryonic Stem Cell Research Advisory Committee

George Q. Daley, Samuel E. Lux IV Chair in Hematology and Director, Stem Cell Transplantation Program, Children's Hospital Boston; Associate Professor of Biological Chemistry and Molecular Pharmacology, Harvard Medical School; Investigator, Howard Hughes Medical Institute; and Past President, International Society for Stem Cell Research

Deborah A. Hursh, Senior Investigator, Division of Cellular and Gene Therapies, Center for Biologics Research and Review, U.S. Food and Drug Administration

Julie Kaneshiro, Team Leader, Policy, Office for Human Research Protections, Department of Health and Human Services

Story Landis, Director, National Institute of Neurological Disorders and Stroke, National Institutes of Health (NIH), and Chair, NIH Stem Cell Task Force

Bernard Lo, Professor of Medicine and Director of the Program in Medical Ethics, University of California, San Francisco

Geoff Lomax, Senior Officer to the Standards Working Group, California Institute for Regenerative Medicine

John McNeish, Executive Director, Pfizer Regenerative Medicine

P. Pearl O'Rourke, Director of Human Research Affairs, Partners Health-Care System, Boston; and Associate Professor of Pediatrics, Harvard Medical School

Sean Tipton, Past-President, Coalition for the Advancement of Medical Research; and Director of Public Affairs, American Society for Reproductive Medicine

Appendix C

National Academies' Guidelines for Human Embryonic Stem Cell Research Amended as of May 2010[7]

1.0 Introduction
2.0 Establishment of an Institutional Embryonic Stem Cell Research Oversight Committee
3.0 Procurement of Gametes, Morulae, Blastocysts or Cells for Generation of hES ~~Generation~~ Cell Lines
4.0 Derivation of hES Cell Lines
5.0 Banking and Distribution of hES Cell Lines
6.0 Research Use of hES Cell Lines
7.0 International Collaboration
8.0 Conclusion

1.0 INTRODUCTION

~~In this chapter we collect all the recommendations made throughout the report and translate them into a series of formal guidelines.~~ These guidelines focus on the derivation, procurement, banking, and use of human embryonic stem (hES) cell lines and some uses of human pluripotent (hPS) cell lines. They provide an oversight process that will help to ensure that research with hES cells is conducted in a responsible and ethically sensitive manner and in compliance with all regulatory requirements pertaining to biomedical research in general. The National Academies ~~are issuing~~ issues these guidelines

[7] New or modified wording is indicated by underlining. Deleted wording is indicated by ~~strikethrough~~.

for the use of the scientific community, including researchers in university, industry, or other private-sector research organizations who are conducting such research with non-federal funding. Researchers conducting federally-funded hES cell research should, however, note that the requirements of the National Institutes of Health (NIH)—available at http://stemcells.nih.gov/policy/2009guidelines.htm—supersede these National Academies' Guidelines for certain sections (as noted below).

1.1 What These Guidelines Cover

1.1(a) These guidelines cover all derivation of hES cell lines and all research that uses hES cells derived from

(i) blastocysts and/or morulae made for reproductive purposes and later obtained for research from *in vitro* fertilization (IVF) clinics,
(ii) blastocysts and/or morulae made specifically for research using IVF,
(iii) somatic cell nuclear transfer (NT) into oocytes or by parthenogensis or androgenesis.

1.1(b) Some of the concerns addressed in this report are common to other types of human stem cell research; as such, certain of these Guidelines should also apply to those other types of research. For example,

(i) research that uses human adult stem cells,
(ii) research that uses fetal stem cells or embryonic germ cells derived from fetal tissue; such research is covered by federal statutory restrictions at 42 U.S.C. 289g-2(a) and federal regulations at 45 CFR 46.210,
(iii) research using hPS cells derived from non-embryonic sources, such as spermatogonial stem cells and "induced pluripotent" stem cells derived from somatic cells by introduction of genes or otherwise (so-called iPS cells), as well as other pluripotent cells yet to be developed; guidelines for hPS cells are collected in Section 7 below.

~~Recommendations as to which guidelines apply to other hPS cells are collected in a new Section 7 below. Institutions and investigators conducting research with adult and fetal stem cells should also consider which individual provisions of these guidelines are relevant to their research.~~

1.1(c) Research supported by NIH funds using NIH-approved hES cell lines is governed by NIH guidelines.

1.1(d) The guidelines do not cover research that uses nonhuman stem cells.

1.2 Reproductive Uses of NT

These guidelines also do not apply to reproductive uses of nuclear transfer, which are addressed in the 2002 report *Scientific and Medical Aspects of Human Reproductive Cloning*, in which the National Academies recommended that "Human reproductive cloning should not now be practiced. It is dangerous and likely to fail." Although these guidelines do not specifically address human reproductive cloning, it continues to be the view of the National Academies that research aimed at the reproductive cloning of a human being should not be conducted at this time.

1.3 Categories of hES Cell Research

These guidelines specify categories of research that:

- Are permissible after currently mandated reviews and proper notification of the relevant research institution.
- Are permissible after additional review by an Embryonic Stem Cell Research Oversight (ESCRO) committee, as described in Section 2.0 of the guidelines.
- Should not be conducted at this time.

Because of the sensitive nature of some aspects of hES cell research, these guidelines in many instances set a higher standard than is required by laws or regulations with which institutions and individuals already must comply.

1.3(a) hES Cell Research Permissible after Currently Mandated Reviews

Purely *in vitro* hES cell research that uses previously derived hES cell lines is permissible provided that the ESCRO committee or equivalent body designated by the investigator's institution (see Section 2.0) receives documentation of the provenance of the cell lines including (i) documentation of the use of an acceptable informed consent process that was approved by an Institutional Review Board (IRB) or foreign equivalent for their derivation (consistent with Section 3.6) and (ii) documentation of compliance with ~~any~~

~~additional required review by~~ an Institutional Animal Care and Use Committee (IACUC), Institutional Biosafety Committee (IBC), or other institutionally mandated review, if necessary. To determine whether the proposed research meets the requirements of this section, the ESCRO committee may choose to conduct an "expedited review" of such research proposals. In this context, *expedited review* means that the ESCRO committee chair or others designated by the committee chair act on behalf of the committee to determine that the hES cells have been acceptably derived (see Section 1.5) and report to the entire committee. All hES cell lines listed on the NIH Registry of approved lines are acceptable for use in research, subject to any restrictions imposed by NIH. Certain other lines may be considered acceptable for research using non-federal funds (see 1.5 below).

1.3(b) hES Cell Research Permissible Only After Additional Review and Approval

(i) Generation of new lines of hES cells by whatever means.

(ii) Research involving the introduction of hES cells into ~~non-human~~ animals other than humans or primates[8] at any stage of embryonic, fetal, or postnatal development. Particular attention should be paid to at least three factors: the extent to which the implanted cells colonize and integrate into the animal tissue; the degree of differentiation of the implanted cells; and the possible effects of the implanted cells on the function of the animal tissue.

(iii) Research involving the introduction of hES cell into nonhuman primates at any stage of fetal or postnatal development. Particular attention should be paid to at least three factors: the extent to which the implanted cells colonize and integrate into the animal tissue; the degree of differentiation of the implanted cells; and the possible effects of the implanted cells on the function of the animal tissue.

(iv) Research in which the identity of the donors of blastocysts, morulae, gametes, or somatic cells from which the hES cells were derived is readily ascertainable or might become known to the investigator.

[8] "Nonhuman animals" has been changed to "animals other than human or primates" as the Guidelines do not permit the introduction of hES cells into humans or nonhuman primates (Section 1.3(c)(ii)).

1.3(c) hES Cell Research That Should Not Be Permitted At This Time

The following types of research should not be conducted at this time:

(i) Research involving *in vitro* culture of any intact human embryo, regardless of derivation method, for longer than 14 days or until formation of the primitive streak begins, whichever occurs first.

(ii) Research in which hES cells are introduced into non-human primate blastocysts or in which any embryonic stem cells are introduced into human blastocysts.

In addition:

(iii) No animal into which hES cells have been introduced such that they could contribute to the germ line should be allowed to breed.

1.4 Obligations of Investigators and Institutions

All scientific investigators and their institutions, regardless of their field, bear the ultimate responsibility for ensuring that they conduct themselves in accordance with professional standards and with integrity. In particular, people whose research involves hES cells should work closely with oversight bodies, demonstrate respect for the autonomy and privacy of those who donate gametes, morulae, blastocysts, or somatic cells and be sensitive to public concerns about research that involves human embryos.

~~**1.5 Use of NIH-approved hES cell lines**~~

~~1.5(a) It is acceptable to use hES cell lines that were approved in August 2001 for use in U.S. federally funded research.~~

~~1.5(b) ESCRO committees should include on their registry a list of NIH-approved cell lines that have been used at their institution in accord with the requirement in section 2.0 of the Guidelines.~~

~~1.5(c) Presence on the list of NIH-approved cell lines constitutes adequate documentation of provenance, as per Section 6.1 of the Guidelines.~~

1.5 Acceptability of research using hES cell lines imported from other institutions or jurisdictions

1.5(a) Before approving use of hES ~~and hPS~~ cell lines imported from other institutions or jurisdictions, ESCRO committees should consider whether such cell lines have been "acceptably derived."

1.5(b) "Acceptably derived" means that the cell lines were derived from gametes or embryos for which

(i) the donation protocol was reviewed and approved by an IRB or, in the case of donations taking place outside the United States, a substantially equivalent oversight body;
(ii) consent to donate was voluntary and informed;
(iii) donation was made with reimbursement policies consistent with these Guidelines; and
(iv) donation and derivation complied with the extant legal requirements of the relevant jurisdiction.

1.5(c) ESCRO committees should include on their registry a list of cell lines that have been imported from other institutions or jurisdictions and information on the specific guidelines, regulations, or statutes under which the derivation of the imported cell lines was conducted. This is in accord with the requirement in section 2.0 of the Guidelines that calls for ESCRO committees to maintain registries listing the cell lines in use at their institutions.

2.0 ESTABLISHMENT OF AN INSTITUTIONAL EMBRYONIC STEM CELL RESEARCH OVERSIGHT COMMITTEE

To provide oversight of all issues related to derivation and use of hES cell lines and to facilitate education of investigators involved in hES cell research, ~~each~~ many institutions currently require that research ~~should have activities~~ involving hES cells should be overseen by an Embryonic Stem Cell Research Oversight (ESCRO) committee. Although not required under the NIH Guidelines on Human Stem Cell Research, institutions conducting federally funded stem cell research are nevertheless likely to decide to maintain their ESCRO committees and use them for consultation, training, and any other functions appropriate to assist the institution and its researchers in evaluating and managing hES cell research. Institutions that conduct both federally funded and non-federally funded hES cell research, particularly if

this research involves the derivation of new cell lines, should maintain and use their ESCRO committees as they did prior to July 7, 2009. An ESCRO committee could be internal to a single institution or established jointly with one or more other institutions. Alternatively, an institution may have its proposals reviewed by an ESCRO committee of another institution, or by an independent ESCRO committee. An ESCRO committee should include independent representatives of the lay public as well as persons with expertise in developmental biology, stem cell research, molecular biology, assisted reproduction, and ethical and legal issues in hES cell research. It must have suitable scientific, medical, and ethical expertise to conduct its own review and should have the resources needed to coordinate the management of the various other reviews required for a particular protocol. A pre-existing committee could serve the functions of the ESCRO committee provided that it has the expertise recommended here and representation to perform the various roles described in this report. For example, an institution might elect to constitute an ESCRO committee from among some members of an IRB. But the ESCRO committee should not be a subcommittee of the IRB, as its responsibilities extend beyond human subject protections. Furthermore, much hES cell research does not require IRB review. The ESCRO committee ~~should~~ would:

(a) Provide oversight over all issues related to derivation ~~and use~~ of hES cell lines.
(b) Provide oversight over issues related to the use of hES cell lines not otherwise covered by NIH guidelines.
(~~b~~c) Review and approve the scientific merit of research protocols.
(~~c~~d) Review compliance of all in-house hES cell research with all relevant regulations and these guidelines.
(~~d~~e) Maintain registries of hES cell research conducted at the institution and hES cell lines derived or imported by institutional investigators. An institution conducting stem cell research should make information from the registries (including, but not necessarily limited to, project abstracts and source of funding) available to the public and the media through the institution's Web site.
(~~e~~f) Facilitate education of investigators involved in hES cell research.

An institution that maintains its own ESCRO committee should also conduct periodic audits of the committee to verify that it is carrying out its responsibilities appropriately. Auditable records include documentation of decisions regarding the acceptability of research proposals and verification that cell

lines in use at the institution were acceptably derived (see Section 1.5). Institutions should make the results of these audits available to the public.

An institution that uses an external ESCRO committee should nevertheless ensure that the registry and educational functions of an internal ESCRO committee are carried out by the external ESCRO committee on its behalf or internally by other administrative units. Institutions that use external ESCRO committees are also responsible for ensuring that these committees are likewise carrying out their responsibilities appropriately.

2.1 For projects that involve more than one institution, review of the scientific merit, justification, and compliance status of the research may be carried out by a single ESCRO committee if all participating institutions agree to accept the results of the review.

3.0 PROCUREMENT OF GAMETES, MORULAE, BLASTOCYSTS OR CELLS FOR GENERATION OF hES CELL LINES ~~GENERATION~~

3.1 An IRB, as described in federal regulations at 45 CFR 46.107, should review all new procurement of all gametes, morulae, blastocysts, or somatic cells for the purpose of generating new hES or hPS cell lines. This includes the procurement of blastocysts and/or morulae in excess of clinical need from infertility clinics, blastocysts made through IVF specifically for research purposes, and oocytes, sperm, and somatic cells donated for development of hES cell lines derived through NT or by parthenogenesis or androgenesis; and hPS cells derived by any means that require human subjects review.

3.2 Consent for donation should be obtained from each donor at the time of donation. Even people who have given prior indication of their intent to donate to research any blastocysts and/or morulae that remain after clinical care should nonetheless give informed consent at the time of donation. Donors should be informed that they retain the right to withdraw consent until the blastocysts and/or morulae are actually used in cell line derivation.

3.3 When donor gametes have been used in the IVF process, resulting blastocysts and/or morulae may not be used for research without consent of all gamete donors. Written agreement at the time of gamete donation that one potential use of the blastocysts and/or morulae is embryo research will constitute sufficient consent.

3.4 Payment and Reimbursement

3.4 (a) No payments, cash or in-kind, may be provided for donating blastocysts and/or morulae in excess of clinical need for research purposes. People who elect to donate stored blastocysts and/or morulae for research should not be reimbursed for the costs of storage prior to the decision to donate.

3.4(b) Women who undergo hormonal induction to generate oocytes specifically for research purposes (such as for NT) should be reimbursed only for direct expenses incurred as a result of the procedure, as determined by an IRB. Direct expenses may include costs associated with travel, housing, child care, medical care, health insurance, and actual lost wages. No payments beyond reimbursements, cash or in-kind, should be provided for donating oocytes for research purposes. Similarly, no payments beyond reimbursements should be made for donations of sperm for research purposes or of somatic cells for use in NT.

3.5 To facilitate autonomous choice, decisions related to the creation of embryos for infertility treatment should be free of the influence of investigators who propose to derive or use hES cells in research. Whenever it is practicable, the attending physician responsible for the infertility treatment and the investigator deriving or proposing to use hES cells should not be the same person.

3.6 In the context of donation of gametes, morulae, blastocysts, or somatic cells for hES cell research or for hPS cell research that requires human subjects review, the informed consent process, should, at a minimum, provide the following information.[9]

(a) A statement that the ~~blastocysts~~, gametes, morulae, blastocysts, or somatic cells will be used to derive hES or hPS cells for research that may include research on human transplantation.

(b) A statement that the donation is made without any restriction or direction regarding who may be the recipient of transplants of the cells derived, except in the case of autologous donation.

(c) A statement as to whether the identities of the donors will be readily ascertainable to those who derive or work with the resulting hES or hPS cell lines.

[9] To be eligible for use in federally-funded research, the NIH guidelines specify specific elements for informed consent that may differ from the elements listed below.

(d) If the identities of the donors are retained (even if coded), a statement as to whether donors wish to be contacted in the future to receive information obtained through studies of the cell lines.
(e) An assurance that participants in research projects will follow applicable and appropriate best practices for donation, procurement, culture, and storage of cells and tissues to ensure, in particular, the traceability of stem cells. (Traceable information, however, must be secured to ensure confidentiality.)
(f) A statement that derived hES or hPS cells and/or cell lines might be kept for many years.
(g) A statement that the hES or hPS cells and/or cell lines might be used in research involving genetic manipulation of the cells or the mixing of human and nonhuman cells in animal models.
(h) Disclosure of the possibility that the results of study of the hES or hPS cells may have commercial potential and a statement that the donor will not receive financial or any other benefits from any future commercial development.
(i) A statement that the research is not intended to provide direct medical benefit to the donor(s) except in the case of autologous donation.
(j) A statement that embryos will be destroyed in the process of deriving hES cells.
(k) A statement that neither consenting nor refusing to donate embryos for research will affect the quality of any future care provided to potential donors.
(l) A statement of the risks involved to the donor.

In addition, donors could be offered the option of agreeing to some forms of hES cell research but not others. For example, donors might agree to have their materials used for deriving new hES cell lines but might not want their materials used, for example, for NT. The consent process should fully explore whether donors have objections to any specific forms of research to ensure that their wishes are honored. Investigators and stem cell banks are, of course, free to choose which cell lines to accept, and are not obligated to accept cell lines for which maintaining information about specific research use prohibitions would be unduly burdensome.

New derivations of stem cell lines from banked tissues obtained prior to the adoption of these guidelines are permissible provided that the original donations were made in accordance with the legal requirements in force at the place and time of donation. This includes gametes, morulae, blastocysts, adult stem cells, somatic cells, or other tissue. In the event that these banked

tissues retain identifiers linked to living individuals, human subjects protections may apply.

3.7 Clinical personnel who have a conscientious objection to hES cell research should not be required to participate in providing donor information or securing donor consent for research use of gametes, morulae, or blastocysts. That privilege should not extend to the care of a donor or recipient.

3.8 Researchers may not ask members of the infertility treatment team to generate more oocytes than necessary for the optimal chance of reproductive success. An infertility clinic or other third party responsible for obtaining consent or collecting materials should not be able to pay for or be paid for the material obtained (except for specifically defined cost-based reimbursements and payments for professional services).

4.0 DERIVATION OF hES CELL LINES

4.1 Requests to the ESCRO committee for permission to attempt derivation of new hES cell lines from donated embryos, morulae, or blastocysts must include evidence of IRB approval of the procurement process (see Section 3.0 above).

4.2 The scientific rationale for the need to generate new hES cell lines, by whatever means, must be clearly presented, and the basis for the numbers of embryos, morulae, and blastocysts needed should be justified.

4.3 Research teams should demonstrate appropriate expertise or training in derivation or culture of either human or nonhuman ES cells before permission to derive new lines is given.

4.4 When NT experiments involving either human or nonhuman oocytes are proposed as a route to generation of hES cells, the protocol must have a strong scientific rationale. Proposals that include studies to find alternatives to donated oocytes in this research should be encouraged.

4.5 Neither blastocysts or morulae made using NT of human nuclei (whether produced with human or nonhuman oocytes) nor parthenogenetic or androgenetic human embryos may be transferred to a human or nonhuman uterus or cultured as intact embryos *in vitro* for longer than 14 days or until formation of the primitive streak, whichever occurs first.

4.6 Investigators must document how they will characterize, validate, store, and distribute any new hES cell lines and how they will maintain the confidentiality of any coded or identifiable information associated with the lines (see Section 5.0 below). Investigators are encouraged to apply the same procedures and standards for characterization, validation, storage, and distribution to hPS cell lines.

5.0 BANKING AND DISTRIBUTION OF hES CELL LINES

There are several models for the banking of human biological materials, including hES cells. The most relevant is the U.K. Stem Cell Bank. The guidelines developed by this and other groups generally adhere to key ethical principles that focus on the need for consent of donors and a system for monitoring adherence to ethical, legal, and scientific requirements. As hES cell research advances, it will be increasingly important for institutions that are obtaining, storing, and using cell lines to have confidence in the value of stored cells—that is, that they were obtained ethically and with the informed consent of donors, that they are well characterized and screened for safety, and that the conditions under which they are maintained and stored meet the highest scientific standards. Institutions engaged in hES research should seek mechanisms for establishing central repositories for hES cell lines—through partnerships or augmentation of existing quality research cell line repositories and should adhere to high ethical, legal, and scientific standards. At a minimum, an institutional registry of stem cell lines should be maintained. Institutions are encouraged to consider the use of the same procedures for banking and distribution of hPS cell lines.

5.1 Institutions that are banking or plan to bank hES cell lines should establish uniform guidelines to ensure that donors of material give informed consent through a process approved by an IRB and that meticulous records are maintained about all aspects of cell culture. Uniform tracking systems and common guidelines for distribution of cells should be established.

5.2 Any facility engaged in obtaining and storing hES cell lines should consider the following standards:

(a) Creation of a committee for policy and oversight purposes and creation of clear and standardized protocols for banking and withdrawals.

(b) Documentation requirements for investigators and sites that deposit cell lines, including

(i) A copy of the donor consent form.
(ii) Proof of Institutional Review Board approval of the procurement process.
(iii) Available medical information on the donors, including results of infectious-disease screening.
(iv) Available clinical, observational, or diagnostic information about the donor(s).
(v) Critical information about culture conditions (such as media, cell passage, and safety information).
(vi) Available cell line characterization (such as karyotype and genetic markers).

A repository has the right of refusal if prior culture conditions or other items do not meet its standards.

(c) A secure system for protecting the privacy of donors when materials retain codes or identifiable information, including but not limited to
(i) A schema for maintaining confidentiality (such as a coding system).
(ii) A system for a secure audit trail from primary cell lines to those submitted to the repository.
(iii) A policy governing whether and how to deliver clinically significant information back to donors.

(d) The following standard practices:
(i) Assignment of a unique identifier to each sample.
(ii) A process for characterizing cell lines.
(iii) A process for expanding, maintaining, and storing cell lines.
(iv) A system for quality assurance and control.
(v) A website that contains scientific descriptions and data related to the cell lines available.
(vi) A procedure for reviewing applications for cell lines.
(vii) A process for tracking disbursed cell lines and recording their status when shipped (such as number of passages).
(viii) A system for auditing compliance.
(ix) A schedule of charges.
(x) A statement of intellectual property policies.
(xi) When appropriate, creation of a clear Material Transfer Agreement or user agreement.
(xii) A liability statement.
(xiii) A system for disposal of material.

(e) Clear criteria for distribution of cell lines, including but not limited to evidence of approval of the research by an embryonic stem cell research oversight committee or equivalent body at the recipient institution.

6.0 RESEARCH USE OF hES CELL LINES

Once hES cell lines have been derived, investigators and institutions, through ESCRO committees and other relevant committees (such as an IACUC, an IBC, or a radiation safety committee) should monitor their use in research.

6.1 Institutions should require documentation of the provenance of all hES cell lines, whether the cells were imported into the institution or generated locally. The institution should obtain evidence of IRB-approval of the procurement process and of adherence to basic ethical and legal principles of procurement as described in Section 1.3(a) and 1.5. In the case of lines imported from another institution, documentation that these criteria were met at the time of derivation will suffice. Listing on the NIH Registry will be sufficient evidence of acceptability of hES cell lines.

6.2 *In vitro* experiments involving the use of already derived and coded hES cell lines will not need review beyond the review described in Sections 1.3(a) and 6.1.

6.3 Each institution should maintain a registry of its investigators who are conducting hES cell research and ensure that all registered users are kept up to date with changes in guidelines and regulations regarding the use of hES cells.

6.4 All protocols involving the combination of hES cells with nonhuman embryos, fetuses, or adult vertebrate animals must be submitted to the local IACUC for review of animal welfare issues and to the ESCRO committee for consideration of the consequences of the human contributions to the resulting chimeras. (See also Section 1.3(c)(iii) concerning breeding of chimeras.)

6.5 Transplantation of differentiated derivatives of hES cells or even hES cells themselves into adult animals will not require extensive ESCRO committee review. If there is a possibility that the human cells could contribute in a major organized way to the brain of the recipient animal, however,

the scientific justification for the experiments must be strong, and proof of principle using nonhuman (preferably primate) cells, is desirable.

6.6 Experiments in which hES cells, their derivatives, or other pluripotent cells are introduced into nonhuman fetuses and allowed to develop into adult chimeras need more careful consideration because the extent of human contribution to the resulting animal may be higher. Consideration of any major functional contributions to the brain should be a main focus of review. (See also Section 1.3(c)(iii) concerning breeding of chimeras.)

6.7 Introduction of hES cells into nonhuman mammalian blastocysts should be considered only under circumstances in which no other experiment can provide the information needed. (See also Sections 1.3(c)(ii) and 1.3(c)(iii) concerning restrictions on breeding of chimeras and production of chimeras with nonhuman primate blastocysts.)

6.8 Research use of existing hES cells does not require IRB review unless the research involves introduction of the hES cells or their derivatives into patients or the possibility that the identity of the donors of the ~~blastocysts~~, gametes, morulae, blastocysts, or somatic cells is readily ascertainable or might become known to the investigator.

7.0 RECOMMENDATIONS FOR RESEARCH USE OF NON-EMBRYO-DERIVED HUMAN PLURIPOTENT STEM CELLS (hPS CELLS)

7.1 Derivation

Because non-embryo-derived hPS cells are derived from human material, their derivation ~~is~~ may be covered by existing IRB regulations concerning review and informed consent, depending on the source of the tissue used. No ESCRO committee review is necessary, although the IRB may always seek the advice of an ESCRO committee if this seems desirable. Where appropriate, t~~T~~he IRB review should consider proper consent for use of the derived hPS cells. Some of the recommendations for informed consent that apply to hES cells also apply to hPS cells (see Section 3.6), including informed consent to genetic manipulation of resulting pluripotent stem cells and their use for transplantation into animals and humans and potentially in future commercial development.

7.2 Use in *in Vitro* Experiments

Use of hPS cells in purely *in vitro* experiments need not be subject to any review beyond that necessary for any human cell line except that any experiments designed or expected to yield gametes (oocytes or sperm) should be subject to ESCRO committee review.

7.3 Use in Experiments Involving Transplantation of hPS Cells into Animals at any Stage of Development or Maturity

~~7.3(a) Research involving transplantation of pluripotent human cells derived from nonembryonic sources into nonhuman animals other than humans or primates at any stage of embryonic, fetal, or postnatal development should be reviewed by ESCRO committees and IACUCs, as are similar experiments that use hES cells.~~

~~7.3(b) ESCRO committees should review the provenance of the hPS cells as they review the provenance of hES cells (see section 1.5) to ensure that the cell lines were derived according to ethical procedures of informed consent as monitored by an IRB or equivalent oversight body.~~

~~7.3()~~ Proposals for use of hPS cells in animals should be considered in one of the following categories:

(i) Permissible after currently mandated reviews and proper documentation [see Section 1.3(a)]: experiments that are exempt from full ESCRO committee review but not IACUC review (experiments that involve only transplantation into postnatal animals with no likelihood of contributing to the central nervous system or germ line).

(ii) Permissible after additional review by an ESCRO committee, as described in Section 2.0 of the guidelines [see Section 1.3(b)]: experiments in which there is a significant possibility that the implanted hPS cells could give rise to neural or gametic cells and tissues. Such experiments need full ESCRO committee and IACUC review and would include generation of all preimplantation chimeras as well as neural transplantation into embryos or perinatal animals. Particular attention should be paid to at least three factors: the extent to which the implanted cells colonize and integrate into the animal tissue; the degree of differentiation

of the implanted cells; and the possible effects of the implanted cells on the function of the animal tissue.

(iii) Should not be conducted at this time [see Section 1.3(c)]

(1) Experiments that involve transplantation of hPS cells into human blastocysts.
(2) Research in which hPS cells are introduced into nonhuman primate embryos, pending further research that will clarify the potential of such introduced cells to contribute to neural tissue or to the germ line.

7.4 Multipotent Neural Stem Cells

It is also relevant to note that neural stem cells, although not pluripotent, are multipotent and may have the potential to contribute to neural tissue in chimeric animals. ESCRO committees should decide whether they wish to review and monitor such experiments with neural stem cells in a similar fashion.

7.5 Prohibition on Breeding

No animal into which hPS cells have been introduced such that they could contribute to the germ line should be allowed to breed.

7.6 Guidance for Banking and Distribution

Institutions should consider the value of banking and distributing hPS cells using the guidance and rules that are already in place for hES cells and the value of including hPS cell lines in their registries.

8.0 INTERNATIONAL COLLABORATION

If a U.S.-based investigator collaborates with an investigator in another country, the ESCRO committee may determine that the procedures prescribed by the foreign institution afford protections consistent with these guidelines, and the ESCRO committee may approve the substitution of some of or all of the foreign procedures for its own.

9.0 CONCLUSION

The substantial public support for hES cell research and the growing trend by many nonfederal funding agencies and state legislatures to support this field

requires a set of guidelines to provide a framework for hES cell research. In the absence of the oversight ~~that would come with unrestricted~~ of hES cell research that falls outside federal funding of this research, these guidelines will continue to offer reassurance to the public and to Congress that the scientific community is attentive to ethical concerns and is capable of self-regulation while moving forward with this important research.

To help ensure that these guidelines are taken seriously, stakeholders in hES cell research—sponsors, funding sources, research institutions, relevant oversight committees, professional societies, and scientific journals, as well as investigators—should develop policies and practices that are consistent with the principles inherent in these guidelines. Funding agencies, professional societies, journals, and institutional review panels can provide valuable community pressure and impose appropriate sanctions to ensure compliance. For example, ESCROs and IRBs should require evidence of compliance when protocols are reviewed for renewal, funding agencies should assess compliance when reviewing applications for support, and journals should require that evidence of compliance accompanies publication of results.

As individual states and private entities move increasingly into hES cell research, it will be important to initiate a national effort to provide a formal context in which the complex moral and oversight questions associated with this work can be addressed on a continuing basis. Both the state of hES cell research and clinical practice and public policy surrounding these topics are in a state of flux and are likely to be so for several years. Therefore, the committee believes that ~~a national body~~ mechanisms should be established to assess periodically the adequacy of the policies and guidelines proposed in this document and elsewhere and to provide a forum for a continuing discussion of issues involved in hES cell research. New policies and standards may be appropriate for issues that cannot now be foreseen. The organization that sponsors this body should be politically independent and without conflicts of interest, should be respected in the lay and scientific communities, and able to call on suitable expertise to support this effort.

Appendix D

Committee Member and Staff Biographies

COCHAIRS

R. Alta Charo, JD, is the Warren P. Knowles Professor of Law and Bioethics at the University of Wisconsin–Madison, on the faculties of both the Law School and the Medical School. On August 31, 2009, she took leave to serve as a senior policy advisor in the Office of the Commissioner of the U.S. Food and Drug Administration. Professor Charo is the author of nearly 100 articles, book chapters, and government reports on such topics as voting rights, environmental law, family planning and abortion law, medical genetics law, reproductive technology policy, science policy, and medical ethics. She has been a member of the boards of the Alan Guttmacher Institute and the Foundation for Genetic Medicine, a member of the National Medical Advisory Committee of the Planned Parenthood Federation of America, and a member of the ethics advisory boards of the International Society for Stem Cell Research, the Juvenile Diabetes Research Foundation, and WiCell. In 1994, Professor Charo served on the National Institutes of Health Human Embryo Research Panel, and from 1996 to 2001, she was a member of the presidential National Bioethics Advisory Commission. She was a member of the National Academies' Board on Life Sciences from 2001 until 2007 and since 2006 has been a member of the Institute of Medicine (IOM) Board on Population Health and Public Health Practices. Professor Charo was elected to IOM in 2006.

Richard O. Hynes, PhD, is the Daniel K. Ludwig Professor for Cancer Research at the David H. Koch Institute for Integrative Cancer Research and Department of Biology at MIT and a Howard Hughes Medical Institute Investigator. He was formerly head of the Biology Department and then director of the Center for Cancer Research at the Massachusetts Institute of Technology. His research focuses on fibronectins and integrins and the molecular basis of cellular adhesion, both in normal development and in pathological situations, such as cancer, thrombosis, and inflammation. Dr. Hynes's current interests are cancer invasion and metastasis, angiogenesis, and animal models of human disease states. He is a member of the National Academy of Sciences and the Institute of Medicine and is a fellow of the Royal Society of London and the American Academy of Arts and Sciences. In 1997, he received the Gairdner International Foundation Award. In 2000, he served as president of the American Society for Cell Biology and testified before Congress about the need for federal support and oversight of embryonic stem cell research. He cochaired the 2005 National Academies *Guidelines for Human Embryonic Stem Cell Research* and is a governor of the Wellcome Trust, UK.

MEMBERS

Eli Y. Adashi, MD, MS, CPE, FACOG, is professor of medical science and the immediate past dean of medicine and biological sciences and the Frank L. Day Professor of Biology at Brown University. Harvard-educated in Health Care Management (MS, 2005), Dr. Adashi previously served as the John A. Dixon Endowed Presidential Professor and Chair of the Department of Obstetrics and Gynecology at the University of Utah Health Sciences Center. A member of the Council on Foreign Relations, the Council on Population Growth of the World Economic Forum, the Association of American Physicians, the Royal College of Obstetricians and Gynaecologists (ad Eundem), and the American Association for the Advancement of Science, Dr. Adashi is a veteran practitioner of women's health. An adviser to the World Health Organization, the World Bank, the Rockefeller Foundation and the Bill and Melinda Gates Foundation, Dr. Adashi is a recent Franklin fellow and Senior Advisor on Global Women's Health to the Secretary of State Office of Global Women's Issues headed by Ambassador-At-Large Melanne Verveer. A long-standing NIH-funded scientist and a Research Career Development Awardee, Dr. Adashi is a former Donna Shalala appointee to the National Advisory Council of the Eunice Kennedy Shriver National Institute of Child

Health and Human Development (NICHD). In addition, Dr. Adashi served the NIH as a member of the Reproductive Sciences 5-Year Planning Forum for NICHD, as a member of the selection committee of the Reproductive Scientist Development Program and as a member of the Reproductive Endocrinology Study Section. A former president of the Society for Reproductive Endocrinologists, the Society for Gynecologic Investigation, and the American Gynecological and Obstetrical Society, Dr. Adashi is the author or co-author of over 250 peer-reviewed publications, over 120 book chapters/reviews, and 13 books focusing on ovarian biology, ovarian cancer and women's reproductive health, freedom and rights. Elected to the Institute of Medicine in 1999, Adashi served on consensus committees on Women's Health Research, Antiprogestins: Assessing the Science and Understanding Premature Birth and Assuring Health Outcomes. Dr. Adashi has also served the IOM as a reviewer of *New Frontiers in Contraceptive Research, A Comprehensive Review of the DHHS Office of Family Planning Title X Program* and *Policy Issues in the Development of Personalized Medicine in Oncology*. Dr. Adashi is presently serving on the Board of Directors of Physicians for Human Rights and Population Connection as well as on the Board of Governors of Tel Aviv University.

A native of Israel, Dr. Adashi received his medical degree in 1973 from the Sackler School of Medicine of Tel Aviv University. After serving a straight medical internship in the same, Dr. Adashi (a naturalized U.S. citizen) completed residency training in obstetrics and gynecology at the New England Medical Center of Tufts University (1974-77). Fellowship training in the subspecialty of reproductive endocrinology and postdoctoral training in reproductive biology followed suit at Johns Hopkins University and at the University of California at San Diego, respectively (1977-81).

Brigid L.M. Hogan, PhD, is the George Barth Geller Professor and chair of the Department of Cell Biology, Duke University Medical Center. Before joining Duke, Dr. Hogan was an investigator of the Howard Hughes Medical Institute and Hortense B. Ingram Professor in the Department of Cell Biology at Vanderbilt University Medical Center. Dr. Hogan earned her PhD in biochemistry at the University of Cambridge. She was then a postdoctoral fellow in the Department of Biology at the Massachusetts Institute of Technology. Before moving to the United States in 1988, Dr. Hogan was head of the Molecular Embryology Laboratory at the National Institute for Medical Research in London. Her research focuses on the genetic control of embryonic development and morphogenesis, using the mouse as a model system. Her laboratory developed methods for deriving mouse pluripotential

embryonic germ cell lines. She was co-chair for science of the 1994 National Institutes of Health Human Embryo Research Panel and a member of the 2001-2002 National Academies Panel on Scientific and Medical Aspects of Human Cloning. Within the last few years, Dr. Hogan has been elected to the Royal Society of London, the American Academy of Arts and Sciences, the Institute of Medicine, and the National Academy of Sciences.

Marcia Imbrescia is the owner of Peartree Design, a landscape design firm, and was previously the media director for Drumbeater, a high-technology advertising agency. She holds BA degrees in marketing and journalism and a graduate certificate in landscape design. Ms. Imbrescia has a passion for health advocacy and helping people with illness and disability. She is a past member of the Board of Trustees of the Arthritis Foundation (AF) (2003-2007), for which she has participated as a volunteer at the chapter and national levels. She served as a member (1996-1998 and 2001) and chairperson (2002-2003) of AF's American Juvenile Arthritis Organization. In 1992, she received the Volunteer of the Year Award from the Massachusetts Chapter of AF. Her volunteer efforts include program development, conference planning, public speaking, fundraising, and advocacy. Currently, Ms. Imbrescia is an active volunteer with New England Disabled Sports. She served on the National Academies Committee on Guidelines for Human Embryonic Stem Cell Research in 2004-2005.

Terry Magnuson, PhD, is Sarah Graham Kenan Professor and chair of the Department of Genetics at the University of North Carolina. He also directs the Carolina Center for Genome Sciences and is the program director of cancer genetics at the Lineberger Comprehensive Cancer Center. Dr. Magnuson's research interests include mammalian genetics, genomics, and development. His laboratory has developed a high-throughput system to study the effects of mutations on mouse development with mouse embryonic stem cells. He is particularly interested in the role of chromatin remodeling complexes in such processes as autosomal imprinting, X-inactivation, and anterior-posterior patterning of axial structures in mammals. He is an elected member of the American Academy of Arts and Sciences and was a member of the Board of Directors of the Genetics Society of America and of the Society for Developmental Biology.

Linda B. Miller, OTR, MS in hospital administration, is president of the Washington, DC–based Volunteer Trustees Foundation, a consortium of not-for-profit hospital governing boards. She has extensive experience in trustee

education, advocacy, and the legal, ethical, and policy issues facing voluntary health care institutions. Recently, she has worked closely with the states' attorneys general in developing guidelines for protecting the community interest in the sale and conversion of nonprofit hospitals and in designing models for practice and legal oversight. She was elected to membership in the Institute of Medicine (IOM) in 1997.

Ms. Miller has been a frequent speaker on health-policy issues and has been published extensively in both the medical and popular press, including the *New England Journal of Medicine*, *Health Affairs*, *USA Today*, the *Washington Post,* and the *New York Times*. She served as a special assistant to the secretary of health, education, and welfare (now the Department of Health and Human Services) and on numerous health-related policy councils and advisory committees, including the National Institutes of Health's Consensus Panel on Liver Transplantation and, most recently, IOM's Committee on Spinal Cord Injury. Ms. Miller serves on the Advisory Board of the University of Louisville–based Institute for Cellular Therapeutics, headed by Suzanne Ildstad, which does research in adult bone marrow transplantation, and has been a member of several academic and health-care institutions' boards of governors, including those of Blythedale Children's Hospital in New York, Capital Hospice in the national capital region, and Cornell University's Alumni Council.

Jonathan D. Moreno, PhD, is the David and Lyn Silfen University Professor of Ethics and professor of medical ethics and of the history and sociology of science at the University of Pennsylvania. He holds a courtesy appointment as professor of philosophy. He is also a senior fellow at the Center for American Progress in Washington, D.C., where edits the magazine *Science Progress* (www.scienceprogress.org). He was a member of President Barack Obama's transition team for the Department of Health and Human Services. Moreno is an elected member of the Institute of Medicine/National Academy of Sciences. In 2008 he was designated a National Associate of the National Research Council. He has served as a senior staff member for two presidential advisory commissions, and has given invited testimony for both houses of congress. He was an Andrew W. Mellon post doctoral fellow, holds an honorary doctorate from Hofstra University, and is a recipient of the Benjamin Rush Medal from the College of William and Mary Law School. Moreno has served as adviser to the Howard Hughes Medical Institute and the Bill and Melinda Gates Foundation, among many other organizations. Moreno is also a faculty affiliate of the Kennedy Institute of Ethics at Georgetown University and a fellow of the Hastings Center and the New York Academy

of Medicine. He is a past president of the American Society for Bioethics and Humanities. His books include *Progress in Bioethics* (2010); *Science Next: Innovation for the Common Good* (2009); *Mind Wars: Brain Research and National Defense* (2006); *Undue Risk: Secret State Experiments on Humans* (1999); *Ethical Guidelines for Innovative Surgery (2006); Is There an Ethicist in the House?* (2005); *In the Wake of Terror: Medicine and Morality in a Time of Crisis* (2003); *Ethical and Regulatory Aspects of Clinical Research* (2003); *Deciding Together: Bioethics and Moral Consensus* (1995); *Ethics in Clinical Practice* (2000); and *Arguing Euthanasia* (1995). Moreno has published more than 300 papers, reviews and book chapters, and is a member of several editorial boards.

Pilar N. Ossorio, PhD, JD, is associate professor of law and bioethics at the University of Wisconsin–Madison and program faculty in the Graduate Program in Population Health at the university. Before taking her position there, she was director of the Genetics Section of the Institute for Ethics at the American Medical Association and taught as an adjunct faculty member at the University of Chicago Law School. For the 2006 calendar year, Professor Ossorio was a visiting professor of law at the University of California, Berkeley Boalt Hall School of Law.

Dr. Ossorio received her PhD in microbiology and immunology in 1990 from Stanford University. She went on to complete a postdoctoral fellowship in cell biology at Yale University School of Medicine. Throughout the early 1990s, Dr. Ossorio worked as a consultant for the federal program on the Ethical, Legal, and Social Implications (ELSI) of the Human Genome Project; in 1994, she took a full-time position with the Department of Energy's ELSI program. In 1993, she served on the Ethics Working Group for President Clinton's Health Care Reform Task Force. Dr. Ossorio received her JD from the Boalt Hall School of Law in 1997. While there, she was elected to the legal honor society Order of the Coif and received several awards for outstanding legal scholarship.

Dr. Ossorio is a fellow of the American Association for the Advancement of Science (AAAS), on the Editorial Board of the *American Journal of Bioethics*, an adviser to the National Human Genome Research Institute on ethical issues in large-scale sequencing, and a member of the University of Wisconsin's institutional review board for health-sciences research. She is a past member of AAAS's Committee on Scientific Freedom and Responsibility, a past member of the National Cancer Policy Board in the Institute of Medicine, and a past member or chair of several working groups on genet-

ics and ethics. She has published scholarly articles in bioethics, law, and molecular biology.

E. Albert Reece, MD, PhD, is dean of the University of Maryland School of Medicine and vice president for medical affairs at the University of Maryland, Baltimore. Previously, he was vice chancellor and dean of the University of Arkansas College of Medicine. Dr. Reece received his undergraduate degree from Long Island University, his MD (Magna Cum Laude) from New York University, his PhD in biochemistry from the University of the West Indies, and his MBA from the Fox School of Business and Management of Temple University. He completed a residency in obstetrics and gynecology at Columbia University–Presbyterian Hospital and a fellowship in maternal-fetal medicine at Yale University School of Medicine. He served on the faculty at Yale for 10 years and was the chairman of the Department of Obstetrics, Gynecology and Reproductive Sciences at Temple University. Dr. Reece has published over 400 journal articles, book chapters, and abstracts and nine textbooks, including *Diabetes in Pregnancy, Medicine of the Fetus & Mother,* and *Fundamentals of Obstetric & Gynecologic Ultrasound*. He is an editor for the *Journal of Maternal-Fetal Medicine* and a reviewer for several other scientific journals. His research focuses on diabetes in pregnancy, birth defects, and prenatal diagnosis. Dr. Reece is a member of the Institute of Medicine.

Joshua R. Sanes, PhD, is professor of molecular and cellular biology and the Paul J. Finnegan Family Director of the Center for Brain Science at Harvard University. He was previously Alumni Endowed Professor of Neurobiology at the Washington University School of Medicine. Dr. Sanes earned a BA in biochemistry and psychology at Yale and a PhD in Neurobiology at Harvard. He studies the formation of the synapses that interconnect nerve cells, including pioneering work on the signals exchanged between nerve cells and their target muscles as new connections are made. He is also using the vertebrate visual system to examine how nerve cells develop and migrate to the right location in the body. He has been elected a fellow of the American Association for the Advancement of Science and a member of the National Academy of Sciences and the American Academy of Arts and Sciences.

Harold T. Shapiro, PhD, is president emeritus of both Princeton University and the University of Michigan and is currently professor of economics and public affairs at Princeton University. His research interests include bioethics, the social role of higher education, hospital and medical- center administra-

tion, university administration, econometrics, statistics, and economics. Dr. Shapiro chaired the Board of Trustees of the Alfred P. Sloan Foundation, was presiding director for the Dow Chemical Company, and is a member of numerous boards, including the Robert Wood Johnson Medical School, HCA, the Merck Vaccine Advisory Board, the Knight Foundation Commission on Intercollegiate Athletics, the U.S. Olympic Committee, and the Stem Cell Institute of New Jersey. He is a former chair of the Association of American Universities and the National Bioethics Advisory Committee and vice chair of the President's Council of Advisors on Science and Technology. He has also served on the Board of Directors of the National Bureau of Economic Research, Inc. and the Board of Trustees of the Universities Research Association, Inc. He has chaired and served on numerous National Academies committees, including the Committee on the Organizational Structure of the National Institutes of Health and the Committee on Particle Physics. Dr. Shapiro was named the 2006 American Association for the Advancement of Science William D. Carey Lecturer for his leadership in science policy. He earned a PhD in economics from Princeton University and holds 14 honorary doctorates.

John E. Wagner, Jr., MD, is a professor of pediatrics at the University of Minnesota Medical School. He is the first recipient of the Children's Cancer Research Fund/Hageboeck Family Chair in Pediatric Oncology and also holds the University of Minnesota McKnight Presidential Chair in Cancer Research. He is the director of the Division of Pediatric Hematology/Oncology and Bone Marrow Transplantation and scientific director of clinical research of the Stem Cell Institute. Dr. Wagner is a member of numerous societies, including the American Society of Hematology, the International Society of Experimental Hematology, and the American Society of Blood and Marrow Transplantation. He is a member of several honorary societies, including Alpha Omega Alpha (1980), the American Society of Clinical Investigation (2000), and the Association of American Physicians (2006). Dr. Wagner holds a patent on the isolation of the pluripotential quiescent stem cell population. Dr. Wagner holds a BA in biological sciences and a BA in psychology from the University of Delaware and an MD from Jefferson Medical College. Dr. Wagner's research has focused on the development of novel cellular therapies for tissue repair and suppression of the immune response using subpopulations of neonatal umbilical cord blood and adult bone marrow and peripheral blood. His projects are funded by the National Institutes of Health and industry. In addition, Dr. Wagner pioneered the use of embryo selection to "create" a perfectly tissue-matched stem cell

donor for the treatment of genetic disease. Dr. Wagner has written more than 250 articles and book chapters in the field of hematopoietic stem cell transplantation. He previously served as a member of the Scientific Board of Directors of the National Marrow Donor Program and on the Institute of Medicine's Committee on Establishing a National Cord Blood Stem Cell Banking Program. He is currently a member of the Scientific and Medical Accountability Standards Working Group of the California Institute of Regenerative Medicine.

STAFF

Adam P. Fagen, PhD, is a senior program officer with the Board on Life Sciences of the National Research Council. He came to the National Academies from Harvard University, where he most recently served as preceptor on molecular and cellular biology. He earned his PhD in molecular biology and education from Harvard, working on issues related to undergraduate science courses; his research focused on mechanisms for assessing and enhancing introductory science courses in biology and physics to encourage student learning and conceptual understanding, including studies of active learning, classroom demonstrations, and student understanding of genetics vocabulary. Dr. Fagen also received an AM in molecular and cellular biology from Harvard, based on laboratory research in molecular evolutionary genetics, and a BA from Swarthmore College with a double-major in biology and mathematics. He served as co-director of the 2000 National Doctoral Program Survey, an on-line assessment of doctoral programs organized by the National Association of Graduate-Professional Students, supported by the Alfred P. Sloan Foundation, and completed by over 32,000 students.

At the National Academies, Dr. Fagen has served as study director for *Bridges to Independence: Fostering the Independence of New Investigators in Biomedical Research* (2005), *Treating Infectious Diseases in a Microbial World: Report of Two Workshops on Novel Antimicrobial Therapeutics* (2006), *2007* and *2008 Amendments to the National Academies' Guidelines for Human Embryonic Stem Cell Research* (2007, 2008), *Understanding Interventions that Encourage Minorities to Pursue Research Careers: Summary of a Workshop* (2007), *Inspired by Biology: From Molecules to Materials to Machines* (2008), *Transforming Agricultural Education for a Changing World* (2009), *Responsible Research with Biological Select Agents and Toxins* (2009), and *Research at the Intersection of the Physical and Life Sciences* (2010). He is currently study director or responsible staff officer for several ongoing projects including the National Academies Summer

Institute on Undergraduate Education in Biology, the National Academies Human Embryonic Stem Cell Research Advisory Committee, and the Special Immunizations Program for Laboratory Personnel Engaged in Research on Countermeasures for Select Agents.

Bruce M. Altevogt, PhD, is a senior program officer in the Board on Health Sciences Policy at the Institute of Medicine (IOM). His primary interests focus on policy issues related to basic research and preparedness for catastrophic events. He received his doctoral thesis from Harvard University's Program in Neuroscience. Following over 10 years of research, Dr. Altevogt joined The National Academies as a science and technology policy fellow with the Christine Mirzayan Science & Technology Policy Graduate Fellowship Program. Since joining the Board on Health Sciences Policy, he has been a program officer on multiple IOM studies including, *Sleep Disorders and Sleep Deprivation: An Unmet Public Health Problem, The National Academies' Guidelines for Human Embryonic Stem Cell Research: 2007 Amendments* and *2008 Amendments, and Research Priorities in Emergency Preparedness and Response for Public Health Systems*. He is currently serving as the director of the Forum on Medical and Public Health Preparedness for Catastrophic Events, the Neuroscience and Nervous System Disorders Forum, and as a co-study director on the National Academies Human Embryonic Stem Cells Research Advisory Committee. He received his BA from the University of Virginia in Charlottesville, where he majored in biology and minored in South Asian studies.

Frances E. Sharples, PhD, has served as director of the National Research Council's Board on Life Sciences since October 2000. Immediately prior to this position, she was a senior policy analyst for the Environment Division of the White House Office of Science and Technology Policy (OSTP) for four years. Dr. Sharples came to OSTP from the Oak Ridge National Laboratory, where she served in various positions in the Environmental Sciences Division between 1978 and 1996, most recently as a Research and Development Section Head. Dr. Sharples received her BA in biology from Barnard College and her MA and PhD in zoology from the University of California, Davis. She served as an American Association for the Advancement of Science (AAAS) Environmental Science and Engineering Fellow at the Environmental Protection Agency during the summer of 1981, and served as a AAAS Congressional Science and Engineering Fellow in the office of Senator Al Gore in 1984-85. She was a member of the National Institutes of Health's

Recombinant DNA Advisory Committee in the mid-1980s, and was elected a Fellow of the AAAS in 1992.

Andrew M. Pope, PhD, is director of the Board on Health Sciences Policy in the Institute of Medicine (IOM). He has a PhD in physiology and biochemistry from the University of Maryland and has been a member of the National Academies staff since 1982 and of the IOM staff since 1989. His primary interests are science policy, biomedical ethics, and environmental and occupational influences on human health. During his tenure at the National Academies, Dr. Pope has directed numerous studies on topics that range from injury control, disability prevention, and biologic markers to the protection of human subjects of research, National Institutes of Health priority-setting processes, organ procurement and transplantation policy, and the role of science and technology in countering terrorism. Dr. Pope is the recipient of IOM's Cecil Award and the National Academy of Sciences President's Special Achievement Award.

Amanda P. Cline, is a senior program assistant with the Board on Life Sciences at the National Academies. She earned a BS in environmental studies from Bucknell University in 2006.

Belmont Report,

The Nat'l Comm'n for the Protection of Human Subjects of Biomedical and Behavioral Research,

Ethical Principles and Guidelines for the Protection of Human Subjects of Research

(Apr. 18, 1979)

The Belmont Report

Office of the Secretary

Ethical Principles and Guidelines for the Protection of Human Subjects of Research

The National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research

April 18, 1979

AGENCY: Department of Health, Education, and Welfare.

ACTION: Notice of Report for Public Comment.

SUMMARY: On July 12, 1974, the National Research Act (Pub. L. 93-348) was signed into law, there-by creating the National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research. One of the charges to the Commission was to identify the basic ethical principles that should underlie the conduct of biomedical and behavioral research involving human subjects and to develop guidelines which should be followed to assure that such research is conducted in accordance with those principles. In carrying out the above, the Commission was directed to consider: **(i)** the boundaries between biomedical and behavioral research and the accepted and routine practice of medicine, **(ii)** the role of assessment of risk-benefit criteria in the determination of the appropriateness of research involving human subjects, **(iii)** appropriate guidelines for the selection of human subjects for participation in such research and **(iv)** the nature and definition of informed consent in various research settings.

The Belmont Report attempts to summarize the basic ethical principles identified by the Commission in the course of its deliberations. It is the outgrowth of an intensive four-day period of discussions that were held in February 1976 at the Smithsonian Institution's Belmont Conference Center supplemented by the monthly deliberations of the Commission that were held over a period of nearly four years. It is a statement of basic ethical principles and guidelines that should assist in resolving the ethical problems that surround the conduct of research with human subjects. By publishing the Report in the Federal Register, and providing reprints upon request, the Secretary intends that it may be made readily available to scientists, members of Institutional Review Boards, and Federal employees. The two-volume Appendix, containing the lengthy reports of experts and specialists who assisted the Commission in fulfilling this part of its charge, is available as DHEW Publication No. (OS) 78-0013 and No. (OS) 78-0014, for sale by the Superintendent of Documents, U.S. Government Printing Office, Washington, D.C. 20402.

Unlike most other reports of the Commission, the Belmont Report does not make specific recommendations for administrative action by the Secretary of Health, Education, and Welfare. Rather, the Commission recommended that the Belmont Report be adopted in its entirety, as a statement of the Department's policy. The Department requests public comment on this recommendation.

National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research

Members of the Commission

Kenneth John Ryan, M.D., Chairman, Chief of Staff, Boston Hospital for Women.
Joseph V. Brady, Ph.D., Professor of Behavioral Biology, Johns Hopkins University.
Robert E. Cooke, M.D., President, Medical College of Pennsylvania.
Dorothy I. Height, President, National Council of Negro Women, Inc.
Albert R. Jonsen, Ph.D., Associate Professor of Bioethics, University of California at San Francisco.
Patricia King, J.D., Associate Professor of Law, Georgetown University Law Center.
Karen Lebacqz, Ph.D., Associate Professor of Christian Ethics, Pacific School of Religion.
**** David W. Louisell, J.D., Professor of Law, University of California at Berkeley.*
Donald W. Seldin, M.D., Professor and Chairman, Department of Internal Medicine, University of Texas at Dallas.
****Eliot Stellar, Ph.D., Provost of the University and Professor of Physiological Psychology, University of Pennsylvania.*
**** Robert H. Turtle, LL.B., Attorney, VomBaur, Coburn, Simmons & Turtle, Washington, D.C.*

**** Deceased.*

Table of Contents

Ethical Principles and Guidelines for Research Involving Human Subjects

A. Boundaries Between Practice and Research

B. Basic Ethical Principles

1. Respect for Persons
2. Beneficence
3. Justice

C. Applications

1. Informed Consent
2. Assessment of Risk and Benefits
3. Selection of Subjects

Ethical Principles & Guidelines for Research Involving Human Subjects

Scientific research has produced substantial social benefits. It has also posed some troubling ethical questions. Public attention was drawn to these questions by reported abuses of human subjects in biomedical experiments, especially during the Second World War. During the Nuremberg War Crime Trials, the Nuremberg code was drafted as a set of standards for judging physicians and scientists who had conducted biomedical experiments on concentration camp prisoners. This code became the prototype of many later codes(1) intended to assure that research involving human subjects would be carried out in an ethical manner.

The codes consist of rules, some general, others specific, that guide the investigators or the reviewers of research in their work. Such rules often are inadequate to cover complex situations; at times they come into conflict, and they are frequently difficult to interpret or apply. Broader ethical principles will provide a basis on which specific rules may be formulated, criticized and interpreted.

Three principles, or general prescriptive judgments, that are relevant to research involving human subjects are identified in this statement. Other principles may also be relevant. These three are comprehensive, however, and are stated at a level of generalization that should assist scientists, subjects, reviewers and interested citizens to understand the ethical issues inherent in research involving human subjects. These principles cannot always be applied so as to resolve beyond dispute particular ethical problems. The objective is to provide an analytical framework that will guide the resolution of ethical problems arising from research involving human subjects.

This statement consists of a distinction between research and practice, a discussion of the three basic ethical principles, and remarks about the application of these principles.

[RETURN TO TABLE OF CONTENTS]

Part A: Boundaries Between Practice & Research

A. Boundaries Between Practice and Research

It is important to distinguish between biomedical and behavioral research, on the one hand, and the practice of accepted therapy on the other, in order to know what activities ought to undergo review for the protection of human subjects of research. The distinction between research and practice is blurred partly because both often occur together (as in research designed to evaluate a therapy) and partly because notable departures from standard practice are often called "experimental" when the terms "experimental" and "research" are not carefully defined.

For the most part, the term "practice" refers to interventions that are designed solely to enhance the well-being of an individual patient or client and that have a reasonable expectation of success. The purpose of medical or behavioral practice is to provide diagnosis, preventive treatment or therapy to particular individuals.(2) By contrast, the term "research' designates an activity designed to test an hypothesis, permit conclusions to be drawn, and thereby to develop or contribute to generalizable knowledge (expressed, for example, in theories, principles, and statements of relationships). Research is usually described in a formal protocol that sets forth an objective and a set of procedures designed to reach that objective.

When a clinician departs in a significant way from standard or accepted practice, the innovation does not, in and

of itself, constitute research. The fact that a procedure is "experimental," in the sense of new, untested or different, does not automatically place it in the category of research. Radically new procedures of this description should, however, be made the object of formal research at an early stage in order to determine whether they are safe and effective. Thus, it is the responsibility of medical practice committees, for example, to insist that a major innovation be incorporated into a formal research project.(3)

Research and practice may be carried on together when research is designed to evaluate the safety and efficacy of a therapy. This need not cause any confusion regarding whether or not the activity requires review; the general rule is that if there is any element of research in an activity, that activity should undergo review for the protection of human subjects.

Part B: Basic Ethical Principles

B. Basic Ethical Principles

The expression "basic ethical principles" refers to those general judgments that serve as a basic justification for the many particular ethical prescriptions and evaluations of human actions. Three basic principles, among those generally accepted in our cultural tradition, are particularly relevant to the ethics of research involving human subjects: the principles of respect of persons, beneficence and justice.

1. Respect for Persons. -- Respect for persons incorporates at least two ethical convictions: first, that individuals should be treated as autonomous agents, and second, that persons with diminished autonomy are entitled to protection. The principle of respect for persons thus divides into two separate moral requirements: the requirement to acknowledge autonomy and the requirement to protect those with diminished autonomy.

An autonomous person is an individual capable of deliberation about personal goals and of acting under the direction of such deliberation. To respect autonomy is to give weight to autonomous persons' considered opinions and choices while refraining from obstructing their actions unless they are clearly detrimental to others. To show lack of respect for an autonomous agent is to repudiate that person's considered judgments, to deny an individual the freedom to act on those considered judgments, or to withhold information necessary to make a considered judgment, when there are no compelling reasons to do so.

However, not every human being is capable of self-determination. The capacity for self-determination matures during an individual's life, and some individuals lose this capacity wholly or in part because of illness, mental disability, or circumstances that severely restrict liberty. Respect for the immature and the incapacitated may require protecting them as they mature or while they are incapacitated.

Some persons are in need of extensive protection, even to the point of excluding them from activities which may harm them; other persons require little protection beyond making sure they undertake activities freely and with awareness of possible adverse consequence. The extent of protection afforded should depend upon the risk of harm and the likelihood of benefit. The judgment that any individual lacks autonomy should be periodically reevaluated and will vary in different situations.

In most cases of research involving human subjects, respect for persons demands that subjects enter into the research voluntarily and with adequate information. In some situations, however, application of the principle is

not obvious. The involvement of prisoners as subjects of research provides an instructive example. On the one hand, it would seem that the principle of respect for persons requires that prisoners not be deprived of the opportunity to volunteer for research. On the other hand, under prison conditions they may be subtly coerced or unduly influenced to engage in research activities for which they would not otherwise volunteer. Respect for persons would then dictate that prisoners be protected. Whether to allow prisoners to "volunteer" or to "protect" them presents a dilemma. Respecting persons, in most hard cases, is often a matter of balancing competing claims urged by the principle of respect itself.

2. Beneficence. -- Persons are treated in an ethical manner not only by respecting their decisions and protecting them from harm, but also by making efforts to secure their well-being. Such treatment falls under the principle of beneficence. The term "beneficence" is often understood to cover acts of kindness or charity that go beyond strict obligation. In this document, beneficence is understood in a stronger sense, as an obligation. Two general rules have been formulated as complementary expressions of beneficent actions in this sense: **(1)** do not harm and **(2)** maximize possible benefits and minimize possible harms.

The Hippocratic maxim "do no harm" has long been a fundamental principle of medical ethics. Claude Bernard extended it to the realm of research, saying that one should not injure one person regardless of the benefits that might come to others. However, even avoiding harm requires learning what is harmful; and, in the process of obtaining this information, persons may be exposed to risk of harm. Further, the Hippocratic Oath requires physicians to benefit their patients "according to their best judgment." Learning what will in fact benefit may require exposing persons to risk. The problem posed by these imperatives is to decide when it is justifiable to seek certain benefits despite the risks involved, and when the benefits should be foregone because of the risks.

The obligations of beneficence affect both individual investigators and society at large, because they extend both to particular research projects and to the entire enterprise of research. In the case of particular projects, investigators and members of their institutions are obliged to give forethought to the maximization of benefits and the reduction of risk that might occur from the research investigation. In the case of scientific research in general, members of the larger society are obliged to recognize the longer term benefits and risks that may result from the improvement of knowledge and from the development of novel medical, psychotherapeutic, and social procedures.

The principle of beneficence often occupies a well-defined justifying role in many areas of research involving human subjects. An example is found in research involving children. Effective ways of treating childhood diseases and fostering healthy development are benefits that serve to justify research involving children -- even when individual research subjects are not direct beneficiaries. Research also makes it possible to avoid the harm that may result from the application of previously accepted routine practices that on closer investigation turn out to be dangerous. But the role of the principle of beneficence is not always so unambiguous. A difficult ethical problem remains, for example, about research that presents more than minimal risk without immediate prospect of direct benefit to the children involved. Some have argued that such research is inadmissible, while others have pointed out that this limit would rule out much research promising great benefit to children in the future. Here again, as with all hard cases, the different claims covered by the principle of beneficence may come into conflict and force difficult choices.

3. Justice. -- Who ought to receive the benefits of research and bear its burdens? This is a question of justice, in the sense of "fairness in distribution" or "what is deserved." An injustice occurs when some benefit to which a person is entitled is denied without good reason or when some burden is imposed unduly. Another way of

conceiving the principle of justice is that equals ought to be treated equally. However, this statement requires explication. Who is equal and who is unequal? What considerations justify departure from equal distribution? Almost all commentators allow that distinctions based on experience, age, deprivation, competence, merit and position do sometimes constitute criteria justifying differential treatment for certain purposes. It is necessary, then, to explain in what respects people should be treated equally. There are several widely accepted formulations of just ways to distribute burdens and benefits. Each formulation mentions some relevant property on the basis of which burdens and benefits should be distributed. These formulations are **(1)** to each person an equal share, **(2)** to each person according to individual need, **(3)** to each person according to individual effort, **(4)** to each person according to societal contribution, and **(5)** to each person according to merit.

Questions of justice have long been associated with social practices such as punishment, taxation and political representation. Until recently these questions have not generally been associated with scientific research. However, they are foreshadowed even in the earliest reflections on the ethics of research involving human subjects. For example, during the 19th and early 20th centuries the burdens of serving as research subjects fell largely upon poor ward patients, while the benefits of improved medical care flowed primarily to private patients. Subsequently, the exploitation of unwilling prisoners as research subjects in Nazi concentration camps was condemned as a particularly flagrant injustice. In this country, in the 1940's, the Tuskegee syphilis study used disadvantaged, rural black men to study the untreated course of a disease that is by no means confined to that population. These subjects were deprived of demonstrably effective treatment in order not to interrupt the project, long after such treatment became generally available.

Against this historical background, it can be seen how conceptions of justice are relevant to research involving human subjects. For example, the selection of research subjects needs to be scrutinized in order to determine whether some classes (e.g., welfare patients, particular racial and ethnic minorities, or persons confined to institutions) are being systematically selected simply because of their easy availability, their compromised position, or their manipulability, rather than for reasons directly related to the problem being studied. Finally, whenever research supported by public funds leads to the development of therapeutic devices and procedures, justice demands both that these not provide advantages only to those who can afford them and that such research should not unduly involve persons from groups unlikely to be among the beneficiaries of subsequent applications of the research.

Part C: Applications

C. Applications

Applications of the general principles to the conduct of research leads to consideration of the following requirements: informed consent, risk/benefit assessment, and the selection of subjects of research.

1. Informed Consent. -- Respect for persons requires that subjects, to the degree that they are capable, be given the opportunity to choose what shall or shall not happen to them. This opportunity is provided when adequate standards for informed consent are satisfied.

While the importance of informed consent is unquestioned, controversy prevails over the nature and possibility of an informed consent. Nonetheless, there is widespread agreement that the consent process can be analyzed as

containing three elements: information, comprehension and voluntariness.

Information. Most codes of research establish specific items for disclosure intended to assure that subjects are given sufficient information. These items generally include: the research procedure, their purposes, risks and anticipated benefits, alternative procedures (where therapy is involved), and a statement offering the subject the opportunity to ask questions and to withdraw at any time from the research. Additional items have been proposed, including how subjects are selected, the person responsible for the research, etc.

However, a simple listing of items does not answer the question of what the standard should be for judging how much and what sort of information should be provided. One standard frequently invoked in medical practice, namely the information commonly provided by practitioners in the field or in the locale, is inadequate since research takes place precisely when a common understanding does not exist. Another standard, currently popular in malpractice law, requires the practitioner to reveal the information that reasonable persons would wish to know in order to make a decision regarding their care. This, too, seems insufficient since the research subject, being in essence a volunteer, may wish to know considerably more about risks gratuitously undertaken than do patients who deliver themselves into the hand of a clinician for needed care. It may be that a standard of "the reasonable volunteer" should be proposed: the extent and nature of information should be such that persons, knowing that the procedure is neither necessary for their care nor perhaps fully understood, can decide whether they wish to participate in the furthering of knowledge. Even when some direct benefit to them is anticipated, the subjects should understand clearly the range of risk and the voluntary nature of participation.

A special problem of consent arises where informing subjects of some pertinent aspect of the research is likely to impair the validity of the research. In many cases, it is sufficient to indicate to subjects that they are being invited to participate in research of which some features will not be revealed until the research is concluded. In all cases of research involving incomplete disclosure, such research is justified only if it is clear that **(1)** incomplete disclosure is truly necessary to accomplish the goals of the research, **(2)** there are no undisclosed risks to subjects that are more than minimal, and **(3)** there is an adequate plan for debriefing subjects, when appropriate, and for dissemination of research results to them. Information about risks should never be withheld for the purpose of eliciting the cooperation of subjects, and truthful answers should always be given to direct questions about the research. Care should be taken to distinguish cases in which disclosure would destroy or invalidate the research from cases in which disclosure would simply inconvenience the investigator.

Comprehension. The manner and context in which information is conveyed is as important as the information itself. For example, presenting information in a disorganized and rapid fashion, allowing too little time for consideration or curtailing opportunities for questioning, all may adversely affect a subject's ability to make an informed choice.

Because the subject's ability to understand is a function of intelligence, rationality, maturity and language, it is necessary to adapt the presentation of the information to the subject's capacities. Investigators are responsible for ascertaining that the subject has comprehended the information. While there is always an obligation to ascertain that the information about risk to subjects is complete and adequately comprehended, when the risks are more serious, that obligation increases. On occasion, it may be suitable to give some oral or written tests of comprehension.

Special provision may need to be made when comprehension is severely limited -- for example, by conditions of immaturity or mental disability. Each class of subjects that one might consider as incompetent (e.g., infants and young children, mentally disable patients, the terminally ill and the comatose) should be considered on its own

terms. Even for these persons, however, respect requires giving them the opportunity to choose to the extent they are able, whether or not to participate in research. The objections of these subjects to involvement should be honored, unless the research entails providing them a therapy unavailable elsewhere. Respect for persons also requires seeking the permission of other parties in order to protect the subjects from harm. Such persons are thus respected both by acknowledging their own wishes and by the use of third parties to protect them from harm.

The third parties chosen should be those who are most likely to understand the incompetent subject's situation and to act in that person's best interest. The person authorized to act on behalf of the subject should be given an opportunity to observe the research as it proceeds in order to be able to withdraw the subject from the research, if such action appears in the subject's best interest.

Voluntariness. An agreement to participate in research constitutes a valid consent only if voluntarily given. This element of informed consent requires conditions free of coercion and undue influence. Coercion occurs when an overt threat of harm is intentionally presented by one person to another in order to obtain compliance. Undue influence, by contrast, occurs through an offer of an excessive, unwarranted, inappropriate or improper reward or other overture in order to obtain compliance. Also, inducements that would ordinarily be acceptable may become undue influences if the subject is especially vulnerable.

Unjustifiable pressures usually occur when persons in positions of authority or commanding influence -- especially where possible sanctions are involved -- urge a course of action for a subject. A continuum of such influencing factors exists, however, and it is impossible to state precisely where justifiable persuasion ends and undue influence begins. But undue influence would include actions such as manipulating a person's choice through the controlling influence of a close relative and threatening to withdraw health services to which an individual would otherwise be entitle.

2. Assessment of Risks and Benefits. -- The assessment of risks and benefits requires a careful arrayal of relevant data, including, in some cases, alternative ways of obtaining the benefits sought in the research. Thus, the assessment presents both an opportunity and a responsibility to gather systematic and comprehensive information about proposed research. For the investigator, it is a means to examine whether the proposed research is properly designed. For a review committee, it is a method for determining whether the risks that will be presented to subjects are justified. For prospective subjects, the assessment will assist the determination whether or not to participate.

The Nature and Scope of Risks and Benefits. The requirement that research be justified on the basis of a favorable risk/benefit assessment bears a close relation to the principle of beneficence, just as the moral requirement that informed consent be obtained is derived primarily from the principle of respect for persons. The term "risk" refers to a possibility that harm may occur. However, when expressions such as "small risk" or "high risk" are used, they usually refer (often ambiguously) both to the chance (probability) of experiencing a harm and the severity (magnitude) of the envisioned harm.

The term "benefit" is used in the research context to refer to something of positive value related to health or welfare. Unlike, "risk," "benefit" is not a term that expresses probabilities. Risk is properly contrasted to probability of benefits, and benefits are properly contrasted with harms rather than risks of harm. Accordingly, so-called risk/benefit assessments are concerned with the probabilities and magnitudes of possible harm and anticipated benefits. Many kinds of possible harms and benefits need to be taken into account. There are, for example, risks of psychological harm, physical harm, legal harm, social harm and economic harm and the corresponding benefits. While the most likely types of harms to research subjects are those of psychological or

physical pain or injury, other possible kinds should not be overlooked.

Risks and benefits of research may affect the individual subjects, the families of the individual subjects, and society at large (or special groups of subjects in society). Previous codes and Federal regulations have required that risks to subjects be outweighed by the sum of both the anticipated benefit to the subject, if any, and the anticipated benefit to society in the form of knowledge to be gained from the research. In balancing these different elements, the risks and benefits affecting the immediate research subject will normally carry special weight. On the other hand, interests other than those of the subject may on some occasions be sufficient by themselves to justify the risks involved in the research, so long as the subjects' rights have been protected. Beneficence thus requires that we protect against risk of harm to subjects and also that we be concerned about the loss of the substantial benefits that might be gained from research.

The Systematic Assessment of Risks and Benefits. It is commonly said that benefits and risks must be "balanced" and shown to be "in a favorable ratio." The metaphorical character of these terms draws attention to the difficulty of making precise judgments. Only on rare occasions will quantitative techniques be available for the scrutiny of research protocols. However, the idea of systematic, nonarbitrary analysis of risks and benefits should be emulated insofar as possible. This ideal requires those making decisions about the justifiability of research to be thorough in the accumulation and assessment of information about all aspects of the research, and to consider alternatives systematically. This procedure renders the assessment of research more rigorous and precise, while making communication between review board members and investigators less subject to misinterpretation, misinformation and conflicting judgments. Thus, there should first be a determination of the validity of the presuppositions of the research; then the nature, probability and magnitude of risk should be distinguished with as much clarity as possible. The method of ascertaining risks should be explicit, especially where there is no alternative to the use of such vague categories as small or slight risk. It should also be determined whether an investigator's estimates of the probability of harm or benefits are reasonable, as judged by known facts or other available studies.

Finally, assessment of the justifiability of research should reflect at least the following considerations: **(i)** Brutal or inhumane treatment of human subjects is never morally justified. **(ii)** Risks should be reduced to those necessary to achieve the research objective. It should be determined whether it is in fact necessary to use human subjects at all. Risk can perhaps never be entirely eliminated, but it can often be reduced by careful attention to alternative procedures. **(iii)** When research involves significant risk of serious impairment, review committees should be extraordinarily insistent on the justification of the risk (looking usually to the likelihood of benefit to the subject -- or, in some rare cases, to the manifest voluntariness of the participation). **(iv)** When vulnerable populations are involved in research, the appropriateness of involving them should itself be demonstrated. A number of variables go into such judgments, including the nature and degree of risk, the condition of the particular population involved, and the nature and level of the anticipated benefits. **(v)** Relevant risks and benefits must be thoroughly arrayed in documents and procedures used in the informed consent process.

3. Selection of Subjects. -- Just as the principle of respect for persons finds expression in the requirements for consent, and the principle of beneficence in risk/benefit assessment, the principle of justice gives rise to moral requirements that there be fair procedures and outcomes in the selection of research subjects.

Justice is relevant to the selection of subjects of research at two levels: the social and the individual. Individual justice in the selection of subjects would require that researchers exhibit fairness: thus, they should not offer potentially beneficial research only to some patients who are in their favor or select only "undesirable" persons for

risky research. Social justice requires that distinction be drawn between classes of subjects that ought, and ought not, to participate in any particular kind of research, based on the ability of members of that class to bear burdens and on the appropriateness of placing further burdens on already burdened persons. Thus, it can be considered a matter of social justice that there is an order of preference in the selection of classes of subjects (e.g., adults before children) and that some classes of potential subjects (e.g., the institutionalized mentally infirm or prisoners) may be involved as research subjects, if at all, only on certain conditions.

Injustice may appear in the selection of subjects, even if individual subjects are selected fairly by investigators and treated fairly in the course of research. Thus injustice arises from social, racial, sexual and cultural biases institutionalized in society. Thus, even if individual researchers are treating their research subjects fairly, and even if IRBs are taking care to assure that subjects are selected fairly within a particular institution, unjust social patterns may nevertheless appear in the overall distribution of the burdens and benefits of research. Although individual institutions or investigators may not be able to resolve a problem that is pervasive in their social setting, they can consider distributive justice in selecting research subjects.

Some populations, especially institutionalized ones, are already burdened in many ways by their infirmities and environments. When research is proposed that involves risks and does not include a therapeutic component, other less burdened classes of persons should be called upon first to accept these risks of research, except where the research is directly related to the specific conditions of the class involved. Also, even though public funds for research may often flow in the same directions as public funds for health care, it seems unfair that populations dependent on public health care constitute a pool of preferred research subjects if more advantaged populations are likely to be the recipients of the benefits.

One special instance of injustice results from the involvement of vulnerable subjects. Certain groups, such as racial minorities, the economically disadvantaged, the very sick, and the institutionalized may continually be sought as research subjects, owing to their ready availability in settings where research is conducted. Given their dependent status and their frequently compromised capacity for free consent, they should be protected against the danger of being involved in research solely for administrative convenience, or because they are easy to manipulate as a result of their illness or socioeconomic condition.

---

(1) Since 1945, various codes for the proper and responsible conduct of human experimentation in medical research have been adopted by different organizations. The best known of these codes are the Nuremberg Code of 1947, the Helsinki Declaration of 1964 (revised in 1975), and the 1971 Guidelines (codified into Federal Regulations in 1974) issued by the U.S. Department of Health, Education, and Welfare Codes for the conduct of social and behavioral research have also been adopted, the best known being that of the American Psychological Association, published in 1973.

(2) Although practice usually involves interventions designed solely to enhance the well-being of a particular individual, interventions are sometimes applied to one individual for the enhancement of the well-being of another (e.g., blood donation, skin grafts, organ transplants) or an intervention may have the dual purpose of enhancing the well-being of a particular individual, and, at the same time, providing some benefit to others (e.g., vaccination, which protects both the person who is vaccinated and society generally). The fact that some forms of practice have elements other than immediate benefit to the individual receiving an intervention, however, should not confuse the general distinction between research and practice. Even when a procedure applied in practice may

benefit some other person, it remains an intervention designed to enhance the well-being of a particular individual or groups of individuals; thus, it is practice and need not be reviewed as research.

(3) Because the problems related to social experimentation may differ substantially from those of biomedical and behavioral research, the Commission specifically declines to make any policy determination regarding such research at this time. Rather, the Commission believes that the problem ought to be addressed by one of its successor bodies.

How Tuskegee Changed Research Practices, Centers for Disease Control, available at http://www.cdc.gov/tuskegee/after.htm (last updated June 15, 2011)

Centers for Disease Control and Prevention
CDC 24/7: Saving Lives. Protecting People.™

Research Implications

How Tuskegee Changed Research Practices

After the Tuskegee Study, the government changed its research practices to prevent a repeat of the mistakes made in Tuskegee.

In 1974, the National Research Act was signed into law, creating the National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research (http://bioethics.georgetown.edu/pcbe/reports/past_commissions/) (http://www.cdc.gov/Other/disclaimer.html). The group identified basic principles of research conduct and suggested ways to ensure those principles were followed.

In addition to the panel's recommendations, regulations were passed in 1974 that required researchers to get voluntary informed consent from all persons taking part in studies done or funded by the Department of Health, Education, and Welfare (DHEW). They also required that all DHEW-supported studies using human subjects be reviewed by Institutional Review Boards, which read study protocols and decide whether they meet ethical standards.

The rules and policies for human subjects research have been reviewed and revised many times since they were first approved. From 1980-1983, the President's Commission for the Study of Ethical Problems in Medicine and Biomedical and Behavioral Research (http://bioethics.georgetown.edu/pcbe/reports/past_commissions/) (http://www.cdc.gov/Other/disclaimer.html) looked at federal rules for doing research on human subjects to see how well those rules were being followed. An Ethics Advisory Board was formed in the late 1970s to review ethical issues of biomedical research. In 1991, federal departments and agencies (16 total) adopted the Federal Policy for the Protection of Human Subjects.

Efforts to promote the highest ethical standards in research are still going on today. In October 1995, President Bill Clinton created a National Bioethics Advisory Commission (http://bioethics.georgetown.edu/nbac/) (http://www.cdc.gov/Other/disclaimer.html), funded and led by the Department of Health and Human Services. The commission's task was to review current regulations, policies, and procedures to ensure all possible safeguards are in place to protect research volunteers. It was succeeded by the President's Council on Bioethics (http://www.bioethics.gov/) (http://www.cdc.gov/Other/disclaimer.html), which was established in 2001.

Page last reviewed: June 14, 2011
Page last updated: September 24, 2013
Content source: National Center for HIV/AIDS, Viral Hepatitis, STD, and TB Prevention, Centers for Disease Control and Prevention

---

Centers for Disease Control and Prevention 1600 Clifton Rd. Atlanta, GA 30333, USA
800-CDC-INFO (800-232-4636) TTY: (888) 232-6348 - Contact CDC–INFO

USA.gov Government Made Easy

The Tuskegee Timeline, Centers for Disease Control,

available at http://www.cdc.gov/tuskegee/timeline.htm (last updated June 15, 2011)

Centers for Disease Control and Prevention
CDC 24/7: Saving Lives. Protecting People.™

The Tuskegee Timeline

The Study Begins

In 1932, the Public Health Service, working with the Tuskegee Institute, began a study to record the natural history of syphilis in hopes of justifying treatment programs for blacks. It was called the "Tuskegee Study of Untreated Syphilis in the Negro Male."

The study initially involved 600 black men – 399 with syphilis, 201 who did not have the disease. The study was conducted without the benefit of patients' informed consent. Researchers told the men they were being treated for "bad blood," a local term used to describe several ailments, including syphilis, anemia, and fatigue. In truth, they did not receive the proper treatment needed to cure their illness. In exchange for taking part in the study, the men received free medical exams, free meals, and burial insurance. Although originally projected to last 6 months, the study actually went on for 40 years.

What Went Wrong?

In July 1972, an Associated Press story about the Tuskegee Study caused a public outcry that led the Assistant Secretary for Health and Scientific Affairs to appoint an Ad Hoc Advisory Panel to review the study. The panel had nine members from the fields of medicine, law, religion, labor, education, health administration, and public affairs.

The panel found that the men had agreed freely to be examined and treated. However, there was no evidence that researchers had informed them of the study or its real purpose. In fact, the men had been misled and had not been given all the facts required to provide informed consent.

The men were never given adequate treatment for their disease. Even when penicillin became the drug of choice for syphilis in 1947, researchers did not offer it to the subjects. The advisory panel found nothing to show that subjects were ever given the choice of quitting the study, even when this new, highly effective treatment became widely used.

The Study Ends and Reparation Begins

The advisory panel concluded that the Tuskegee Study was "ethically unjustified"--the knowledge gained was sparse when compared with the risks the study posed for its subjects. In October 1972, the panel advised stopping the study at once. A month later, the Assistant Secretary for Health and Scientific Affairs announced the end of the Tuskegee Study.

In the summer of 1973, a class-action lawsuit was filed on behalf of the study participants and their families. In 1974, a $10 million out-of-court settlement was reached. As part of the settlement, the U.S. government promised to give lifetime medical benefits and burial services to all living participants. The Tuskegee Health Benefit Program (THBP) was established to provide these services. In 1975, wives, widows and offspring were added to the program. In 1995, the program was expanded to include health as well as medical benefits. The Centers for Disease Control and Prevention was given responsibility for the program, where it remains today in the National Center for HIV/AIDS, Viral Hepatitis, STD, and TB Prevention (http://www.cdc.gov/nchhstp/). The last study participant died in January 2004. The last widow receiving THBP benefits died in January 2009. There are 15 offspring currently receiving medical

and health benefits.

Timeline

1895 Booker T. Washington at the Atlanta Cotton Exposition, outlines his dream for black economic development and gains support of northern philanthropists, including Julius Rosenwald (President of Sears, Roebuck and Company).

1900 Tuskegee educational experiment gains widespread support. Rosenwald Fund provides monies to develop schools, factories, businesses, and agriculture.

1915 Booker T. Washington dies; Robert Motin continues work.

1926 Health is seen as inhibiting development and major health initiative is started. Syphilis is seen as major health problem. Prevalence of 35 percent observed in reproductive age population.

1929 Aggressive treatment approach initiated with mercury and bismuth. Cure rate is less than 30 percent; treatment requires months and side effects are toxic, sometimes fatal.

1929 "Wall Street Crash"--economic depression begins.

1931 Rosenwald Fund cuts support to development projects. Clark and Vondelehr decide to follow men left untreated due to lack of funds in order to show need for treatment program.

1932 Follow-up effort organized into study of 399 men with syphilis and 201 without. The men would be given periodic physical assessments and told they were being treated. Motin agrees to support study if "Tuskegee Institute gets its full share of the credit" and black professionals are involved (Dr. Dibble and Nurse Rivers are assigned to study).

1934 First papers suggest health effects of untreated syphilis.

1936 Major paper published. Study criticized because it is not known if men are being treated. Local physicians asked to assist with study and not to treat men. Decision was made to follow the men until death.

1940 Efforts made to hinder men from getting treatment ordered under the military draft effort.

1945 Penicillin accepted as treatment of choice for syphilis.

1947 USPHS establishes "Rapid Treatment Centers" to treat syphilis; men in study are not treated, but syphilis declines.

1962 Beginning in 1947, 127 black medical students are rotated through unit doing the study.

1968 Concern raised about ethics of study by Peter Buxtun and others.

1969 CDC reaffirms need for study and gains local medical societies' support (AMA and NMA chapters officially support continuation of study).

1972 First news articles condemn studies.

1972 Study ends.

1973 Congress holds hearings and a class-action lawsuit is filed on behalf of the study participants.

1974 A $10 million out-of-court settlement is reached and the U.S. government promised to give lifetime medical benefits and burial services to all living participants. The Tuskegee Health Benefit Program (THBP) was established to provide these services.

1975 Wives, widows and offspring were added to the program.

1995 The program was expanded to include health as well as medical benefits.

1997 On May 16th President Clinton apologizes on behalf of the Nation.

1999 Tuskegee University National Center for Bioethics in Research and Health Care hosts 1st Annual Commemoration of the Presidential Apology.

2001 President's Council on Bioethics (http://www.bioethics.gov/) (http://www.cdc.gov/Other/disclaimer.html) was established.

2004 CDC funds 10 million dollar cooperative agreement to continue work at Tuskegee University National Center for Bioethics in Research and Health Care.

2004 The last U.S. Public Health Service Syphilis Study at Tuskegee participant dies on January 16.

2006 Tuskegee University holds formal opening of Bioethics Center.

2007 CDC hosts Commemorating and Transforming the Legacy of the United States Public Health Service (USPHS) Syphilis Study at Tuskegee.

2009 The last widow receiving THBP benefits dies on January 27.

Page last reviewed: June 14, 2011
Page last updated: September 24, 2013
Content source: National Center for HIV/AIDS, Viral Hepatitis, STD, and TB Prevention, Centers for Disease Control and Prevention

---

Centers for Disease Control and Prevention 1600 Clifton Rd. Atlanta, GA 30333, USA
800-CDC-INFO (800-232-4636) TTY: (888) 232-6348 - Contact CDC–INFO

Dolgin,

Time to ditch stand-alone stem cell oversight panels, experts say,

NATURE MEDICINE, vol. 19, p. 250 (Mar. 2013)

Time to ditch stand-alone stem cell oversight panels, experts say

Research involving human embryonic stem cells (hESCs) has always been a controversial subject in the US, but never more so as when George W. Bush was president. At the time, federal support for such research was limited to fewer than two dozen cell lines, and up through his first term in office, studies involving hESCs in the country were often conducted under a patchwork of inconsistent, and sometimes nonexistent, oversight.

To encourage responsible practices, the US National Academies issued a report in 2005 calling for the establishment of stand-alone institutional oversight committees, and within two years, at least 25 so-called 'embryonic stem cell research oversight committees', or ESCROs, cropped up across the country. Most of these were created voluntarily, except in the few states—California and Connecticut, as well as New York if the research is performed with state funding—that made the practice mandatory. Now, however, with hESC research widely practiced and accepted, some experts are questioning whether stand-alone ESCROs are still needed.

"As that early period of figuring out ways to implement good ethical guidelines has shifted into the more normal science of applying those concerns, the need for ESCROs has gotten smaller," says Henry Greely, a bioethicist at the Stanford Law School and chair of California's Human Stem Cell Research Advisory Committee. "It's not rocket science any more. A lot of [hESC research] is pretty routine, and it doesn't necessarily need a unique institution to deal with it."

In a commentary published in the January issue of the *American Journal of Bioethics* (**13**, 44–52, 2013), Greely argues that ESCROs were invaluable throughout the past decade because they kept early investigations involving hESCs above the ethical fray. Plus, they provided political cover for this contentious topic. However, the committees also came at a cost in the form of time, resources and manpower that could have been spent elsewhere, especially when studies involving human subjects were also being reviewed by institutional review boards (IRBs) and studies that used laboratory animals needed the approval of institutional animal care and use committees (IACUCs). Some protocols involving hESCs were even overseen by all three. "It may well make sense to cut down on the number of committees that people have to file applications to," Greely says.

Looking ahead, Greely contends that the vital role ESCROs have played in recent years can now be taken over by IRBs and IACUCs—both of which have existed at universities and research institutions for decades. Those two review bodies would only have to expand their remits slightly to cover hESC-specific considerations. For example, IACUCs would be tasked with weighing whether specific embryonic stem cell experiments might confer "human characteristics" to bioengineered animals, as well as enforcing the no-breeding requirements for chimeras dictated by the 2005 guidelines, both of which the IACUCs currently don't do.

Likewise, IRBs would have to consider cell line experiments that don't involve human subjects at all. "It's a bit of an extension of what IRBs have done," notes Greely, "but it should be within their comfort zone." Doing so, he asserts, would require little more than adding committee members with special expertise in stem cell research to handle the new oversight duties—a smaller burden, perhaps, than maintaining separate ESCROs.

"I'm very sympathetic to this view," says David Magnus, director of the Stanford Center for Biomedical Ethics and the editor-in-chief of the *American Journal of Bioethics*. Magnus, who, together with Greely, sits on the Stanford Stem Cell Oversight Committee, argues that the ethical debates are now settled and that "anybody who's at least moderately sophisticated scientifically and ethically could be trained" to assess the validity of hESC research.

Put it to a committee

Julie Aultman, a bioethicist at Northeast Ohio Medical University in Rootstown, likes Greely's proposal—she's seen too many inconsistencies between ESCROs nationwide. However, she worries about filling in the expertise gap that would disappear. For that reason, she argues that the dissolution of ESCROs should coincide with the creation of a 'National Ethics Committee for Scientific Advancement' (*Am. J. Bioeth.* **13**, 61–62, 2013). Such a committee would oversee all controversial emerging areas of research, not just the science of pluripotent stem cells—thereby eliminating the notion of hESC exceptionalism. By providing a direct resource and policy forum for institutional oversight panels, it could also offer more on-the-ground assistance than other national bodies such as the Presidential Commission for the Study of Bioethical Issues.

Not all ethicists agree with Greely's proposal, though. "I don't think the particular solution he provides is the right solution moving forward," says Jason Scott Robert, director of the Bioethics, Policy and Law Program at Arizona State University in Tempe. "If we were to transition to a model that relies on IRBs and IACUCs, we might jeopardize either the scientific progress or the careful oversight [provided by ESCROs], and I would rather not see either of those sacrificed."

"This is one of those areas where we have to be careful," adds Geoffrey Lomax, who oversees medical and ethical standards at the California Institute for Regenerative Medicine in San Francisco. Keeping ESCROs around, he maintains, is "conservative without really putting a lot of baggage on the research."

Unlike in the US, all research in Canada involving hESCs that is funded by that country's federal government or that takes place at an institution that receives federal support must be reviewed by a national ESCRO convened by the Canadian Institutes of Health Research (CIHR). Such research must additionally be approved by local IRBs and IACUCs where appropriate. (Canada uses different titles and acronyms for all these committees.)

John Williams, a medical ethicist at the University of Ottawa in Ontario who chaired the CIHR's Stem Cell Oversight Committee from 2007 to 2012, says that such a national policy, with its unique stem cell oversight body, helps ensure that federal granting agencies maintain the most up-to-date and scientifically informed regulations. "Over the years in reviewing protocols we have been faced with new questions that have been translated into changes in the guidelines," he says. "So, if the research protocols are dealt with simply at the local level, it would be pretty difficult to see how the policy issues would be kept up to date."

Ultimately, the decision to maintain ESCROs or not all comes down to a cost-benefit ratio, says Greely. Streamlining committee structures won't bring "enormous benefits," he admits, "but I don't think they're trivial benefits, and what are the benefits of continuing to have ESCROs after this breaking in and bureaucratization process has worked itself out? They're not very big."

"It's not saying these guys have been failures and we should get rid of them," he adds. "It's almost saying, 'These guys have been so successful that they worked themselves out of a job'—and that's not a bad thing."

Elie Dolgin

James et al.,

Contribution of Human Embryonic Stem Cells to Mouse Blastocysts,

DEVELOPMENTAL BIOLOGY vol. 295, pp. 90-102 (2006)

Available online at www.sciencedirect.com
SCIENCE DIRECT
Developmental Biology 295 (2006) 90–102
DEVELOPMENTAL BIOLOGY
www.elsevier.com/locate/ydbio

Contribution of human embryonic stem cells to mouse blastocysts

Daylon James [1], Scott A. Noggle [1], Tomasz Swigut, Ali H. Brivanlou *

Laboratory of Molecular Embryology, The Rockefeller University, 1230 York Avenue, New York, NY 10021, USA

Received for publication 18 January 2006; revised 13 February 2006; accepted 16 March 2006
Available online 12 June 2006

Abstract

In addition to their potential for cell-based therapies in the treatment of disease and injury, the broad developmental capacity of human embryonic stem cells (hESCs) offers potential for studying the origins of all human cell types. To date, the emergence of specialized cells from hESCs has commonly been studied in tissue culture or in teratomas, yet these methods have stopped short of demonstrating the ESC potential exhibited in the mouse (mESCs), which can give rise to every cell type when combined with blastocysts. Due to obvious barriers precluding the use of human embryos in similar cell mixing experiments with hESCs, human/non-human chimeras may need to be generated for this purpose. Our results show that hESCs can engraft into mouse blastocysts, where they proliferate and differentiate in vitro and persist in mouse/human embryonic chimeras that implant and develop in the uterus of pseudopregnant foster mice. Embryonic chimeras generated in this way offer the opportunity to study the behavior of specialized human cell types in a non-human animal model. Our data demonstrate the feasibility of this approach, using mouse embryos as a surrogate for hESC differentiation.

Keywords: Chimera; Mouse blastocyst; hESC; Human embryonic stem cells; Derivation

Introduction

Embryonic stem cells (ESCs) are a population of self-renewing, pluripotent cells that are derived from the inner cell mass (ICM) of mammalian blastocyst stage embryos and are able to differentiate into all the cell types of the adult (Rossant, 2001). In recent years, human embryonic stem cells (hESCs) have generated tremendous enthusiasm for the promise they provide to both revolutionize cell-based therapies and regenerative medicine, as well as provide a vehicle for the study of early human embryology. While clinical application of hESCs does not necessarily depend on their ability to mimic natural development, the capacity for hESCs to model human embryogenesis, either as a whole or in part, depends largely on the ability of these cells to faithfully parallel their cognate population in a developing human embryo.

To date, hESC differentiation has mostly been assayed by two means: formation of embryoid bodies (EBs) and teratomas, both of which contain representative cell types from all three primary germ layers (Conley et al., 2004). Yet, while the timing of gene expression in differentiating mouse EBs can mirror embryonic gene expression (Keller et al., 1993; Leahy et al., 1999), neither mESCs nor hESCs have been shown to undergo axial morphogenesis within EBs. And though hESCs can give rise to relatively organized tissue rudiments within teratomas (Przyborski, 2005), they differentiate in response to an environment that does not reflect the developmental context of embryogenesis. For these reasons, engraftment of hESCs into an embryonic environment may be better suited to the study of specialized human cell types in a live animal model.

Numerous studies have demonstrated the viability and developmental potency of human stem cells within interspecies chimeras: human mesenchymal stem cells injected into e11.5 rat embryos have been shown to give rise to complex functional structures of the kidney (Yokoo and Kawamura, 2005); hESCs injected into an organogenesis stage chick embryo have been shown to proliferate and contribute to neural cell types

* Corresponding author. Fax: +1 212 327 8685.
E-mail address: brvnlou@rockefeller.edu (A.H. Brivanlou).
[1] Both authors contributed equally to the work.

0012-1606/$ - see front matter © 2006 Published by Elsevier Inc.
doi:10.1016/j.ydbio.2006.03.026

(Goldstein et al., 2002); and recently, when hESCs were injected directly into the lateral ventricle of e14 fetal mouse brains, they were shown to give rise to functional human neurons within the adult mouse brain (Muotri et al., 2005). In the present study, we use the mouse blastocyst as a host for engraftment of hESCs. The advantages of using the blastocyst stage mouse embryo as a host for engraftment of hESCs are twofold: first, like the ICM and mESCs, hESCs are theoretically capable of differentiating to any cell type; second, engraftment into mouse embryos at the blastocyst stage allows for integration at a point that is relatively close in developmental timing.

As these types of experiments are currently prohibited by the material transfer agreements attached to the NIH-registry cell lines, none of these hESC lines could have been used. We therefore isolated a new hESC line from human blastocysts donated from IVF clinics, named "RUES1." RUES1 had a stable karyotype, expressed markers of pluripotency and differentiated to derivatives of all three primary germ layers in embryoid bodies as well as teratomas. Using both unmodified and genetically marked RUES1, we show first that RUES1 injected into mouse blastocysts proliferated, intermingled and differentiated along with host cells in cultured blastocyst outgrowths. Strikingly, hESCs that engrafted to mouse embryos localized to their niche of origin, the ICM, despite a hundred million years of evolutionary distance. When chimeric blastocysts were implanted transiently into pseudo-pregnant foster mice, most of the resultant embryos were developmentally abnormal, though RUES1 derivatives persisted in rare embryos that proceeded through gastrulation and displayed normal morphology at e8. This study establishes the feasibility of adapting classic embryonic stem cell mixing experiments for use with hESCs. These approaches can be extended to take advantage of the large collection of mutant mice for use as host, and genetically modified and/or diseased hESCs as graft, to address both basic embryological properties of hESCs as well as shed light on their potential application for cell-based therapies.

Materials and methods

RUES1 derivation and culture

Derivation, culture and embryoid body formation were performed as previously described (Thomson et al., 1998). Blastocysts frozen at day 6 post-fertilization were donated with informed consent from embryos in excess of clinical need according to institutional guidelines. Identifying information was removed before receipt of the vials, and blastocysts were thawed by stepwise removal of cryoprotectant. Blastocysts were washed two times in recovery medium and incubated for 2 h before immunosurgery to allow for blastocoel expansion and morphological grading. Recovery medium consisted of 10% Plasmanate, 1× non-essential amino acids, 1× essential amino acids and 1× GlutaMAX in M16 medium (Specialty Media). The blastocysts were treated with 2 mg/ml pronase to remove the zona pellucida and then incubated in a 1:10 dilution of anti-human placental alkaline phosphatase antibody (DAKO). The embryos were washed three times in recovery medium and incubated in a 1:10 dilution of guinea pig complement (Sigma) and monitored for trophectoderm lysis. Lysed trophectoderm was removed by pipetting through a pulled Pasteur pipette, and isolated ICMs were washed 2× in HUESM medium. HUESM consisted of DMEM supplemented with 20% KSR, 1× non-essential amino acids, 1× essential amino acids, 1× GlutaMAX and 20 ng/ml bFGF (Invitrogen). Human LIF was added (12 ng/ml) during the initial outgrowth but was excluded from subsequent culture. ICMs were plated on an MEF feeder layer, and outgrowths were micro-dissected and transferred to fresh feeder layers for three passages for expansion. Stable culture of RUES1 was maintained as previously described (Sato et al., 2004; Xu et al., 2001). Embryoid bodies were generated by incubation of cultures in dispase until colonies detatched from the substrate. This was followed by culture of the aggregates in DMEM supplemented with 20% FCS, 1× penicillin–streptomycin, 1× GlutaMAX (all from Gibco) on non-tissue-culture-treated Petri dishes coated with a thin layer of agarose to prevent attachment.

Teratoma formation

To generate teratomas, 1–2 × 10^6 hESCs were injected into the rear leg muscle of SCID/beige mice. Teratomas were allowed to develop for 6 weeks and were then excised and fixed in neutral-buffered formalin and analyzed histologically by trained pathologist. Some teratomas were fixed, equilibrated in 30% sucrose and embedded for cryosectioning. Sections were processed immunohistochemically for markers of germ layers as described above.

Immunofluorescence

Undifferentiated hESCs plated on thermanox™ coverslips coated with MEFs or Matrigel, hESC-derived EBs, teratomas and chimeric embryos were analyzed by immunofluorescence staining for markers of pluripotency and/or differentiation. Briefly, samples were fixed in 4% paraformaldehyde, washed in PBST and blocked in 5% donkey or goat serum. Samples were exposed to primary antibodies in blocking solution overnight at 4°C, washed 3 times in PBST and exposed to fluorescent-conjugated secondary antibodies. Primary antibodies included Oct-3/4 (Signal Transduction labs), SSEA4, Tra-1-60, and nestin (Chemicon), β-tubulinIII/Tuj1 (Sigma), Alpha-1-Fetoprotein (DAKO) HNF3β, Sox2, Oct-3/4 (Santa Cruz), Muscle MHC/MF20 (Developmental Studies Hybridoma Bank), Neurofilament Heavy Chain, Phospho-HistoneH3, Desmin (Abcam), and Cdx2 (BioGenex). Alexa-conjugated secondary antibodies, SytoxGreen and SytoxOrange nuclear counterstains were purchased from Molecular Probes. Endogenous alkaline phosphatase was assayed using manufacturer's instructions (Vector Labs). All imaging was performed using a Zeiss Pascal confocal microscope.

Lentiviral vectors and infections

Supernatants containing infectious particles were collected 36 h after calcium phosphate co-transfection of HEK 293 (Graham and van der Eb, 1973; Graham et al., 1977) cells with pTrip (Sirven et al., 2000, 2001), psPAX2 (D. Trono Swiss Institute of Technology Lausanne), and pL-VSV-G (Bartz and Vodicka, 1997; Yee et al., 1994). hESCs were infected at 5 × 10^5 ifu/ml.

Blastocyst injections and embryonic outgrowth culture

RUES1 hESCs were manually dissected into 10–15 cells clumps using finely drawn glass Pasteur pipettes and injected into embryonic day 3.5 mouse blastocysts flushed from the uterine horns of Swiss Webster mice. hESC clumps were drawn into custom pulled transfer pipettes with a 25 μm bore (Eppendorf™) and injected into the blastocoel cavity of mouse embryos. hESC-injected blastocysts were either fixed 24 h post-injection or cultured on Matrigel™-coated tissue culture plastic in culture medium containing 15% fetal bovine serum for 6 days. Resultant embryonic outgrowths were fixed and processed immunocytochemically as described above.

RUES1 aggregation with mouse blastomere embryos

Embryonic day 2.5 mouse embryos were flushed from the oviduct of superovulated CBA/B6 mice and treated with acid tyrodes to remove their

zona pellucidae. In conical bottomed wells of a 96-well plate, one embryo was placed with a dispase-dissociated hESC clump of ~10–15 cells, and the plate was centrifuged briefly in order to combine them (adapted from Nagy, 2003). Embryos were allowed to recover for 48 h, when they were fixed and processed immunocytochemically as described above. Under these conditions, mESCs aggregated in parallel showed contribution to host ICM after 48 h.

Chimeric blastocyst implantation

Embryonic day 3.5 mouse blastocysts that were injected with hESCs and allowed to recover for 6 h post-injection were transferred to the uterine horns of pseudopregnant foster mice as previously described. Five days following transfer, implanted embryos were recovered from the uterus of foster mothers and examined for hESC contribution.

Results

Derivation and characterization of RUES1

We isolated an hESC line on mouse embryonic fibroblasts by immunosurgery from thawed blastocysts that had been frozen at day six of in vitro development after in vitro fertilization (Cowan et al., 2004; Thomson et al., 1998, Figs. 1A and B). Upon plating of the ICMs isolated from blastocysts, one expanded with continued culture and gave rise to colonies with tightly packed cells with a high nuclear to cytoplasmic ratio (Fig. 1C). These could be maintained on MEFs (Fig. 1E) by manual dissection (Mitalipova et al.,

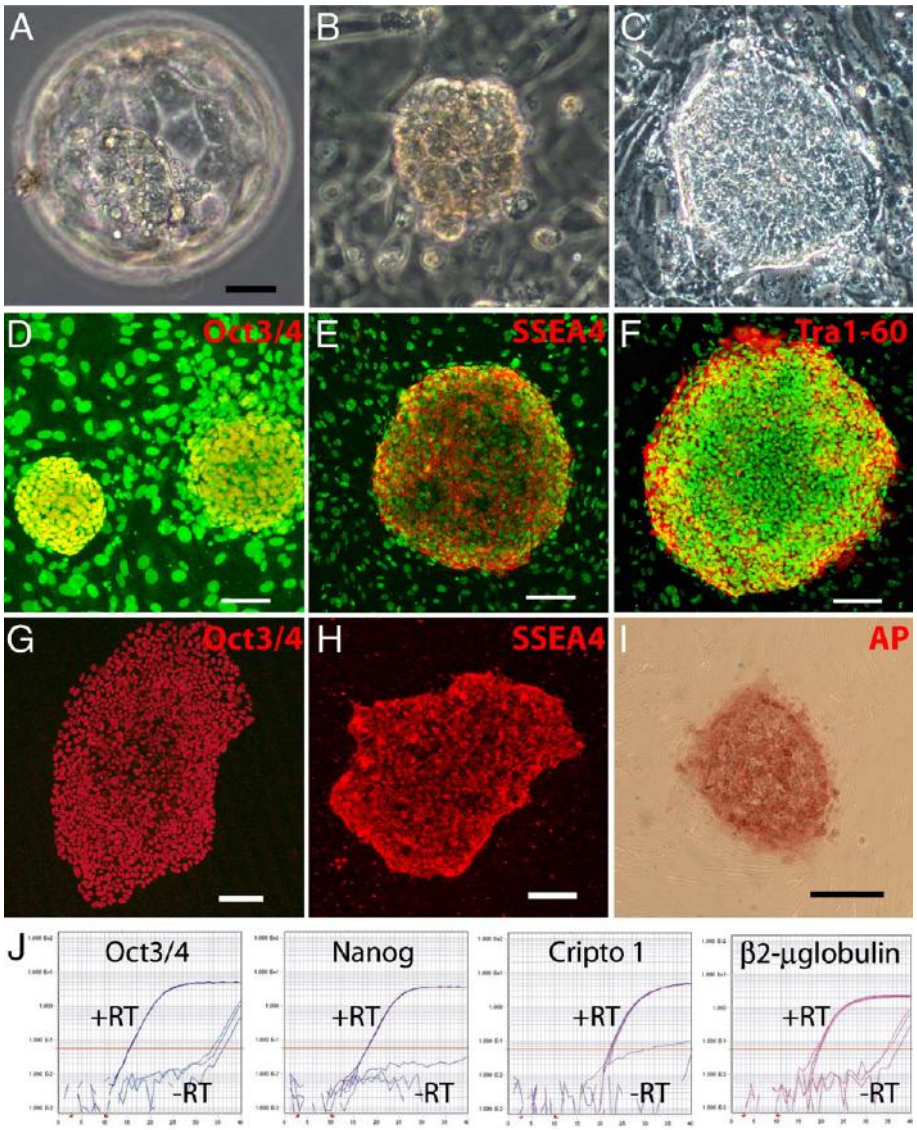


Fig. 1. Derivation and pluripotent marker expression of RUES1 hESCs. RUES1 was derived from a frozen 6-day-old blastocyst (A). The ICM after isolation by immunosurgery is shown (B). This ICM attached to the MEF feeder layer and produced a primary outgrowth of small ICM-like cells with a high nuclear to cytoplasmic ratio (C). Colonies on MEFs (D, E, F, and I) and on Matrigel (G and H) were analyzed for the presence of pluripotency markers Oct-3/4 (D and G), SSEA4 (E and H), and TRA-1-60 (F) by immunofluorescence. Colonies on MEFs were also positive for alkaline phosphatase (I) by cytochemistry. Real-time RT-PCR analysis of pluripotency markers (J). Shown are amplification plots of relative fluorescence vs. cycle number for Oct-3/4, Nanog, and Cripto-1. β-2-microglobulin as an amplification control. The no-RT controls are indicated for each primer. Red lines indicate the threshold cycle of amplification used to determine the level of expression. Scale bars are: 20 μm in panel A; and 50 μm in panels D–I.

2005; Oh et al., 2005) or transferred to and maintained on Matrigel-coated plates in MEF conditioned medium (Xu et al., 2001) (Fig. 1F) for more than 38 passages. This line was named RUES1 (for *R*ockefeller *U*niversity *E*mbryonic *S*tem-cell line *1*). Karyotype analysis revealed that the line was male [46, XY], and most cells had a normal karyotype (26/30) after 6 passages (Supplemental Fig. 1). Time-lapse video-microscopy established that the RUES1 cell cycle is about 24 h (data not shown). This is equivalent to the rate of most hESCs, reported to be about 24 to 36 h, with a range of 12 h to 72 h (Cowan et al., 2004).

RUES1 expressed previously described molecular markers of pluripotency (Thomson et al., 1998; Brivanlou et al., 2003). The markers Oct-3/4 (POU5F1, Figs. 1D and G), SSEA4 (Figs. 1E and H), TRA-1-60 (Fig. 1F), and alkaline phosphatase (Fig. 1I) were readily detected. By real-time RT-PCR, expression of Oct-3/4, Nanog, and Cripto-1 (Fig. 1J) was also detected. We have recently reported the identification of a set of genes that are consistently enriched in undifferentiated hESCs across several independent microarray studies (Suárez-Fariñas et al., 2005). We verified the enrichment of 91 of these markers in RUES1 hESCs by real-time RT-PCR (Supplementary Table 1). Together, these data demonstrate that RUES1 is similar to previously reported cell lines in origin, growth properties, and marker expression.

Differentiation of RUES1 into derivatives of all three embryonic germ layers

RUES1 also formed complex and cystic embryoid bodies when aggregated and cultured in suspension in vitro (Figs. 2A and B). Embryoid bodies could be maintained in suspension culture for at least 5 months. After prolonged in vitro culture or after reattachment to adhesive substrates, embryoid bodies generated multiple cell types indicative of the three embryonic germ layers (Figs. 2C–F). Neural cell types were evident in outgrowths from the EBs (data not shown). Immunostaining for nestin and Neurofilament Heavy Chain (NFH) confirmed the presence of ectoderm derivatives (Fig. 2C) in these cultures. During culture, beating cardiac myocytes were observed, indicating the presence of functional mesoderm differentiation (arrow in Fig. 2B). Staining for Desmin confirmed the presence of mesoderm (Fig. 2D); staining for HNF3β demonstrated the presence of endoderm derivatives (Fig. 2E). We also found an early marker of trophectoderm, Cdx2 (Strumpf et al., 2005), in EB cultures (Fig. 2F). These data indicated that RUES1 could be induced to form derivatives of all three primary germ layers in vitro.

To further demonstrate the differentiation potential of RUES1, we generated teratomas in SCID/beige mice and analyzed for tissue derivatives of the three embryonic germ layers (Figs. 2G–M and Supplementary Fig. 2). Several teratomas were analyzed including a single teratoma from which we could identify representatives of ectoderm, mesoderm, and endoderm by histology (Figs. 2K–M and Supplementary Figs. 2E–G). We immunostained a separate teratoma for germ layer markers to verify these results and identified neuroepithelium that stained positively for nestin (Fig. 2G), Tuj1, and NFH (Supplementary Figs. 2A and B); mesodermal tissue stained positively for Desmin (Fig. 2H) and Muscle MHC (Supplementary Fig. 2C); and endoderm tissue stained positively for HNF3β (Fig. 2I) and AFP (Supplementary Fig. 2D). We also identified trophectoderm, as marked by Cdx2 (Fig. 2J). Taken together, the results presented above establish that RUES1 is a bona fide new hESC line meeting the current criteria of prolonged undifferentiated proliferation while maintaining the ability to differentiate into trophectoderm and germ layer derivatives.

hESCs incorporate and differentiate in mouse blastocyst outgrowths

Although the functional qualities of mESCs and hESCs are very much the same, mouse and human ESCs show significant differences. For example, cell cycle length and the signaling factors that mediate self-renewal have been shown to be different between the two cell types (James et al., 2005; Sato et al., 2004; Xu et al., 2002a,b). The fact that hESCs are grown on top of MEFs in culture experiments clearly demonstrates that embryonic cell types from the two species can coexist. But factors secreted by MEFs are important for the maintenance of self-renewal in hESCs, so it is possible that paracrine signaling between mouse cells and hESCs within mosaic embryos could affect the differentiation process of one or both cell types. In order to assess the ability of hESCs to proliferate, integrate, and differentiate in mouse embryos, we injected e3.5 blastocysts with 10–15 cell clumps of RUES1 and cultured the embryos in vitro for 6 days (Fig. 3). These experiments described below were designed to minimize hESC input into host embryos, in accordance with policies in place at the Rockefeller University, and are also in line with guidelines recommended by the National Academy of Sciences (http://www.books.nap.edu/catalog/11278.html). Figs. 3A and B show the injection protocol.

As RUES1 hESCs do not tolerate trypsin-passaging, two independent means of RUES1 dissociation, trypsin and micro-dissection into cell clumps, were compared for their ability to integrate into host blastocysts. In each case, a total of 10 to 15 cells, either as individual cells or in clumps, were microinjected into the blastocoel cavity of a mouse blastocyst. When trypsin-dissociated cells were compared to cell clumps, micro-dissected colonies showed the best quality and quantity of contribution (data not shown). Other available cell lines have shown poor recovery from trypsin-passaging in tissue culture (Amit et al., 2000), and this enzymatic treatment may account for poor contribution of trypsin-dissociated RUES1 hESCs to mouse blastocysts. But another cell line HUES#6 (Cowan et al., 2004; generously provided by Doug Melton), which is routinely trypsin-passaged in cell culture, also showed poor contribution (data not shown). For this reason, the embryonic chimeras in these experiments were generated by injection of manually dissociated clumps.

In order to determine whether human cells would proliferate and mix with the mouse host, injected blastocysts were cultured

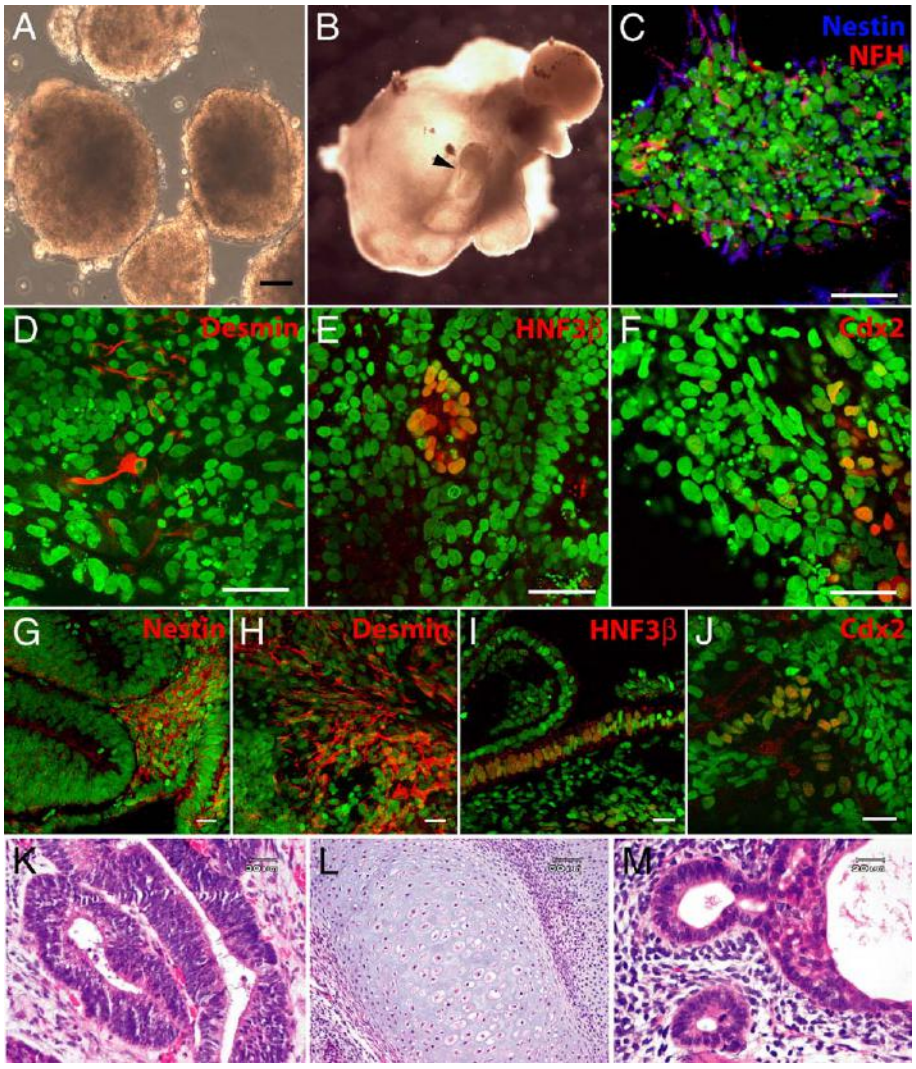


Fig. 2. Germ layer differentiation of RUES1 in embryoid bodies and teratomas. RUES1 generated complex aggregates after 14 days of in vitro differentiation in suspension (A). These subsequently formed complex embryoid bodies during 2 months of culture (B). The arrow in panel B indicates an area of contracting cardiac muscle after 2 months of culture, indicating mesoderm differentiation. When plated on adhesive substrates, the EBs generated multiple differentiated cell types, including neural tissue (C). The neural cell types can be propagated in vitro and stain for molecular markers of ectoderm: nestin (C, blue) and Neurofilament Heavy Chain (C, red). Mesoderm, marked by Desmin (D, red), and endoderm, marked by HNF3β (E, red), as well as trophectoderm, marked by Cdx2 (F, red), can also be found in EBs. RUES1 at passage 11 was also injected intramuscularly into SCID/beige mice and allowed to develop for 6 weeks to generate teratomas. Germ layer markers were verified by immunofluorescence and histology on cryosections of the teratoma (G–M). Examples of ectoderm: nestin (G, red) and retinal pigmented epithelium (K), mesoderm: Desmin (H, red) and cartilage (L), and endoderm: HNF3β (I, red) and glandular tissue (M) are shown. In addition, trophectoderm: Cdx2 (J, red) is present. SytoxGreen nuclear counterstain is shown in green. Scale bars are: 50 μm in panels A–J; 30 μm in panels K and M; and 60 μm in panel L.

on Matrigel™ for 6 days in vitro (Figs. 3C–I). RUES1 derivatives persisted in 14% of cultured chimeric blastocysts, and resultant embryonic outgrowths showed a complex and disorganized three-dimensional structure with human cells present in significant numbers (>500 nuclei in some cases). Human nuclei were predominantly concentrated in the suspended body of the outgrowth, while the "stalk" by which the outgrowth adhered to the extracellular matrix (star in Fig. 3F) was devoid of hESC derivatives. Cells were actively proliferating, as evidenced by the co-localization of Phospho-HistoneH3 (Gurley et al., 1978; green in Fig. 3G) and human nuclear antigen (red in Fig. 3G). Relative to host cells, however, human nuclei were underrepresented in all chimeric outgrowths (Fig. 3F and data not shown). This evidence established that hESCs could proliferate and intermingle with their mouse embryonic counterparts in cultured blastocyst outgrowths.

Mouse embryonic fibroblasts have commonly been used to maintain the undifferentiated state of hESCs, so it is possible that the mouse embryonic environment may impede the differentiation of hESCs. In order to address this, we examined whether human cells within the outgrowths expressed markers of the differentiated state. Human cells derived from all three germ layers were detected (Figs. 4E–J). Furthermore, we concluded that all the human cells were differentiated as no RUES1 nuclei were positive for the pluripotency marker Oct-3/4. In fact, a cluster of Oct-3/4-positive mouse cells that was retained in one outgrowth provided a valuable internal control for the absence of Oct-3/4 in human cells (Figs. 4A–D).

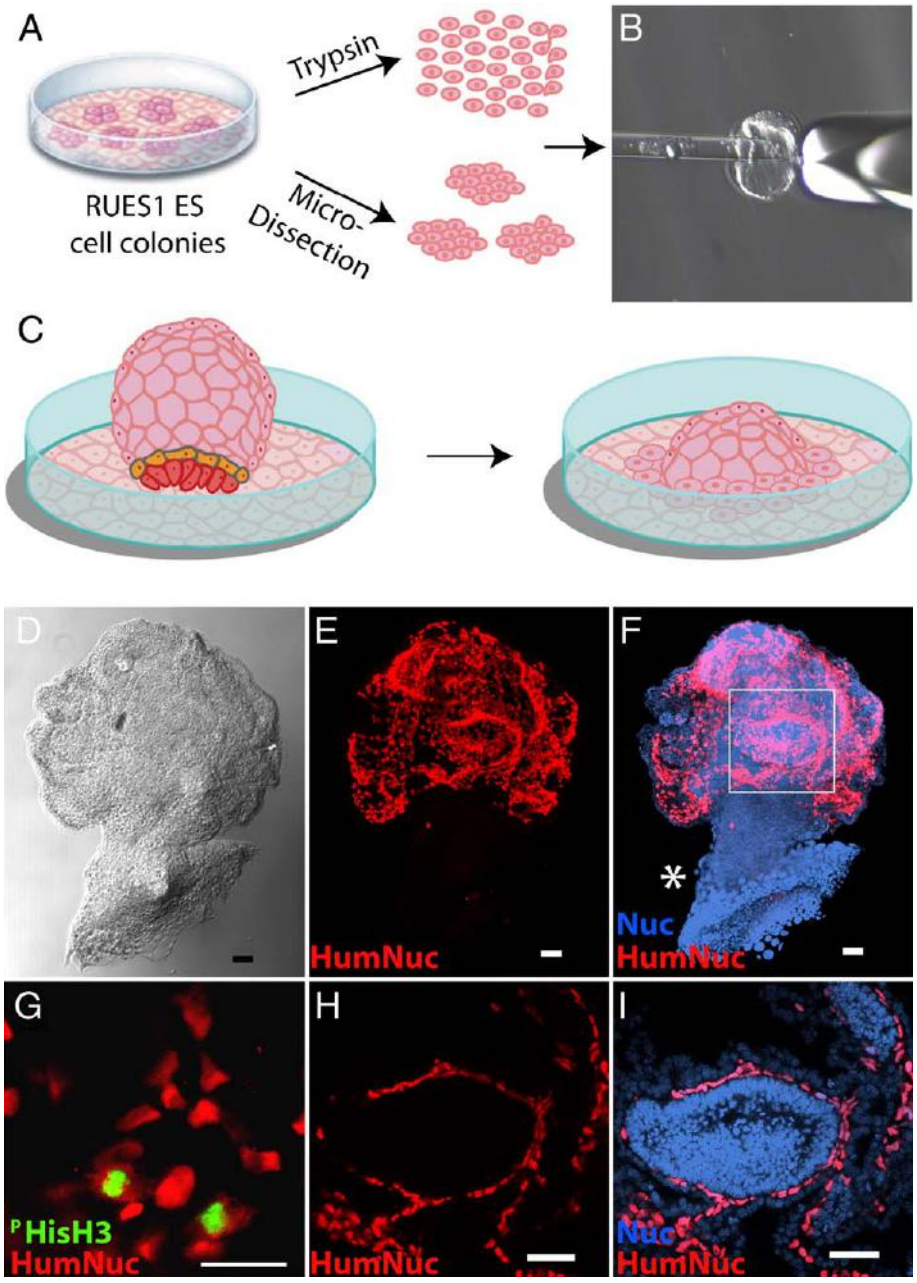


Fig. 3. hESCs survive, proliferate, and incorporate into cultured mosaic embryos. RUES1 was dissociated enzymatically by trypsin or manually by micro-dissection and injected into the blastocoel of e3.5 mouse embryos. The injection scheme is shown in panels A and B. hESC-injected embryos were cultured in vitro on Matrigel-coated tissue culture plastic (C) for 6 days. Resultant outgrowths showed complex three-dimensional structure, and human cells were present in significant numbers (D–I). Panel G shows human cells near the end of mitosis; Phospho-HistoneH3, which is a marker of mitosis, is shown in green. The inset in panel F is magnified as a single optical section in panels H and I, which show intermingling of human cells with the host. Human nuclei are represented in red in panels E–I. SytoxGreen nuclear counterstain is shown in blue. Scale bars—C, D, F, H, I—50 μm; G—20 μm.

Stable expression of a genetic marker in RUES1

In order to localize RUES1 derivatives within chimeric blastocysts and cultured outgrowths, we performed immunocytochemistry on our samples using an antibody specific for human nuclear antigen. While these methods were adequate for our purposes, secondary detection by immunofluorescence can result in signal artifacts that arise from non-specific binding of primary or secondary antibodies. To avoid this possibility in our subsequent experiments, we set out to generate hESCs that stably express green fluorescent protein (GFP). Because methods commonly used to generate stably expressing mESCs by lipofection were not as effective in hESCs (data not shown), we used lentiviral transduction to stably integrate GFP into RUES1 (Fig. 5). We first transfected HEK 293 cells with a lentiviral vector containing eGFP and used the supernatant from these cells to infect RUES1 at 5×10^5 infectious units/ml. After manually selecting for regions of strong GFP expression through two passages, homogenous, GFP-expressing RUES1 colonies were obtained (Figs. 5B and

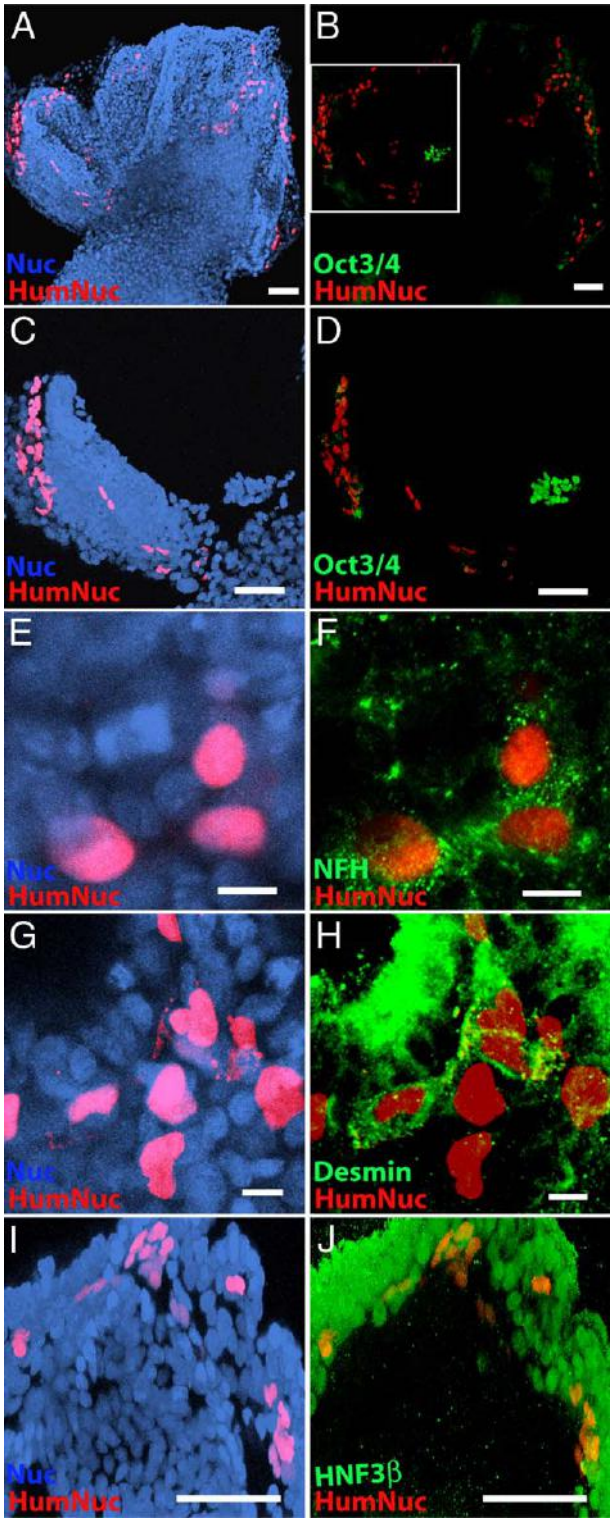


Fig. 4. hESCs differentiate into three primary germ layer derivatives within mosaic outgrowths. RUES1 cells were injected into e3.5 mouse blastocysts and cultured for 6 days on Matrigel. Resulting outgrowths were fixed and processed immunohistochemically using antibodies specific for Oct-3/4 (green in panels B and D), Neurofilament Heavy Chain (green in panel F), Desmin (green in panel H), and HNF3β (green in panel J). Human nuclei are stained by an antibody to human nuclear antigen in red and SytoxGreen nuclear counterstain in blue. Panels C and D represent a magnified view of the inset in B. Scale bars—A–D, I and J—50 μm; E–H—10 μm).

C). GFP expression remained stable in these hESCs for more than 10 passages.

hESCs can maintain their pluripotency within the mouse ICM niche

Because in vitro culture of blastocyst outgrowths cannot begin to recapitulate the dynamic process of early embryonic development in vivo, it is unclear from these experiments whether hESCs and mouse ICM derivatives would combine to form a coherent embryo. It is possible that human cells are growing ectopically in the host embryo and only become intermingled with mouse cells by virtue of their proximity. This possibility prompted us to ask whether RUES1 would integrate into and maintain the identity of the host ICM shortly after injection. In order to address this, we repeated the injection protocol and examined the expression of Oct-3/4 after 24 h of culture. Fig. 6 shows that RUES1 cells that incorporated generated a small niche of Oct-3/4-positive cells among their Oct-3/4 mouse counterparts in the ICM (Figs. 6A–H). In contrast, we consistently observed that hESCs that did not incorporate into the ICM were Oct-3/4-negative and showed unhealthy nuclear morphology (arrow in Fig. 6C). In these settings, we never found contribution of human cells to the trophectoderm. Human cells that integrated into host ICM maintained Oct-3/4 levels and were negative for the trophectoderm marker Cdx2 (Figs. 6F–H). From this evidence, we concluded that the differentiated cells seen in the mosaic outgrowths originated from the hESCs that engrafted into the host ICM.

hESCs aggregated with blastomere stage mouse embryos engraft into ICM

A common alternative to blastocyst injection for the generation of embryonic chimeras entails the aggregation of ESCs with pre-compacted blastomere stage embryos (Zeilmaker, 1973; Nagy et al., 1993). Given the technical difficulty and physical stress involved in the injection of hESC clumps into mouse blastocysts, adapting aggregation protocols to suit hESCs would not only allow for an increase in the scale of chimera generation, but would also ensure reduced trauma to engrafted hESCs. Furthermore, prior to compaction, mammalian embryos have yet to make the cell fate distinction between ICM, which gives rise to the embryo proper, and trophectoderm, which mediates invasion of uterine epithelium during implantation and gives rise to extraembryonic tissues. While mESCs do not normally differentiate to trophectoderm lineages in vitro, hESCs have demonstrated this potential (Xu et al., 2002b). Hence, engraftment of hESCs into mouse blastomere stage embryos provides a means of testing the ability of hESCs to take on dual cell fates (trophectoderm vs. ICM) concomitantly with the cells of the host. To address these questions, we combined dispase-dissociated RUES1 clumps with pre-compaction embryos in conical bottomed wells followed by mild centrifugation (Schematic shown in Fig. 6I). After 48 h,

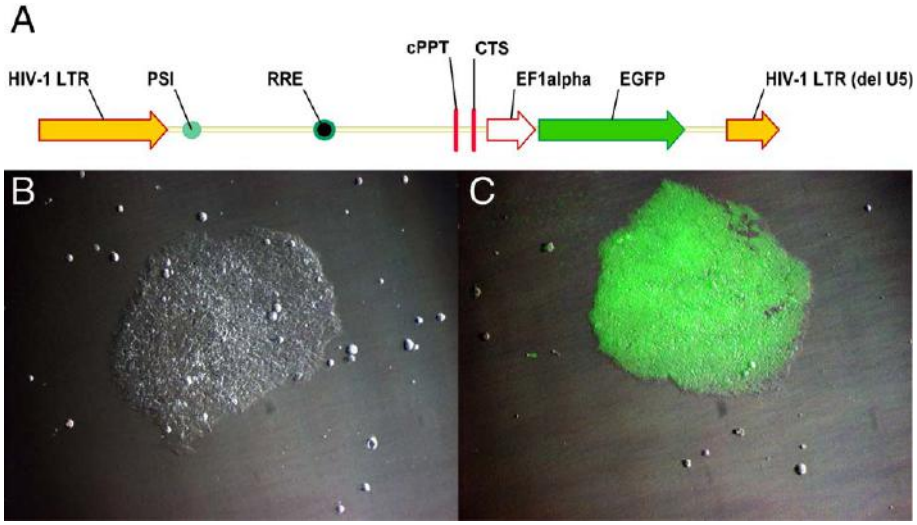


Fig. 5. eGFP transduction of RUES1 by lentivirus. (A) Schematic map of the pTrip-eGFP vector (Sirven et al., 2000, 2001). Panels B and C show RUES1 cells viewed with DIC (B), and composite of DIC and fluorescence in RUES1 transduced (at 5×10^5 ifu/ml) with pTrip-eGFP vector, after two rounds of manual passaging.

an average of 39% of resultant blastocysts contained hESCs in three independent experiments. Engrafted cells were localized to the ICM of a vast majority (97%) of embryonic chimeras that formed, and they retained the Oct-3/4-positive identity of adjacent mouse cells (Figs. 6J–N). As was the case for RUES1 hESCs injected into mouse blastocysts, human cells did not exhibit the trophectoderm marker Cdx2 in successfully aggregated embryos (Figs. 6L–N). However, in one individual case, aggregation resulted in a chimeric blastocyst in which hESCs were not localized to the host ICM (arrow in Fig. 6J). In this embryo, some human cells retained Oct-3/4 expression, but surprisingly, a minority exhibited Cdx2 expression (arrowhead in Fig. 6O), though at a reduced level relative to host trophectoderm (Figs. 6O–Q); and upon further differentiation in outgrowths after 6 days, Cdx2-positive human cells were also evident (data not shown). From these observations, we concluded that hESCs localized to host ICM in embryonic chimeras whether they were generated by blastocyst injection or morula aggregation.

hESCs persist in implanted embryonic chimeras in vivo

Given the strikingly disparate developmental schedules for mouse and human embryogenesis, it is unexpected that embryonic cell types from the two species could be combined within chimeras to form a coherent embryo. To determine whether embryonic chimeras generated by blastocyst injection would give rise to developmentally viable embryos in vivo, we transiently implanted hESC-injected blastocysts into the uterus of pseudopregnant foster mice and harvested them, along with uninjected controls, at embryonic day 8 (Fig. 7). Of 28 chimeric embryos that were implanted, 24 formed deciduae that contained embryos. Thirteen of these embryos were phenotypically normal and did not contain any GFP-positive RUES1 derivatives (Fig. 7A, left); 7 of the embryos were developmentally delayed and did not contain RUES1 derivatives (Fig. 7A, right); and 3 embryos contained GFP-positive RUES1 derivatives but showed aberrant morphology (Figs. 7B and C). Strikingly, one embryo of the 28 was morphologically similar to normal littermates but contained 10 GFP-positive hESC derivatives localized to the prospective foregut endoderm and neuroepithelium (Figs. 7D–H). The persistence of human cells in implanted chimeras was supported by a separate data set, in which human cells were shown by immunocytochemistry to persist in rare embryos, specifically in the anterior neural folds of embryonic chimeras at e8.5 (Figs. 7I–K). From these experiments, we conclude that hESCs can engraft in embryonic chimeras implanted in vivo and furthermore that they can be maintained in an embryo that proceeds normally through gastrulation.

Discussion

Embryonic cell mixing and recombination experiments between related species are a traditional approach of experimental embryology, used for more than a hundred years to understand embryonic processes at the cellular level. The origin of these methods can be traced to experiments in which early embryonic explants were transplanted between frog and newt gastrulae—an approach that allowed the identification of the origins of inductive signals during embryogenesis (Spemann, 1918, 1921; Spemann and Mangold, 1923). In the past few decades, pioneering experiments have contributed greatly to the understanding of vertebrate embryogenesis, but relative to other model species, a similar understanding of our own development has been elusive. hESCs have the potential to resolve this. In this study, we chose to test the capacity of a new hESC line, RUES1, to incorporate into the closely related embryonic environment of the mouse ICM. We showed that hESCs engrafted into pre-implantation stage mouse embryos and proliferated into differentiated human derivatives in the context of host tissue

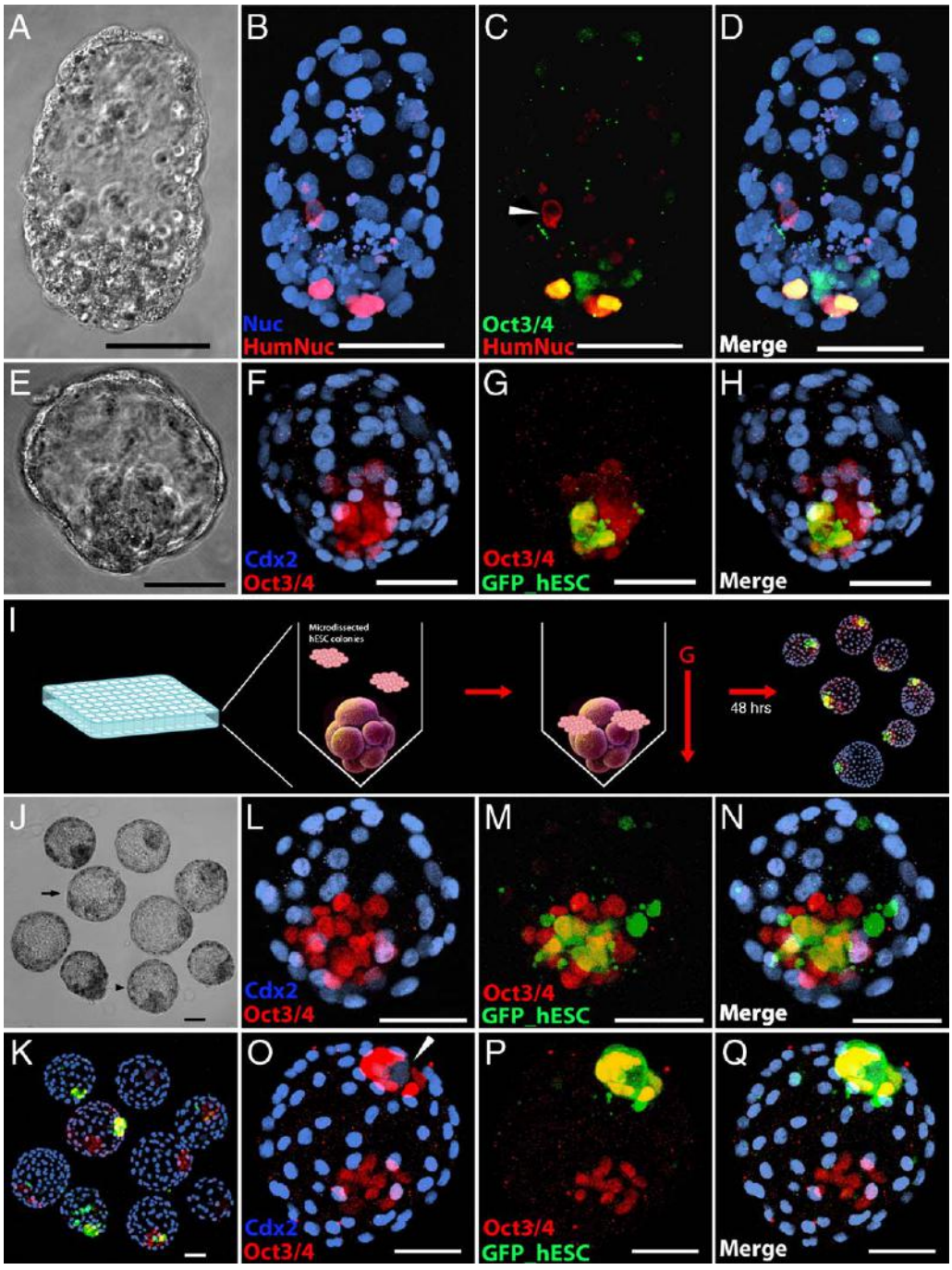


Fig. 6. RUES1 integrate into host ICM and retain pluripotent identity. Embryonic chimeras were generated by blastocyst injection (A–H) or aggregation (I–P) and fixed 24 h or 48 h post-injection/aggregation, respectively, for immunohistochemical analysis. (A–D) Human cells detected by anti-human nuclear antibody (red in panels B–D) that integrated into the host ICM showed healthy nuclear morphology (blue in panels B and D) and maintained Oct-3/4 (green in panels C and D). Human cells that did not incorporate into host ICM exhibited unhealthy, apoptotic morphology and did not retain Oct-3/4 (arrowhead in panel C). (E–H) GFP expressing hESCs (green in panels G and H) were negative for the trophectoderm marker Cdx2 (blue in panels F and H) in embryonic chimeras generated by blastocyst injection. A schematic diagram showing methods used to generate chimeric embryos by aggregation of blastomere stage embryos with hESCs is shown in panel I. (J–N) A majority of embryonic chimeras generated from morula aggregation showed localization of hESCs (green in panels K, M, and N) to host ICM with retention of Oct-3/4 (red in K–N) and absence of Cdx2 (blue in panels K, L, and N). (O–Q) One embryo contained hESC derivatives (green in panels P and Q) that were positive for Cdx2 (arrowhead in panel O). Nuclear counterstain is shown in blue in panels B and D. Panels L–N and O–Q show magnified views of embryonic chimeras indicated by the arrowhead and arrow, respectively, in panel J. Scale bars—50 μm.

in vitro. In addition, we showed that there is biological compatibility between both human and mouse cells in the ICM of mouse blastocysts as well as following implantation into pseudopregnant foster mice. These data establish for the first time that hESCs can integrate into the mouse embryo, validating the potential for non-human embryos to serve as a surrogate environment in which to study hESCs and their derivatives.

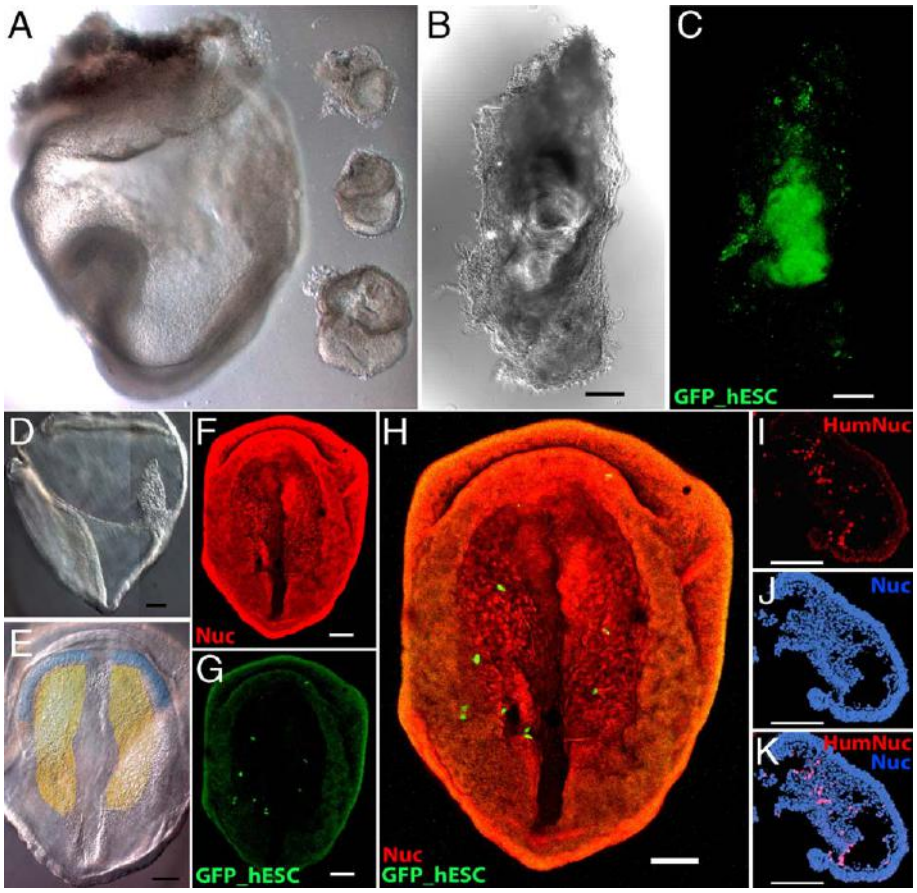


Fig. 7. hESC derivatives are retained in embryonic chimeras following implantation in vivo. RUES1-injected blastocysts were implanted into the uterus of pseudopregnant foster mice and recovered after 5 days of development. (A) Examples of implanted blastocysts that resulted in wild type phenotype (left) and aberrant/delayed phenotype without GFP-positive hESC contribution (right). (B and C) Bright field and fluorescent images of abnormal embryos containing GFP-positive hESC contribution. (D–H) Morphologically normal embryo containing 10 GFP-positive hESC derivatives. (D) Sagittal view; panel E shows a color-coded fate map specifying prospective foregut region in yellow and prospective neuroepithelium in blue (Nagy, 2003). (F–H) Anterior view. (I–K) A section of the neural fold region of an embryonic chimera at e8.5 was labeled by immunocytochemistry with the anti-human nuclear antigen antibody. Many RUES1 cells that were positive for the anti-human nuclear stain are shown in red in panels I and K; pan-nuclear counterstain is shown in red in panels F and H and in blue in panels J and K; GFP-positive cells are shown in green in panels C, G, and H. Scale bars—100 μm.

Our study complements previous evidence establishing that ESCs have the capacity to integrate into extra-species hosts—mESCs differentiated in vitro to motor neurons innervate chick hind legs (Wichterle et al., 2002); and hESCs differentiate into neuronal cell types in ovo in the context of the chick embryo (Goldstein et al., 2002) or when injected directly into lateral ventricles of e14 fetal mouse brains (Muotri et al., 2005). While these studies recombine late stage or stage-mismatched tissue, our embryonic chimeras are recombined from cells of the same stage as the one from which they were derived: the blastocyst. Twenty four hours after injection into mouse blastocysts, hESCs that were not incorporated into the ICM showed fragmented nuclear morphology suggestive of apoptosis or necrosis. And those hESCs that did incorporate into ICM maintained Oct-3/4 expression at levels similar to adjacent mouse cells, while those that did not incorporate were negative for Oct-3/4. Embryonic chimeras were also generated by aggregation of hESCs with blastomere stage embryos, and 48 h after aggregation, hESCs were again localized to the host ICM and maintained Oct-3/4 levels similar to adjacent host cells.

Localization of hESCs to host ICM is relevant to the ability of embryonic chimeras to implant and develop in vivo. Chimeras generated from aggregation of rat and mouse embryos show varied developmental progress depending on the methods used to combine them. When rat and mouse embryos are combined at the blastomere stage, rat cells contribute to the trophectoderm of the chimeras and they fail to implant into mouse uterus, presumably due to an immune response against the foreign rat component (Rossant, 1976). However, when isolated rat ICM is injected into mouse blastocyst, rat cells do not contribute to trophectoderm, and these chimeras are able to implant (Gardner and Johnson, 1973, 1975). Our observation that injected or aggregated hESCs engraft into host ICM and do not contribute to host trophectoderm suggests that chimeras generated by either method should at least be able to implant into the uterus of mouse foster mothers. Indeed, embryonic chimeras generated by blastocyst injection were able to implant and develop within foster mice, though the influence of hESCs seemed to disrupt embryogenesis in most cases.

The generation of interspecific chimeras using mouse pre-implantation embryos was first accomplished in 1973, when

chimeric blastocysts were generated from mouse and rat embryos (Mulnard, 1973; Stern, 1973; Zeilmaker, 1973). Since then, embryonic chimeras have been generated from combinations of mouse and vole (Mystkowska, 1975), the variant mouse species *Mus musculus* and *Mus caroli* (Rossant and Frels, 1980), the variant cow species *Bos taurus* and *Bos indicus* (Williams et al., 1990), and sheep and goat (Fehilly et al., 1984). Not surprisingly, chimeras generated from the more evolutionarily distant species were not viable, presumably due to irreconcilable differences between developmental programs—chimeras between mouse and vole or mouse and rat, for instance, did not come to term and only rarely developed to advanced stages in utero. On the other hand, chimeras generated from mixing embryos of closely related species (*M. musculus* and *M. caroli*, *B. taurus*, and *B. indicus*, or sheep and goat) resulted in successful development to adulthood. Considering these results, it seems unlikely that chimeras generated from engraftment of hESCs into mouse blastocysts would develop into viable chimeric embryos. Our results show that the majority of embryonic chimeras that implanted and retained hESC derivatives were developmentally abnormal/delayed. Rarely, however, hESCs persisted in morphologically normal embryos, demonstrating that hESC engraftment is not irreconcilable with mouse embryogenesis. In fact, the differences between mouse and human embryogenesis may account for these rare morphologically normal embryos. In particular, the difference in cell cycle between mouse and human ESCs may explain the relative scarcity of hESC derivatives in our embryonic chimeras at e8: considering our experimental design, in which hESC contribution was intentionally minimized, combined with the relatively slow pace of hESC proliferation and/or human embryogenesis, it makes sense that hESC derivatives should be underrepresented; and if human contribution is minimized, the relatively brisk pace of mouse development may allow the host cells to out-compete the hESC derivatives, resulting in "pockets" of human cells in a morphologically normal mouse embryo.

The observation that RUES1 localized to the ICM of host blastocysts indicated that hESCs preferentially occupied a niche that parallels that of their origin, the ICM. Yet, the emergence of functional neurons from undifferentiated hESCs injected directly into the lateral ventricles of e14 mouse brains (Muotri et al., 2005) establishes that pluripotent human cells and their derivatives can also respond appropriately to the inductive signals of an evolutionarily distant niche. In the rare instance where hESCs engrafted "ectopically" into regions of trophectoderm upon blastocyst formation (Figs. 6O–Q), most of the hESC derivatives retained Oct-3/4 expression, but in a subset of the engrafted cells, Oct-3/4 was completely lost, and a weak Cdx2 signal was observed. While little can be concluded as to the timing of differentiation in mouse vs. human cells from these observations, they did provide some indication that human cells were capable of taking on the molecular identity of the niche into which they engrafted. Following from our work, it is feasible that mouse/human chimeras could be generated in which hESCs are engrafted into pre-implantation stage mouse embryos and distributed throughout the host anatomy through gastrulation. This may allow for chimeras in which hESC derivatives are "seeded" into an array of developmental niches within a viable mouse, which would be of considerable value for the modeling of human development and disease in live animals.

While these experiments are in line with ethical guidelines set forth by the National Academy of Sciences (Committee on Guidelines for Human Embryonic Stem Cell Research and National Research Council, 2005) (http://www.books.nap.edu/catalog/11278.html), we purposefully restricted our analysis to early developmental stages and minimized hESC engraftment in order to affirm the feasibility of these assays. As such, the current study is lacking in some respects. First, in order to further explore the utility of embryonic chimeras as a vehicle for examining the emergence and behavior of human cell types, we must characterize the extent to which human cells can contribute to a viable mouse–human chimera. Allowing progression of chimeras to later developmental time points would indicate whether hESC derivatives are capable of integrating functionally into host anatomy. Second, in the rare instances in which hESC derivatives persisted in morphologically normal chimeras, the observed GFP-positive cells could have been a result of cell fusion rather than persistence of bona fide human cells. Given the rarity of this phenotype and the scarcity of presumed human cells, it is difficult to rule out the possibility of cell fusion without increasing the scale of the experiments and allowing further proliferation of hESC derivatives within chimeras left to develop to later stages.

Regardless of whether human cells can accommodate the spatiotemporal signaling environment and/or developmental schedule of mouse embryogenesis in any or all instances, the generation and culture of mouse/human chimeras in vitro may at least allow for a study of the murine embryonic explant's influence on hESC differentiation, yet engraftment of hESC derivatives into live chimeric animal models would be a much more valuable tool. Provided that hESCs can be reconciled with mouse embryogenesis in vivo, engrafting hESCs into host anatomy before gastrulation may provide an accessible platform for studying the emergence of many human cell types; and with the expansion of available hESCs to include genetically diseased lines, mouse/human chimeras may allow us to elucidate the bases of disease by examining the behavior of such hESC lines in live animal models. In addition to their contribution to the basic understanding of human embryology, the advances reported here provide a foundation for future work towards an understanding of human disease.

Acknowledgments

The authors wish to acknowledge the leadership of the Rockefeller University administration, especially Dr. Paul Nurse, for establishing policies at the university to allow these experiments. We are grateful for the advice from the Rockefeller University Bioethics Committee, as well as the general council's office for their guidance before and during this work. We would also like to thank Brivanlou laboratory

members for advice and technical support, especially Sandra Wellington. Additional thanks to Dr. Douglas Melton (Harvard University) for his gift of non-registry cell lines and Dr. D. Trono (Swiss Institute of Technology Lausanne) for the psPAX2 vector. The derivation of RUES1 was supported by private funds from the Juvenile Diabetes Research Foundation and private funds from the Rockefeller University. Cell mixing experiments were supported entirely by private funds provided by the Rockefeller University. All experiments were approved by the Rockefeller University Bioethics Committee.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ydbio.2006.03.026.

References

Amit, M., Carpenter, M.K., Inokuma, M.S., Chiu, C.P., Harris, C.P., Waknitz, M.A., Itskovitz-Eldor, J., Thomson, J.A., 2000. Clonally derived human embryonic stem cell lines maintain pluripotency and proliferative potential for prolonged periods of culture. Dev. Biol. 227, 271–278.

Bartz, S.R., Vodicka, M.A., 1997. Production of high-titer human immunodeficiency virus type 1 pseudotyped with vesicular stomatitis virus glycoprotein. Methods 12, 337–342.

Brivanlou, A.H., Gage, F.H., Jaenisch, R., Jessell, T., Melton, D., Rossant, J., 2003. Stem cells. Setting standards for human embryonic stem cells. Science 300, 913–916.

Committee on Guidelines for Human Embryonic Stem Cell Research, National Research Council and Institute of Medicine of the National Academies, 2005. Guidelines for human embryonic stem cell research, National Academies Press.

Conley, B.J., Trounson, A.O., Mollard, R., 2004. Human embryonic stem cells form embryoid bodies containing visceral endoderm-like derivatives. Fetal Diagn. Ther. 19, 218–223.

Cowan, C.A., Klimanskaya, I., McMahon, J., Atienza, J., Witmyer, J., Zucker, J.P., Wang, S., Morton, C.C., McMahon, A.P., Powers, D., Melton, D.A., 2004. Derivation of embryonic stem-cell lines from human blastocysts. N. Engl. J. Med. 350, 1353–1356.

Fehilly, C.B., Willadsen, S.M., Tucker, E.M., 1984. Interspecific chimaerism between sheep and goat. Nature 307, 634–636.

Gardner, R.L., Johnson, M.H., 1973. Investigation of early mammalian development using interspecific chimaeras between rat and mouse. Nat. New Biol. 246, 86–89.

Gardner, R.L., Johnson, M.H., 1975. Investigation of cellular interaction and deployment in the early mammalian embryo using interspecific chimaeras between the rat and mouse. Ciba Found. Symp. 0, 183–200.

Goldstein, R.S., Drukker, M., Reubinoff, B.E., Benvenisty, N., 2002. Integration and differentiation of human embryonic stem cells transplanted to the chick embryo. Dev. Dyn. 225, 80–86.

Graham, F.L., van der Eb, A.J., 1973. A new technique for the assay of infectivity of human adenovirus 5 DNA. Virology 52, 456–467.

Graham, F.L., Smiley, J., Russell, W.C., Nairn, R., 1977. Characteristics of a human cell line transformed by DNA from human adenovirus type 5. J. Gen. Virol. 36, 59–74.

Gurley, L.R., D'Anna, J.A., Barham, S.S., Deaven, L.L., Tobey, R.A., 1978. Histone phosphorylation and chromatin structure during mitosis in Chinese hamster cells. Eur. J. Biochem. 84, 1–15.

James, D., Levine, A.J., Besser, D., Hemmati-Brivanlou, A., 2005. TGFbeta/activin/nodal signaling is necessary for the maintenance of pluripotency in human embryonic stem cells. Development 132, 1273–1282.

Keller, G., Kennedy, M., Papayannopoulou, T., Wiles, M.V., 1993. Hematopoietic commitment during embryonic stem cell differentiation in culture. Mol. Cell. Biol. 13, 473–486.

Leahy, A., Xiong, J.W., Kuhnert, F., Stuhlmann, H., 1999. Use of developmental marker genes to define temporal and spatial patterns of differentiation during embryoid body formation. J. Exp. Zool. 284, 67–81.

Mitalipova, M.M., Rao, R.R., Hoyer, D.M., Johnson, J.A., Meisner, L.F., Jones, K.L., Dalton, S., Stice, S.L., 2005. Preserving the genetic integrity of human embryonic stem cells. Nat. Biotechnol. 23, 19–20.

Mulnard, J., 1973. Formation of chimeric blastocysts by fusion of rat and mice embryos at stage 8. C. R. Acad. Sci. Hebd. Seances Acad. Sci. D. 276, 379–381.

Muotri, A.R., Nakashima, K., Toni, N., Sandler, V.M., Gage, F.H., 2005. Development of functional human embryonic stem cell-derived neurons in mouse brain. Proc. Natl. Acad. Sci. U. S. A. 102, 18644–18648.

Mystkowska, E.T., 1975. Preimplantation development in vivo and in vitro in bank voles, *Clethrionomys glareolus*, treated with PMSG and HCG. J. Reprod. Fertil. 42, 287–292.

Nagy, A., 2003. Manipulating the Mouse Embryo: A Laboratory Manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Nagy, A., Rossant, J., Nagy, R., Abramow-Newerly, W., Roder, J.C., 1993. Derivation of completely cell culture derived mice from early passage embryonic stem cells. Proc. Natl. Acad. Sci. U. S. A. 90, 8424–8428.

Oh, S.K., Kim, H.S., Park, Y.B., Seol, H.W., Kim, Y.Y., Cho, M.S., Ku, S.Y., Choi, Y.M., Kim, D.W., Moon, S.Y., 2005. Methods for expansion of human embryonic stem cells. Stem Cells 23, 605–609.

Przyborski, S.A., 2005. Differentiation of human embryonic stem cells after transplantation in immune-deficient mice. Stem Cells 23, 1242–1250.

Rossant, J., 1976. Investigation of inner cell mass determination by aggregation of isolated rat inner cell masses with mouse morulae. J. Embryol. Exp. Morphol. 36, 163–174.

Rossant, J., 2001. Stem cells from the mammalian blastocyst. Stem Cells 19, 477–482.

Rossant, J., Frels, W.I., 1980. Interspecific chimeras in mammals: successful production of live chimeras between *Mus musculus* and *Mus caroli*. Science 208, 419–421.

Sato, N., Meijer, L., Skaltsounis, L., Greengard, P., Brivanlou, A.H., 2004. Maintenance of pluripotency in human and mouse embryonic stem cells through activation of Wnt signaling by a pharmacological GSK-3-specific inhibitor. Nat. Med. 10, 55–63.

Sirven, A., Pflumio, F., Zennou, V., Titeux, M., Vainchenker, W., Coulombel, L., Dubart-Kupperschmitt, A., Charneau, P., 2000. The human immunodeficiency virus type-1 central DNA flap is a crucial determinant for lentiviral vector nuclear import and gene transduction of human hematopoietic stem cells. Blood 96, 4103–4110.

Sirven, A., Ravet, E., Charneau, P., Zennou, V., Coulombel, L., Guetard, D., Pflumio, F., Dubart-Kupperschmitt, A., 2001. Enhanced transgene expression in cord blood CD34(+)-derived hematopoietic cells, including developing T cells and NOD/SCID mouse repopulating cells, following transduction with modified trip lentiviral vectors. Mol. Ther. 3, 438–448.

Spemann, H., 1918. Uber die determination der ersten organanlagen des amphibien embryo, I–VI. Arch. Entwicklungsmech. 43, 448–555.

Spemann, H., 1921. Die erzeugung tierischer chimaren durch heteroplastiche transplantation zwischen *Triton cristatus* und *taeniatus*. Wilhelm Roux' Arch. Entwickl.Mech. Org. 48, 533–570.

Spemann, H., Mangold, H., 1923. Induction of embryonic primordia by implantation of organizers from a different species. 1923. Int. J. Dev. Biol. 45, 13–38.

Stern, M.S., 1973. Letter: chimaeras obtained by aggregation of mouse eggs with rat eggs. Nature 243, 472–473.

Strumpf, D., Mao, C.A., Yamanaka, Y., Ralston, A., Chawengsaksophak, K., Beck, F., Rossant, J., 2005. Cdx2 is required for correct cell fate specification and differentiation of trophectoderm in the mouse blastocyst. Development 132, 2093–2102.

Suárez-Fariñas, M., Noggle, S., Heke, M., Hemmati-Brivanlou, A., Magnasco, M.O., 2005. Comparing independent microarray studies: the case of human embryonic stem cells. BMC Genomics 6, 99.

Thomson, J.A., Itskovitz-Eldor, J., Shapiro, S.S., Waknitz, M.A., Swiergiel, J.J., Marshall, V.S., Jones, J.M., 1998. Embryonic stem cell lines derived from human blastocysts. Science 282, 1145–1147.

Wichterle, H., Lieberam, I., Porter, J.A., Jessell, T.M., 2002. Directed differentiation of embryonic stem cells into motor neurons. Cell 110, 385–397.

Williams, T.J., Munro, R.K., Shelton, J.N., 1990. Production of interspecies chimeric calves by aggregation of *Bos indicus* and *Bos taurus* demi-embryos. Reprod. Fertil. Dev. 2, 385–394.

Xu, C., Inokuma, M.S., Denham, J., Golds, K., Kundu, P., Gold, J.D., Carpenter, M.K., 2001. Feeder-free growth of undifferentiated human embryonic stem cells. Nat. Biotechnol. 19, 971–974.

Xu, C., Police, S., Rao, N., Carpenter, M.K., 2002a. Characterization and enrichment of cardiomyocytes derived from human embryonic stem cells. Circ. Res. 91, 501–508.

Xu, R.H., Chen, X., Li, D.S., Li, R., Addicks, G.C., Glennon, C., Zwaka, T.P., Thomson, J.A., 2002b. BMP4 initiates human embryonic stem cell differentiation to trophoblast. Nat. Biotechnol. 20, 1261–1264.

Yee, J.K., Miyanohara, A., LaPorte, P., Bouic, K., Burns, J.C., Friedmann, T., 1994. A general method for the generation of high-titer, pantropic retroviral vectors: highly efficient infection of primary hepatocytes. Proc. Natl. Acad. Sci. U. S. A. 91, 9564–9568.

Yokoo, T., Kawamura, T., 2005. Ex vivo regeneration of the murine kidney from human mesenchymal stem cells. Kidney Int. 68, 1967.

Zeilmaker, G.H., 1973. Fusion of rat and mouse morulae and formation of chimaeric blastocysts. Nature 242, 115–116.

ENCLOSURE 2

ALDF'S 2015 PETITION FOR RULEMAKING SUPPLEMENT

April 9, 2015

By U.S. Mail

Secretary Sylvia Mathews Burwell
U.S. Dep't of Health & Human Servs.
200 Independence Avenue, S.W.
Washington, D.C. 20201

RE: FIRST SUPPLEMENT AND AMENDED RULE TO ANIMAL LEGAL DEFENSE FUND'S DECEMBER 2013 CITIZEN PETITION FOR RULEMAKING TO PROTECT HUMANIZED CHIMERAS UNDER THE PUBLIC HEALTH SERVICES ACT

Dear Secretary,

In light of recent scientific progress, the Animal Legal Defense Fund (ALDF) hereby submits its first supplement and amendment to its rulemaking petition to protect humanized chimeras under the Public Health Services Act (PHSA). ALDF submitted its original rulemaking petition in December 2013 asking HHS to promulgate regulations requiring Internal Review Board (IRB) review of research involving human-animal chimeras with a substantial possibility of resulting in humanized high-level cognitive capacity. ALDF also asked HHS to recognize that chimeras with high-level cognitive capacity qualified as individual research subjects in accordance with the PHSA and common rule. 45 C.F.R. §§ 46.101, *et seq.*

Subsequently, HHS has not even acknowledged receiving ALDF's original rulemaking petition. Meantime, rapid scientific progress demonstrates a more urgent need than ever for HHS to not only adopt the rulemaking petition, but to expand the scope to include transgenic human-animals. The important studies demonstrating this need to act are described in the enclosed "First Supplement and Amended Rule to Protect Modified Human-Animal Beings."

Thank you for your time and attention to this matter. Please feel free to contact me should you have any questions about this important issue.

Christopher A. Berry, Esq.
Staff Attorney
ANIMAL LEGAL DEFENSE FUND
cberry@aldf.org
(707) 795-2533 ext. 1041

Animal Legal Defense Fund

170 East Cotati Avenue
Cotati, California 94931

T 707.795.2533
F 707.795.7280

info@aldf.org
aldf.org

BEFORE THE UNITED STATES DEPARTMENT OF HEALTH AND HUMAN SERVICES

CITIZEN PETITION FOR RULEMAKING TO PROTECT HUMANIZED CHIMERAS UNDER THE PUBLIC HEALTH SERVICES ACT

FIRST SUPPLEMENT AND AMENDED RULE TO PROTECT MODIFIED HUMAN-ANIMAL BEINGS

ANIMAL LEGAL DEFENSE FUND,

Citizen petitioner,

170 E. Cotati Ave.

Cotati, CA 94931

Filing with:

SYLVIA MATHEWS BURWELL,

In her official capacity as Secretary of the

United States Department of Health and Human Services,

200 Independence Avenue S.W.

Washington, D.C. 20201

TABLE OF CONTENTS

I. ALDF's Original 2013 Rulemaking Petition To Protect Human-Animal Chimeras As Individuals Under The Public Health Services Act 1

II. Recent Advances Involving Cognitively-Enhanced Chimeric And Transgenic Human-Animal Beings Heightens The Need For HHS To Adopt And Extend ALDF's Rulemaking Petition 2

A. Chimeric Mice With Entirely Humanized Glial Progenitor (Brain) Cells 3

B. Advancements Involving Transgenic Human-Animals 3

1. Transgenic mice with humanized language gene outperformed other mice on memory task. 4

2. Transgenic mice with humanized gene had "marked acceleration of neural progenitor cell cycle and increased brain size" 4

III. Amended Rule To Protect Modified Human-Animal Beings 5

§ 1 Scope. 5

§ 2 Definitions. 5

§ 3 IRB review. 6

§ 4 Protection for modified human-animal beings with high-level cognitive capacity. 7

IV. Discussion Of Amended Rule 7

A. Scope 7

B. Definitions 8

C. IRB Review 9

V. Conclusion 10

I. ALDF'S ORIGINAL 2013 RULEMAKING PETITION TO PROTECT HUMAN-ANIMAL CHIMERAS AS INDIVIDUALS UNDER THE PUBLIC HEALTH SERVICES ACT

ALDF submitted a rulemaking petition on December 3, 2013, asking the Department of Health and Human Services (HHS) to regulate studies involving human-animal chimeras – i.e. animals with some human cells or tissue – under the common rule of the Public Health Services Act (PHSA).[1] Specifically, ALDF asked HHS to require Institutional Review Board (IRB) monitoring of all studies with a "substantial possibility" of generating human-animal chimeras with human-like intelligence.[2] Moreover, ALDF's proposed rule recognized that the humanized chimeras found to *actually acquire* human-like intelligence would qualify as "individuals" with all the protections afforded by the common rule under the PHSA.[3]

In support of this rulemaking petition, ALDF appended extensive evidence that the creation of cognitively-enhanced human-animal chimeras is already occurring, and that the creation of such a chimera with human-like intelligence is already possible.[4] Leading bioethicists have expressed well-reasoned concern that such beings would deserve – but might not receive – similar protection afforded to human research subjects.[5] Current HHS regulation of this type of research is under-inclusive and insufficient to prevent such a morally disastrous situation.[6]

After submitting its rulemaking petition in 2013, ALDF has not even received an acknowledgement from HHS that the agency received the petition. Meanwhile, scientific progress continues to advance and heighten the need for HHS to act swiftly to protect human-

---

[1] *See [Original] Citizen Petition for Rulemaking to Protect Humanized Chimeras Under the Public Health Services Act,* ANIMAL LEGAL DEFENSE FUND BEFORE THE UNITED STATES DEPARTMENT OF HEALTH AND HUMAN SERVICES (Dec. 3, 2013), citing 42 U.S.C. § 289(a) (PHSA) and 45 C.F.R. §§ 46.101, *et seq.* (common rule).

[2] *[Original] Citizen Petition for Rulemaking*, *supra* n.1 at pp.21-23.

[3] *Id.*

[4] *Id.* at pp.5-9.

[5] *Id.* at pp.9-15.

[6] Id. at pp.15-21.

animal chimeras – *and also transgenic human-animals* – due to new studies demonstrating enhanced cognitive capacity for both types of beings.

II. RECENT ADVANCES INVOLVING COGNITIVELY-ENHANCED CHIMERIC AND TRANSGENIC HUMAN-ANIMAL BEINGS HEIGHTENS THE NEED FOR HHS TO ADOPT AND EXTEND ALDF'S RULEMAKING PETITION

As HHS failed to act on ALDF's rulemaking petition to protect human-animal chimeras, three important studies have been published that highlight the need for comprehensive regulation of research involving modified human-animal beings. One of these studies involved the creation of chimeric mice with humanized glial progenitor cells that completely took over the mice brains.[7] Two other studies involved transgenic mice with human genetic material thought to affect cognition that did in fact relate to cognition and brain size – one study involving mice with a humanized Foxp2 gene associated with language[8] and another study involving mice with a humanized HARE5 gene associated with the neocortex and brain size.[9] *In toto*, these studies demonstrate that HHS should not only adopt ALDF's original rulemaking petition to protect cognitively-enhanced human-animal chimeras, but should extend the scope to include similarly enhanced transgenic human-animals. (Due to a lack of generic terminology that encompasses both chimeras and transgenics, human-animal chimeras and transgenic human-animals will be collectively referred to as "modified human-animal beings" throughout this petition.)

---

[7] Windrem et al., *A Competitive Advantage by Neonatally Engrafted Human Glial Progenitors Yields Mice Whose Brains Are Chimeric for Human Glia*, THE JOURNAL OF NEUROSCIENCE, vol. 34 no. 48 pp.16153-16161 (Nov. 26, 2014) ("Attachment A").

[8] Schreiweis et al., *Humanized Foxp2 accelerates learning by enhancing transitions from declarative to procedural performance*, PROCEEDINGS OF THE NATIONAL ACADEMIES OF SCIENCE, vol. 111 no. 39 pp.14253-14258 (Sept. 30, 2014) ("Attachment B").

[9] Boyd et al., *Human-Chimpanzee Differences in a FZD8 Enhancer Alter Cell-Cycle Dynamics in the Developing Neocortex*, CURRENT BIOLOGY 25, pp.772-770 (March 16, 2014) ("Attachment C").

A. Chimeric Mice With Entirely Humanized Glial Progenitor (Brain) Cells

One widely-reported study published in November 2014 involved mice with a humanized type of brain cell that entirely took over the mice brains.[10] In that study, researchers transplanted human glial progenitor cells (hGPCs) in newborn mouse pup brains.[11] Within one year, the hGPCs – cells that support neurons in the brain and contribute to cognitive function – entirely or almost entirely displaced the mouse glial cells in the brain, resulting in mice with a totally humanized glial progenitor population.[12] The researchers in that article noted that similar studies involving less dramatic changes to a mouse's brain have caused mice to perform significantly better on cognition tasks than standard mice.[13]

A news story in a popular science magazine contained an interview with one of the researchers indicating that *mere self-restraint* prevented the scientists from performing the same experiment with monkeys instead of mice.[14]

B. Advancements Involving Transgenic Human-Animals

Transgenic human-animals are generally similar to human-animal chimeras in the sense that both involve manipulation of an individual's basic biology to make an animal more human. But whereas human-animal chimeras receive human cells or tissues, *transgenic* human-animals contain human genetic material resulting from insertion of human genetic material, or genetic alteration of an animal's genetic sequence designed to resemble human genes.[15]

---

[10] Windrem et al., *supra* n.7 (Attachment A) at pp.16153-16161.

[11] *Id.*

[12] *Id.* at p.16159.

[13] *Id.* at p.16153.

[14] Coghlan, *The smart mouse with the half-human brain*, NEWSCIENTIST (Dec. 1, 2014), available at http://www.newscientist.com/article/dn26639-the-smart-mouse-with-the-halfhuman-brain.html#.VSbV6PnnRVM ("Attachment D").

[15] *Animals containing human material*, THE ACADEMY OF MEDICAL SCIENCES, p.18 (§ 2.2.1) (July 2011) ("Attachment E").

ALDF's original rulemaking petition did not include transgenic human-animals because there was insufficient evidence at the time of *actual* cognitive enhancement of transgenic human-animals. However, two recent studies prove that transgenic human-animals are in fact becoming cognitively enhanced in current research. ALDF's original proposed rule should be amended and extended to transgenic human-animals accordingly.

1. Transgenic mice with humanized language gene outperformed other mice on memory task.

The title of a widely publicized study in 2014 declared that "Humanized Foxp2 accelerated learning [in mice] by enhancing transitions from declarative to procedural performance."[16] In that study, researchers substituted the endogenous version of the Foxp2 gene in mice with the *humanized* Foxp2 gene which is understood to be an important gene "firmly linked to [human] speech and language development."[17] Astonishingly, researchers reported "marked effects of this humanization of Foxp2 on learning and striatal neuroplasticity." Specifically, the researchers found that the humanized mice learned stimulus-response associations significantly faster than their standard littermates in certain situations.[18] This cognitive enhancement was, of course, entirely attributable to the humanized genetic material.

2. Transgenic mice with humanized gene had "marked acceleration of neural progenitor cell cycle and increased brain size"

In another study, researchers created transgenic mice were given human or chimpanzee HARE5 enhancer gene to determine the effect on the neocortex.[19] The authors found that even compared to "chipmanzeed" mice with the HARE5 gene, the humanized mice developed brains that were *twelve percent larger*, and had noticeably larger neocortex size that was detectable by

[16] Schreiweis et al., *supra* n.8 (Attachment B) at pp.14253-14258.

[17] *Id.* at p.14253.

[18] *Id.* at pp.14253-14258.

[19] Boyd et al., *supra* n.9 (Attachment C) at pp.772-770.

the naked eye.[20] Although the mice in this study were killed before maturing, one news report noted that some of the researchers planned to let the mice mature in future studies to test if the bigger brains made them smarter.[21]

III. AMENDED RULE TO PROTECT MODIFIED HUMAN-ANIMAL BEINGS

As explained in ALDF's original petition, HHS has ample authority to promulgate comprehensive regulations that would protect modified human-animal beings pursuant to the Administrative Procedures Act,[22] the HHS implementing regulations,[23] and the PHSA duty to protect human subjects.[24] Accordingly, ALDF submits the following amended rule to be codified at 42 C.F.R. Pt. 45 Sub. E that would replace the original proposed rule from 2013 (changes are underlined):

§ 1 Scope.

This subpart applies to all research involving modified human-animal beings that is conducted or otherwise supported by the federal government.

§ 2 Definitions.

(a) "High-level cognitive capacity" means mental ability that is substantially similar to a normal adult human considering the individual's:

(i) linguistic ability;

(ii) degree of self-awareness;

---

[20] *Id.*

[21] Pennisi, *Human DNA enlarges mouse brains*, SCIENCE (Feb. 19, 2015), available at http://news.sciencemag.org/biology/2015/02/human-dna-enlarges-mouse-brains ("Attachment F").

[22] 5 U.S.C. § 553(e).

[23] 45 C.F.R. §§ 160.101, *et seq.*

[24] 42 U.S.C. §§ 201, *et seq.*; 42 U.S.C. § 289(a).

(iii) sense of past and future self;

(iv) moral agency; and

(v) rational agency.

(b) "Human-animal chimera" means a nonhuman animal implanted with human tissue such as stem cells, or cells derived therefrom, during any stage of life.

(c) "Modified human-animal being" means any human-animal chimera or transgenic human-animal.

(d) "Transgenic human-animal" means a nonhuman animal containing any amount of human genetic material as a result of:

(i) transgenic insertion of human genetic material; or

(ii) gene-targeting manipulation designed to resemble human genetic material.[25]

§ 3 IRB review.

(a) An IRB shall conduct a preliminary review of all research proposals involving modified human-animal beings to determine if there is a substantial risk of the research subject obtaining high-level cognitive capacity.

(b) In determining whether there is a substantial risk of a human-animal chimera obtaining high-level cognitive capacity, the IRB shall consider:

(i) proportion of engrafted human cells;

(ii) stage of neural development;

(iii) species of animal;

(iv) brain size;

(v) degree of integration; and

(vi) brain pathology.[26]

---

[25] *Animals containing human material*, *supra* n.15 at p.18 (§ 2.2.1).

[26] Greene et al., *ETHICS: Moral Issues of Human-Non-Human Primate Neural Grafting*, SCIENCE, vol. 309, no. 5733, pp.385-386 (July 15, 2005) (Appendix to *[Original] Citizen Petition for Rulemaking*).

(c) In determining whether there is a substantial risk of a transgenic human-animal obtaining high-level cognitive capacity, the IRB shall consider:

(i) amount of human genetic material;

(ii) known or suspected attributes of the human genetic material; and

(iii) species of animal.

§ 4 Protection for modified human-animal beings with high-level cognitive capacity.

(a) Any individual who has high-level cognitive capacity under Section 2(a) and is a modified human-animal being as defined by Section 2(c) shall have the same protection as other human research subjects under this part.

(b) If at any point in the review process described in Section 3 the IRB finds there is a substantial risk that the modified human-animal being subject will obtain or has obtained high-level cognitive capacity status, the IRB shall require the researchers to reduce the risks to a non-substantial level. If the risks cannot be reduced to a non-substantial level then the IRB shall ensure that the individual is protected as a research subject under this part.

(c) If the IRB determines there is no substantial risk of a modified human-animal being obtaining high-level cognitive capacity, it shall nonetheless continue to monitor the research and ensure the individual's protection as a human research subject upon the existence of plausible evidence that high-level cognitive capacity has been obtained.

IV. DISCUSSION OF AMENDED RULE

A. Scope

In light of the rapid pace of scientific progress previously discussed, ALDF is amending the proposed rule it originally submitted with the rulemaking petition in 2013. The key change in this amended proposed rule compared to the original rule is an expansion of the scope to include *transgenic* human-animals in addition to human-animal chimeras. The underlying

framework nonetheless remains the same: IRBs must monitor and review all experiments that might generate a humanized animal with high-level cognitive capacity, and humanized animals who obtain high-level cognitive capacity must be recognized as "individual" research subjects under the PHSA and common rule. This expansion of the proposed rule's scope required additions to the section on "definitions" and section on "IRB review" of studies involving transgenics, discussed below.

B. Definitions

This amended rule encountered two semantic challenges. First, there is no commonly used word that generically refers to both transgenic and chimeric animals. A review of literature found one source that invented the term "animals containing human material" to refer to both humanized transgenic and chimeric animals,[27] another developed the term "CHIMBRID",[28] and an entry on Wikipedia used the term "parahuman" without further citation.[29] The amended rule coins its own phrase, "modified human-animal beings", to collectively refer to modified animals containing either human cells/tissues (chimeric) or human genetic information (transgenic).

A second semantic challenge was finding a term that encompasses all types of genetic manipulation that humanize an animal. This amended rule uses the term "transgenic", but some understandings of that term limit it to situations where human DNA is *actually spliced* into an animal's genetic code.[30] This would exclude other instances where, for example, researchers directly manipulate an animal's genes to resemble human genes without actually inserting human genetic material.[31] Thus, definition of "transgenic animal-being" at Section 2(d) is designed to

---

[27] *Animals containing human material*, *supra* n.15 (Attachment E) at p.5.

[28] Taupitz and Wescheka (eds.), *CHIMBRIDS – Chimeras and Hybrids in Comparative European and International Research*, © SPRINGER-VERLAG BERLIN HEIDELBERG (2009).

[29] *Parahuman*, Wikipedia, available at http://en.wikipedia.org/wiki/Parahuman (last accessed April 9, 2015).

[30] *Animals containing human material*, *supra* n.15 (Attachment E) at p.18 (§ 2.2.1).

[31] *Id.*

broadly and explicitly encompass all situations where an animal's genetic material is humanized by any method.

C. IRB Review

Enhanced cognition of transgenic human-animals has not received as much consideration from bioethicists as enhanced cognition of human-animal chimeras. As a result, little guidance could be found enumerating the risk factors to consider when assessing the risk that an experiment creating a transgenic human-animal might cause the transgenic to acquire high-level cognitive capacity. (By contrast, the Greene study discussed in the original rulemaking petition enumerated several risk factors in the context of neural grafting of human-animal (primate) chimeras.[32])

Due to the lack of published guidance on risk factors for cognitive enhancement of transgenics, Section 3(c) of the proposed rule borrows generally from the factors listed in the Greene study.[33] Specifically, Section 3(c) requires the IRB to consider (i) the amount of human genetic material, (ii) known or suspected effects of the human genetic material, and (iii) the host species of animal. Alternatively, IRBs could make determinations without guidelines on a case-by-case basis provided that there is sufficient expertise on the IRB.[34] More specific guidelines could be created in the future based on early experiences.

In any event, the critical essence of Section 3 is that there is *some IRB oversight* of research that could potentially enhance human-animal cognition. As explained at length in the

---

[32] Greene et al., *supra* n.26 (Appendix to *[Original] Citizen Petition for Rulemaking*) at pp.385-386.

[33] *Id.*

[34] See *Animals containing human material*, *supra* n.15 (Attachment E) at p.9 fn.1 (recommending that in high-risk studies involving the mixing human and non-human primate embryonic or pluripotent stem cells, that the reviewing body carefully study the proposed research on a case-by-case basis).

original rulemaking petition, such oversight is necessary to both avoid an unacceptable risk of creating a modified human-animal being with high-level cognitive capacity, and to ensure that being who actually obtains high-level cognitive capacity is recognized as an individual entitled to all the rights of a human research subject under the PHSA and common rule.

V. CONCLUSION

Petitioner ALDF submits that the Secretary should initiate rulemaking to clarify that protection for human subjects under the PHSA and accompanying regulations applies, at a minimum, to both human-animal chimeras and transgenic human-animals with cognitive capacity substantially similar to a normal adult human. Rapidly progressing science cited in this supplement demonstrates that the risk of creating a chimeric or transgenic being with humanized intelligence is significant, and HHS must make clear that those individuals are fully protected in accordance with the ethical principles, guidelines, and laws governing research on human subjects.

Dated: April 9, 2015

Respectfully submitted,

Christopher A. Berry, Esq.

ANIMAL LEGAL DEFENSE FUND
cberry@aldf.org
(707) 795-2533 ext. 1041
170 E. Cotati Ave.
Cotati, CA 94931

ATTACHMENT A

Windrem et al.,

A Competitive Advantage by Neonatally Engrafted Human Glial Progenitors Yields Mice Whose Brains Are Chimeric for Human Glia,

THE JOURNAL OF NEUROSCIENCE, vol. 34 no. 48 pp.16153-16161
(Nov. 26, 2014)

Development/Plasticity/Repair

A Competitive Advantage by Neonatally Engrafted Human Glial Progenitors Yields Mice Whose Brains Are Chimeric for Human Glia

Martha S. Windrem,[1] Steven J. Schanz,[1] Carolyn Morrow,[1] Jared Munir,[1] Devin Chandler-Militello,[1] Su Wang,[1] and Steven A. Goldman[1,2]

[1]Center for Translational Neuromedicine, University of Rochester Medical Center, Rochester, New York 14642, and [2]Center for Basic and Translational Neuroscience, University of Copenhagen Faculty of Medicine, 2200 Copenhagen N, Denmark

Neonatally transplanted human glial progenitor cells (hGPCs) densely engraft and myelinate the hypomyelinated *shiverer* mouse. We found that, in hGPC-xenografted mice, the human donor cells continue to expand throughout the forebrain, systematically replacing the host murine glia. The differentiation of the donor cells is influenced by the host environment, such that more donor cells differentiated as oligodendrocytes in the hypomyelinated *shiverer* brain than in myelin wild-types, in which hGPCs were more likely to remain as progenitors. Yet in each recipient, both the number and relative proportion of mouse GPCs fell as a function of time, concomitant with the mitotic expansion and spread of donor hGPCs. By a year after neonatal xenograft, the forebrain GPC populations of implanted mice were largely, and often entirely, of human origin. Thus, neonatally implanted hGPCs outcompeted and ultimately replaced the host population of mouse GPCs, ultimately generating mice with a humanized glial progenitor population. These human glial chimeric mice should permit us to define the specific contributions of glia to a broad variety of neurological disorders, using human cells *in vivo*.

Key words: **cell transplant; chimera; demyelinating disease; glial progenitor; neural stem cell; oligodendrocytic progenitor**

Introduction

In an effort to develop human cellular vectors for therapeutic remyelination, we have developed efficient methods by which to identify and isolate human glial progenitor cells (hGPCs), in quantities and purities appropriate for transplantation (Goldman et al., 2012). Using immune-deficient mice as hosts, we established a neonatal multisite delivery procedure that results in widespread hGPC engraftment throughout the brain and spinal cord, with infiltration of the forebrain, brainstem, and cerebellum, and ultimately the spinal cord and roots (Windrem et al., 2008). When delivered to myelin-deficient *shiverer* mice ($MBP^{shi/shi}$), these donor hGPCs, whether isolated from tissue (Windrem et al., 2004, 2008) or generated from human embryonic stem cells or induced pluripotential stem cells (Wang et al., 2013), exhibited efficient oligodendrocyte differentiation and myelination, as well as fibrous astrocyte production, permitting the clinical rescue of these otherwise lethally hypomyelinated mice.

Yet in contrast to their bilineal oligodendrocytic and astrocytic fate competence in a hypomyelinated host, these xenografted hGPCs either remained as progenitors or differentiated into astrocytes in wild-type mice, revealing little oligodendrocytic differentiation, and thus suggesting the context dependence of their fate choice (Goldman et al., 2008). As a result, when hGPCs were xenografted into immunodeficient but otherwise wild-type neonatal mice, the recipient brains were effectively colonized by hGPCs and their derived astroglia (Han et al., 2013). Indeed, the resultant occupation of these brains by human glia was so robust that it prompted us to investigate the functional and behavioral consequences of this interspecific chimerization. We found that the glial chimeric mice exhibited both increased synaptic plasticity and improved cognitive performance, manifested by both enhanced long-term potentiation and improved performance in a variety of learning tasks (Han et al., 2013). In the context of that study, we were surprised to note that the forebrains of these animals were often composed primarily of human glia and their progenitors, with overt diminution in the relative proportion of resident mouse glial cells.

On the basis of these observations, we asked here whether neonatal human glial chimerization can yield the large-scale replacement of resident murine glial progenitor cells by hGPCs, whether this process can result in the effective humanization of the adult mouse with respect to its glial phenotypes, and if so, by what kinetics this process proceeds, and with what context-

Received April 13, 2014; revised Sept. 24, 2014; accepted Oct. 18, 2014.

Author contributions: M.S.W., S.W., and S.A.G. designed research; M.S.W., S.J.S., C.M., J.M., D.C.-M., and S.W. performed research; M.S.W., S.J.S., C.M., J.M., and S.A.G. analyzed data; M.S.W. and S.A.G. wrote the paper.

This work was supported by the Mathers Charitable Foundation, National Institute of Neurological Disorders and Stroke R01NS75345, the National Institute of Mental Health Grants R01MH099578-01 and R01MH104701, the National Multiple Sclerosis Society, the Adelson Medical Research Foundation, the New York Stem Cell Research Program, and the Novo Nordisk Fondation.

S.A.G. and M.S.W. have a patent on chimeric mouse models (U.S. patent no. US7524491B2); the patent is owned by the University of Rochester, and the authors receive no income from it. The remaining authors declare no competing financial interests.

Correspondence should be addressed to either Dr. Martha Windrem or Dr. Steven Goldman, Center for Translational Neuromedicine, University of Rochester Medical Center, 601 Elmwood Avenue, Rochester, NY 14642. E-mail: martha_windrem@urmc.rochester.edu or steven_goldman@urmc.rochester.edu.

DOI:10.1523/JNEUROSCI.1510-14.2014

 0270-6474/14/3416153-09$15.00/0

Table 1. Antibodies and dilutions used for histological analysis in this study

Antigen	Name	Dilution	Catalog	Company
BrdU	Rat anti-BrdU	1:200	MCA2060	Serotec
CNPase	Mouse anti-CNPase	1:1000	SMI-91R	Covance
GFAP	Rabbit anti-GFAP	1:800	ab5804	Millipore
hGFAP	Mouse antihuman (specific) GFAP	1:500	SMI-21R	Covance
hN	Mouse antihuman nuclei, clone 235-1	1:800	MAB1281	Millipore
hNG2	Mouse anti-NG2, clone 9.2.27	1:200	MAB2029	Millipore
Ki67	Rabbit anti-Ki67, clone SP6	1:200	RM-9106-S1	LabVision
MBP	Rat anti-MBP	1:25	ab7349	Abcam
mNG2	Rabbit anti-NG2	1:200	AB5320	Millipore
Olig2	Rabbit anti-Olig2	1:500	RA25017	Neuromics
PDGFRα	Rabbit anti- PDGFRα, clone D13C6	1:300	5241S	Cell Signaling
PDGFRα	Rabbit anti- PDGFRα, clone D1E1E	1:300	3174S	Cell Signaling
Transferrin	Transferrin antibody	1:800	ab9538	Abcam
EGFP	EGFP, 3E6	1:400	A11120	Invitrogen
Secondary antibodies	AlexaFluor-568 goat anti-mouse IgG (H + L)	1:400	A-11031	Invitrogen
	AlexaFluor-568 goat anti-mouse IgG1	1:400	A-21124	Invitrogen
	AlexaFluor-488 goat anti-mouse IgG (H + L)	1:400	A-11029	Invitrogen
	AlexaFluor-488 goat anti-mouse IgG1	1:400	A-21121	Invitrogen
	DyLight 649 goat anti-mouse IgG1	1:400	115-495-205	The Jackson Laboratory
	Biotin-SP goat anti-mouse IgG (H + L)	1:250	115-065-166	The Jackson Laboratory
	AlexaFluor-568 goat anti-rabbit IgG (H + L)	1:400	A-11036	Invitrogen
	AlexaFluor-488 goat anti-rabbit IgG (H + L)	1:400	A-11034	Invitrogen
	Cy5 goat anti-rat	1:400	A10525	Invitrogen
	AlexaFluor-568 goat anti-rat IgG (H + L)	1:400	A-11077	Invitrogen
	AlexaFluor-488 goat anti-rat IgG (H + L)	1:400	A-11006	Invitrogen
	Streptavidin, AlexaFluor-568	1:1000	S-11226	Invitrogen
	Avidin, AlexaFluor-488	1:500	A-21370	Invitrogen

dependent determination of cell lineage and fate. In doing so, we have found that hGPCs exhibit a competitive dominance when xenografted into the mouse brain that results in the effective, and often complete, replacement of mouse glial progenitors by their human counterparts, with subsequent astrocytic differentiation, thereby yielding murine brains in which human glial cells predominate.

Materials and Methods

Human and mouse cell dissociation. For xenograft of human fetal GPCs, cells were extracted from second-trimester human fetuses (18–22 weeks gestation age) obtained at abortion. The forebrain ventricular/subventricular zones were dissected from the brain, the samples chilled on ice, minced and dissociated using papain/DNase, as described previously (Roy et al., 1999, 2000), always within 3 h of extraction. The dissociates were maintained overnight in minimal media of DMEM/F12/N1 with 10 ng/ml bFGF. Samples were deidentified and obtained with the approval of the University of Rochester Research Subjects Review Board.

As controls, allografted mouse cells were obtained from *Tg(CAG-EGFP)B5Nagy/J* mice (The Jackson Laboratory). P1 pups were cryoanesthetized, their forebrains removed, and dissociated as above; like their human counterparts, the mouse cells were also maintained overnight in DMEM/F12/N1 with 10 ng/ml bFGF before sorting.

Sorting. Glial progenitor cells were isolated the day after tissue dissociation, using immunomagnetic sorting (MACS, Miltenyi Biotec), as described previously (Windrem et al., 2008). The human cells were incubated with mouse anti-PSA-NCAM (clone 2–2B, Millipore; clone 5A5, DSHB), then washed and labeled with microbead-tagged rat anti-mouse IgM (Miltenyi), and the PSA-NCAM$^+$ cells removed by MACS depletion. The PSA-NCAM-depleted remainder was then incubated with mAb A2B5 supernatant (clone 105; ATCC) for 20 min, then washed and tagged with microbead-tagged rat anti-mouse IgM (Miltenyi), and the A2B5$^+$ cells separated by MACS selection. The bound cells were then eluted, yielding a highly enriched population of PSA-NCAM$^-$/A2B5$^+$ cells. After sorting, the cells were either maintained *in vitro* up to 2 weeks in DMEM/F12/N1 with 10 ng/ml bFGF and 20 ng/ml PDGF-AA, or frozen and stored in liquid N_2 at 2×10^6 cells/ml in 7.5% DMSO/50% media (DMEM/F12/N1)/42.5% ProFreeze-CDM (Lonza).

Mouse cells were incubated with mAb A2B5 supernatant for 20 min, then washed and labeled with microbead-tagged anti-mouse IgM, and separated by MACS. The bound cells were then eluted, yielding a highly enriched population of A2B5$^+$ cells. After sorting, the cells were maintained for 1–2 d in DMEM/F12/N1 with 10 ng/ml bFGF and 20 ng/ml PDGF-AA.

Transplantation. Myelin-deficient, immunodeficient *shiverer* (MBP$^{shi/shi}$) × *rag2*$^{-/-}$ mice were generated as previously described (Windrem et al., 2008). *Shiverer* × *rag2*$^{-/-}$ and myelin wild-type *rag2*$^{-/-}$ newborn pups of either sex were both transplanted within a day of birth, using a total of 300,000 cells dispersed over five injection sites, also as described previously (Windrem et al., 2008). The myelin wild-type mice were killed for histology at 3, 4.5, 6, 8, or 12 months of age ($n = 3$ per time-point; 15 total), whereas engrafted shiverer brains were analyzed at 3, 4.5, 6, 8, or 12 months ($n = 3$ per time-point, except single mice at 6 and 8 months; 11 total). As allograft controls, *EGFP*$^+$ mouse GPCs were prepared and sorted as above and transplanted into *rag2*$^{-/-}$ ($n = 22$) or *shiverer* × *rag2*$^{-/-}$ ($n = 8$) mice on P1, at 300,000 cells/mouse in 5 sites, using the same procedure as that for hGPC xenografts. The A2B5-sorted mouse GPCs (mGPCs) were cultured for a week in DMEM/F12/N1 with 5% FBS and then immunostained for the neuron-specific protein MAP2AB, so as to assess the incidence of neurons in this pool. Among 14 cultures derived from two separate sorts, an average of 2.1 ± 0.5% expressed MAP2AB, consistent with the minor incidence of neuronal contaminants in these GPC sorts, as we had previously reported for A2B5$^+$/PSA-NCAM-based isolation of hGPCs (Windrem et al., 2004).

Immunolabeling. Mice were given barbiturate anesthesia, perfusion fixed with HBSS followed by 4% PFA. Brains were removed and post-fixed for 2 h in cold PFA. Brains were cryopreserved in 6% and 30% sucrose (w/v), embedded sagittally in OCT, and cryosectioned at 20 μm. Cells were labeled with antibodies as listed in Table 1.

BrdU tagging. To estimate the mitotic indices of each donor-derived phenotype at the time of death, the thymidine analog BrdU (150 mg/kg, i.p.) was given once daily for 5 consecutive days in the terminal week, and

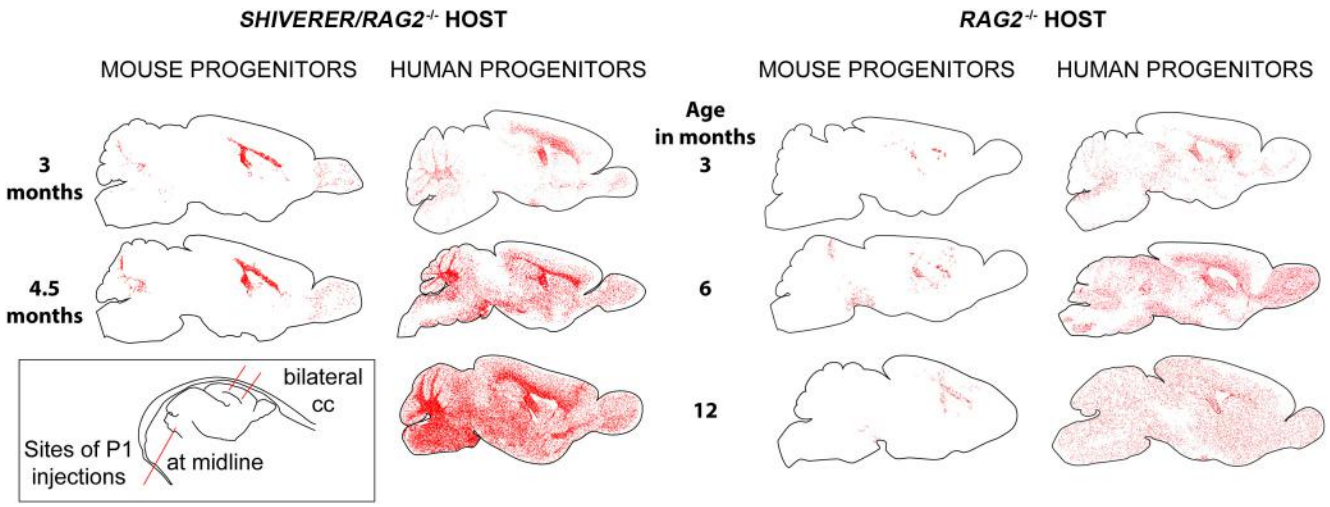


Figure 1. hGPCs colonize both wild-type and myelin-deficient immunodeficient host brain. hGPCs neonatally transplanted into either congenitally hypomyelinated *shiverer* × *rag2*$^{-/-}$ mice (left columns) or normally myelinated *rag2*$^{-/-}$ mice (right columns) disperse and expand broadly throughout the brain as a function of age, and do so more aggressively than allografted mouse GPCs. hGPCs reach higher density in white matter than gray matter of the hypomyelinated *shiverer*, in contrast to their relatively uniform distribution in normally myelinated brain (right). Same-species neonatal allografts of EGFP-expressing mouse GPCs migrate and expand substantially less. Red dots indicate individual donor GPCs, as labeled by human nuclear antigen (human GPCs) or anti-GFP (mouse GPCs). Cells were mapped in 20 μm sections using Stereo Investigator. Inset, Bottom left, Sites of neonatal injection, given anteriorly and posteriorly into the corpus callosum bilaterally, and as a single injection into the cerebellar peduncle.

the mice killed 2 d later so as to allow sufficient time for phenotype-specific antigenic expression. In BrdU-labeled samples, PDGFRα was used instead of NG2 to identify GPCs because NG2 proved incompatible with our BrdU labeling protocol. In control sections, we observed complete overlap of the NG2 and PDGFRα-immunoreactive populations, except for a minor pool of morphologically apparent NG2$^+$ pericytes that did not express PDGFRα.

Transplant mapping, cell counts, and phenotypic analysis. Montages for whole-section mapping of donor cells were generated on a Leica DM6000B equipped with a Leica DFC360FX high-speed camera system, using the HCX PL FLUOTAR 20× objective.

Quantification of callosal engraftment and donor cell phenotype in mice younger than 1 year were based on counts of the corpus callosum in three equally spaced sections of each mouse. In the 1-year-old mice whose brains had substantial donor cell engraftment, quantification of donor cell phenotypes in the corpus callosum was performed stereologically, by optical fractionator (West, 1999). We used a stereology system (StereoInvestigator; MicroBrightField), consisting of an Olympus BX-51 microscope equipped with a Ludl motorized stage, Heidenhain *z*-axis encoder, and Optronics QuantiFire video camera. Within each corpus callosum, from a random starting point, six sections equidistantly spaced 576 μm apart were selected for analysis. After outlining the boundaries of the corpus callosum and establishing upper and lower exclusion zones of 10% of section thickness, a set of counting frames was placed by the software in a systematic random fashion to cover the corpus callosum of each section at ~40 sites. At each sampling site, the system acquired photographs at 1 μm intervals along the *z*-axis through the sample rectangular prism (xyz = 80 μm × 80 μm × 16 μm). Photographs were taken at 400× or higher magnification. Cells were counted in the optical section in which they first came into focus.

Results

Neonatally engrafted hGPCs progressively expand in the murine forebrain

Using both homozygous *shiverer* (MBP$^{shi/shi}$) and myelin wild-type mice, each crossed to *rag2*$^{-/-}$ immunodeficients, we evaluated the absolute numbers, relative proportions, and geographic distributions of human donor cells in neonatally engrafted recipients. In both cases, to generate mice chimeric for hGPCs, we transplanted newborn mouse pups with hGPCs isolated from second-trimester fetal human brain tissue, using immunomagnetic isolation of the A2B5$^+$/PSA-NCAM$^-$ phenotype. The cells were then delivered to the test mice using a five site intracerebral injection protocol that targeted the corpus callosum and cerebellar peduncle, as we have previously described (Windrem et al., 2008). As graft hosts, we used either newborn *rag2*$^{-/-}$ immunodeficient myelin wild-type pups or hypomyelinated homozygous *shiverer* × *rag2*$^{-/-}$ pups. Each mouse was transplanted with 300,000 GPCs delivered at five forebrain sites (Windrem et al., 2008), either with xenografted hGPCs, or as allograft controls, with EGFP$^+$ mGPCs, isolated via A2B5-based sorting from the cortices of P1 EGFP knock-in transgenic mice. The hGPC-engrafted myelin wild-type mice were assessed for histology at 3, 4.5, 6, 8, or 12 months of age, whereas engrafted shiverer brains were analyzed at 3, 4.5, 6, 8, or 12 months (generally n = 3 mice/time-point/genotype).

In the first 3 months following transplantation, hGPCs migrated widely to progressively engraft the forebrain white matter tracts (Fig. 1). During this period, the distribution of hGPCs in hypomyelinated and normally myelinated mouse brain were analogous. By 4.5 months of age, the dispersal pattern of hGPCs in wild-type mice was noted to differ from that in hypomyelinated *shiverers*, in that whereas hGPCs infiltrated in a relatively uniform fashion in both the gray as well as the white matter in myelin wild-types, hGPCs transplanted into *shiverer* mice preferentially expanded in the callosal and capsular white matter, in which they gave rise to new oligodendrocytes as well as additional GPCs and astrocytes (Fig. 1). Nonetheless, in both surviving *shiverers* and myelin wild-types, infiltration of the cortical and subcortical gray by migrating hGPCs ensued such that, by 1 year of age, donor hGPCs were distributed in a relatively uniform manner throughout both the white and gray matter.

In contrast to the aggressive expansion of xenografted hGPCs, EGFP$^+$ mouse GPCs allografted into myelin wild-type *rag1*$^{-/-}$ mice dispersed but did not expand substantially over time, nor did they migrate substantially beyond white matter tracts (Figs. 1 and 2*A*). Nonetheless, those EGFP-identified mGPCs allografted into *shiverer* brains did indeed expand as NG2$^+$ progenitors within the hypomyelinated white matter (Fig. 2*C*,*D*,*G*), matur-

ing therein into transferrin- and MBP-expressing oligodendroglia (Fig. 2*E*,*F*,*H*) and ultimately forming mature myelin (Fig. 2*B*), just as did hGPCs delivered into *shiverer*. However, allografted mGPCs manifested little dispersal beyond the major white matter tracts, compared with the widespread dispersal of hGPCs in shiverer as well as myelin wild-type hosts (Figs. 1 and 2*A*). Thus, the hypomyelinated shiverer brain could be myelinated by allografted murine as well as by xenografted hGPCs, but only hGPCs manifested preferential expansion and dominant colonization of the murine subcortical and neocortical gray matter.

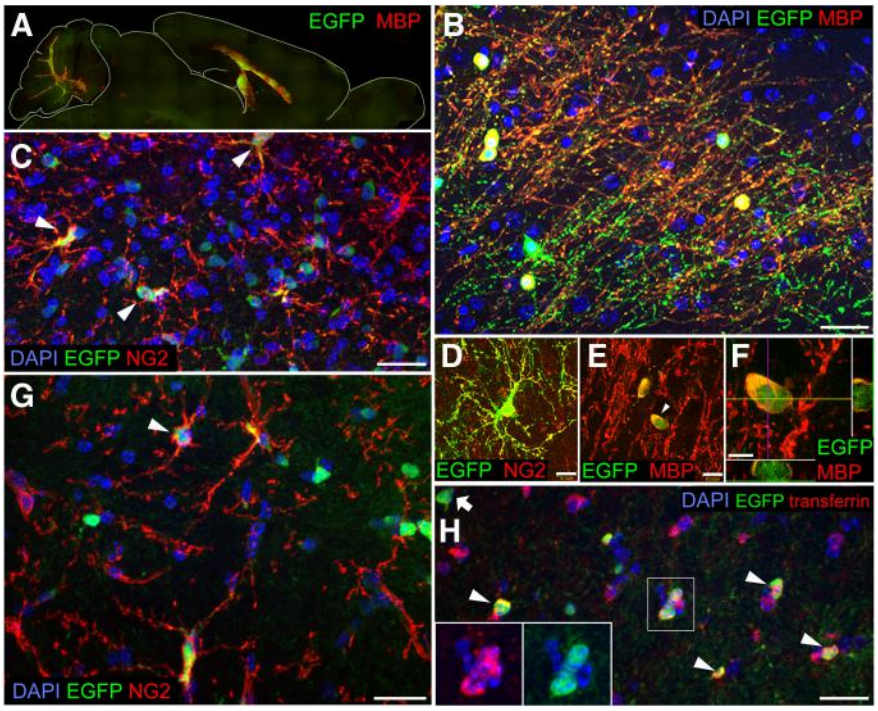


Figure 2. Allografted mouse GPCs engraft host brain without selective expansion. ***A***, ***B***, Mouse EGFP$^+$ GPCs densely engraft and myelinate the white matter of the *shiverer* corpus callosum and cerebellum by 4.5 months: green represents EGFP; red represents MBP. ***B***, Higher-magnification view of ***A***. ***C***, EGFP-defined mGPCs persistent in the *shiverer* host corpus callosum, 4.5 months. Arrowheads indicate EGFP$^+$/NG2$^+$ donor mGPCs. ***D***, Donor EGFP NG2$^+$ cells: green represents EGFP; red represents NG2. ***E***, Donor-derived MBP$^+$ oligodendrocytes in the cortex: green represents EGFP; red represents MBP. ***F***, Confocal *z*-stack with orthogonal views of indicated donor-derived oligodendrocyte shown in ***E***. ***G***, At 6 months after neonatal delivery, mouse EGFP$^+$ cells allografted into myelin wild-type corpus callosum have either integrated as NG2-defined progenitors (***G***) or (***H***) have differentiated as either astrocytes or transferrin-expressing oligodendrocytes (arrows). Scale bars: ***B***, ***C***, ***G***, ***H***, 20 μm; ***D–E***, 10 μm; ***F***, 5 μm.

hGPCs actively excluded resident murine glial progenitors

To define the dynamics of hGPC dispersal in the mouse brain, we used species-specific antibodies against the glial progenitor proteoglycan NG2 to map the respective locations of human donor and murine host NG2$^+$ cells, as a function of time after neonatal transplant. Analyzing 300-μm-wide columns of callosal wall extending from the lateral ventricle to the pial surface, we noted a progressive expansion of the hGPC pool relative to that of the host, with a serially expanding border between the two at all time-points. In both myelin wild-type and *shiverer* hosts, we noted that by 3 months of age, human NG2$^+$ cells typically replaced mouse NG2$^+$ cells in the corpus callosum (Fig. 3*A*,*B*). At that relatively early time-point, donor progenitors begin to advance into the lower layers of cortex, whereas more superficial cortical layers remain inhabited principally by murine NG2$^+$ GPCs (Fig. 3*C*). By 8 months, the human hGPCs have invaded the superficial cortex, whereas endogenous murine GPCs have become sequestered in the most superficial cortical layers (Fig. 3*D*). This process of hGPC expansion in the host forebrains continued, such that, by 1 year of age, the murine progenitors were largely replaced by transplanted human cells, often completely so (Fig. 2*E*,*F*). Indeed, in 3 of 4 animals assessed at 1 year, no remaining mouse GPCs could be identified in the sampled forebrain sections. The geographical advance of donor progenitor cells is typically characterized by a discrete advancing front, which demarcates their border with host murine cells (Fig. 3*C–E*). Remarkably, isolated GPCs of either species were rarely noted behind these borders, suggesting the potency of the repulsive interactions likely characterizing the relationship of these analogous but heterospecific phenotypes.

The concurrent expansion of human cells from the callosum and elimination of endogenous progenitors proceeded with approximately exponential decay kinetics, and did so in both the cortical and subcortical gray matter, with striatal and basal forebrain infiltration by hGPCs occurring concurrently with neocortical invasion. Importantly, while the geographic patterns and timing of GPC migration proved analogous in both hypomyelinated *shiverer* and myelin wild-type hosts (Fig. 1), the relative degrees of local intracompartmental expansion differed, in that hGPCs expanded in the callosal environment of *shiverer* mice to a notably greater extent than their myelin wild-type counterparts (compare callosal hGPC densities between *shiverer* and wild-type hosts in Fig. 1). These observations suggested that the dominant colonization of the mouse brain by hGPCs reflected not only a species-selective competitive advantage of human over mouse GPCs, but also a context-dependent instruction of relative expansion and phenotypic differentiation.

hGPCs differentiate in a context-dependent fashion

On that basis, we next sought to assess the responsiveness of engrafted hGPCs to the host environment, by comparing their phenotypic differentiation in congenitally hypomyelinated and normally myelinated murine recipients. To that end, we used stereological analysis of progenitor-derived human astrocytes and oligodendrocytes to define the relative representation of each phenotype in the corpus callosum of neonatally xenografted *shiverer* and wild-type mice. In one year-old *shiverer* recipients, >40% of human cells in the callosum expressed the oligodendrocytic protein CNP. In contrast, <10% of human callosal cells in myelin wild-type mice did so at that point ($p < 0.001$ by 2-way ANOVA with Bonferroni *t* test; Fig. 4*A*). Accordingly, >50% of the human donor-derived cells remained as glial progenitors in the myelin wild-type chimeras, while <15% do in the shiverer ($p < 0.001$, Bonferroni *t* test). Yet in contrast to the marked difference in oligodendrocytic differentiation by hGPCs between *shiverer* and wild-type mice, the relative proportions of engrafted human cells that developed GFAP$^+$ astrocytic phenotype proved no different in the two recipient models (Fig. 4*A*).

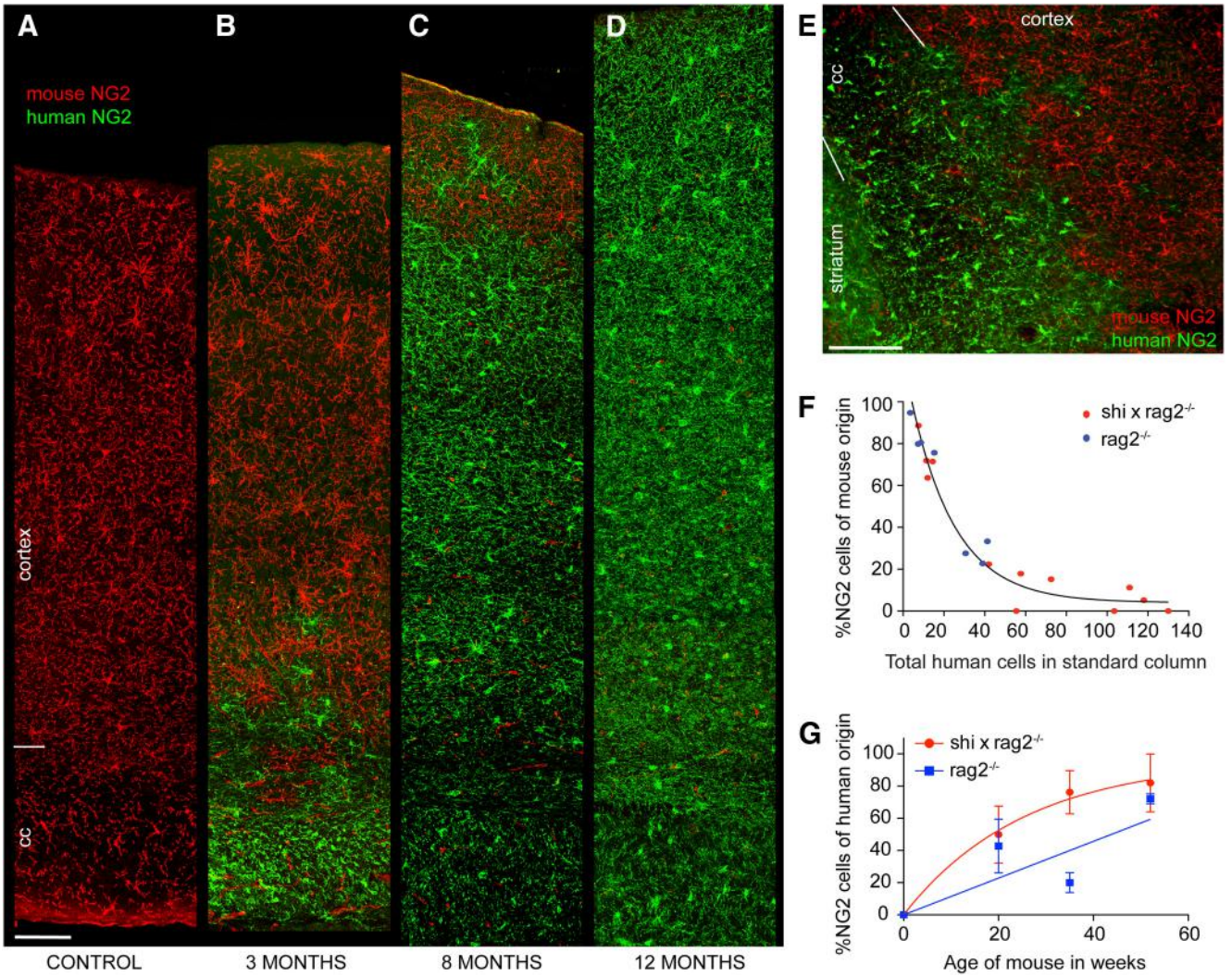


Figure 3. Progressive domination of murine forebrain by human glial progenitors. hGPC replacement of mouse glial progenitor cells (mGPCs) in both *shiverer* and normally myelinated *rag2* $^{-/-}$ mice, visualized using species-specific antibodies to the GPC chondroitin sulfate proteoglycan NG2. ***A***, An unengrafted control showing the normal distribution of mouse NG2 $^{+}$ cells (red), spanning the distance from the ventricular ependyma to the pial surface; 3 months of age. ***B–D***, Analogous radial strips spanning the ventricular to the pial surface, including the corpus callosum and cortex, of *shiverer* × *rag2* $^{-/-}$ mice engrafted neonatally with hGPCs. These images, taken at 3 (***B***), 8 (***C***), and 12 (***D***) months of age, show the systematic expansion of hNG2-defined hGPCs from the callosum to the cortical mantle, and the concurrent displacement of endogenous murine NG2 $^{+}$ cells. Red represents mouse NG2; green represents human NG2. ***E***, Higher-magnification image of the mGPC-hGPC border in a 3-month-old myelin wild-type *rag2* $^{-/-}$ mouse demonstrates the sharp demarcation between the advancing human and retreating mouse glial progenitors. In myelin wild-type mice, by this time-point, hGPCs have replaced mGPCs throughout the corpus callosum, the hippocampus, and lower layers of cortex; mouse NG2 $^{+}$ cells still dominate the superficial layers of the cortex. ***F***, The replacement of host mouse cells by transplanted hGPCs in both *shiverer/rag2* $^{-/-}$ (red dots) and normally myelinated *rag2* $^{-/-}$ (blue dots) mouse callosal-cortical strips, plotted as the percentage of mouse NG2 $^{+}$ cells versus the number of human NG2 $^{+}$ cells/radial column. Across all time-points, as the total number of human cells in a radial column increased, the proportion of host mouse GPCs fell. ***G***, The kinetics of hGPC replacement of endogenous mGPCs, as a function of time, in both *shiverer* and myelin wild-type × *rag2* null hosts. In both cases, colonization by hGPCs eventually yielded the substantial replacement and, in some cases, the apparent elimination of the endogenous mouse progenitor population. Scale bars: ***A–E***, 100 μm.

In absolute numbers, stereological estimation revealed that many times more human cells engrafted into the callosa of *shiverer* than in myelin wild-type mice (2-way ANOVA, $p < 0.0001$) (Fig. 4*B*). In particular, the numbers of donor-derived oligodendrocytes and progenitors were each significantly higher in the *shiverer* than the myelin wild-type brains ($p < 0.001$ and <0.01, respectively, by Bonferroni *t* tests), suggesting that the hypomyelinated environment encouraged selective expansion of donor hGPCs, as well as facilitating their oligodendrocytic differentiation (Fig. 4*B*).

It is important to note that the selective expansion of donor hGPCs in the *shiverer* brains appeared to be a product of the hypomyelinated environment, and not of xenograft per se, because neither the number nor relative proportion of hGPCs in neonatally xenografted wild-type callosa differed from the corresponding numbers of mouse progenitors in untransplanted wild-type controls (Fig. 4*C*,*D*). At baseline, 3.5 ± 0.2% of callosal cells in untransplanted 1-year-old *rag2*-null mice were identified as GPCs by their expression of mouse NG2. Yet when 1-year-old hGPC engrafted *rag2*-nulls were similarly evaluated, using species-specific anti-NG2 antibodies, 3.6 ± 0.3% of callosal cells were found to express human NG2. Similarly, in xenografted *shiverers* that survived to 1 year by virtue of neonatal transplantation, 4.1 ± 0.2% of all callosal cells expressed human NG2 (Fig. 4*D*). As noted previously, by this late time-point, few, if any, mouse NG2 cells were noted to persist in the xenografted callosum, whether of *shiverer* or myelin wild-type hosts (Fig. 3*F*,*G*). In each case, the net density of parenchymal GPCs was unchanged despite the complete or near-complete replacement of mGPCs by hGPCs; by 1 year of age, 3%–4% of all cells expressed NG2, regardless of species or host genotype.

In a similar vein, we noted that the proportion of human astrocytes increased as a function of time in the corpus callosum of the *shiverer* mice and was matched by a corresponding decrease in the proportion of mouse astrocytes (Fig. 4*E*); the net density of astrocytes was approximately preserved, despite the slow replacement of mouse by human astroglia. As with host colonization by hGPCs, we found that xenograft influenced only the species of resident astroglia and not their overall density. Together, these data indicated the context-dependent expansion and differentiation of hGPCs, with the species-independent preservation of phenotype-specific callosal cell densities for GPCs and astroglia alike.

hGPCs remain mitotically active longer than allografted mGPCs

We next asked whether species-selective differences in proliferative activity might contribute to the relative expansion of hGPCs. To that end, we treated matched cohorts of human and allografted mouse glial chimeric myelin wild-type mice with the DNA replication marker BrdU for 5 d before death at either 4 or 8 months of age. Death was followed by immunohistochemistry for BrdU together with phenotype-selective markers, followed by estimation of the respective mitotic indices of human and mouse GPCs. At both 4 and 8 months of age, we noted that the mitotic fraction of human donor cells was significantly higher than that of allografted mouse GPCs (Fig. 5*A*) (two-way ANOVA, $p < 0.0005$). These data indicate that hGPCs remain mitotically active long after the mitotic expansion of allografted murine glial progenitors has slowed, just as the xenografted hGPCs remain mitotically active long after endogenous GPC expansion has abated (Windrem et al., 2008); this in turn suggests that the selective expansion of hGPCs may contribute, at least in part, to their dominance over time in xenografted mice.

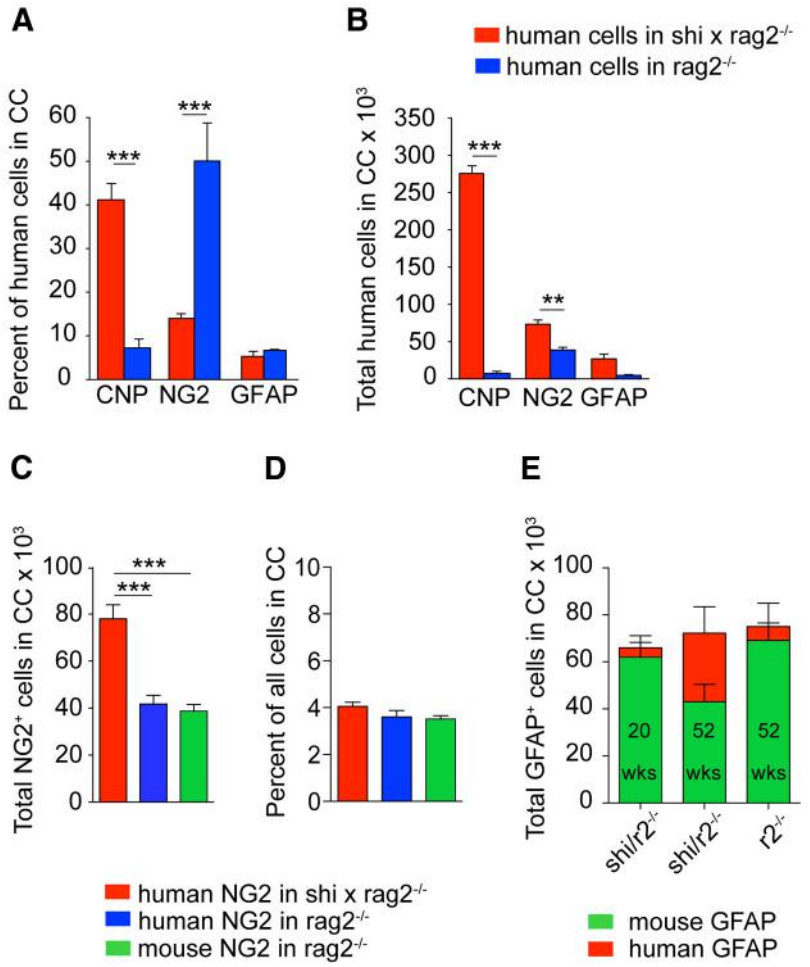


Figure 4. Phenotypic differentiation by engrafted hGPCs is context-dependent. ***A***, Differentiation at 1 year of hGPCs in the corpus callosum of congenitally hypomyelinated, transplant-rescued *shiverer* (red) and myelin wild-type mice (blue), each *rag2* $^{-/-}$ immunodeficient. In the *shiverer* callosum, >40% of human donor cells had differentiated as CNP $^{+}$ oligodendrocytes; in contrast, in myelin wild-types, most remained as progenitors. ***B***, Total number of human cells in the corpus callosum of *shiverer* × *rag2* $^{-/-}$ versus myelin wild-type *rag2* $^{-/-}$ mice. The higher number of human cells engrafted in *shiverer* white matter may be attributed to the greater number of CNP-defined oligodendrocytes engrafting in *shiverer*. ***C***, At 1 year, the total number of human NG2 $^{+}$ cells in the corpus callosum of engrafted *shiverer* × *rag2* $^{-/-}$ mice is almost double that of myelin wild-type *rag2* $^{-/-}$ mice. ***D***, The proportion of hNG2-defined hGPCs is the same in both backgrounds, consistent with the higher density of GPCs in the *shiverer* callosum (Bu et al., 2004). ***E***, Human GFAP $^{+}$ cells slowly replace mouse GFAP $^{+}$ cells in the *shiverer* corpus callosum, such that, by 1 year, almost half of all mouse callosal astrocytes have been replaced by hGPC-derived human astroglia, yielding white matter substantially chimeric for human astroglia as well as for hGPCs. $^{**}p < 0.001$ (ANOVA with Bonferroni *t* tests). $^{***}p < 0.0001$ (ANOVA with Bonferroni *t* tests).

On that basis, we next asked whether the difference in expansion between human and mouse glia might also reflect phenotype-selective differences in the mitotic rate of progenitor-derived daughters, relative to those derived from resident mouse GPCs. To this end, we immunostained BrdU-labeled sections of neonatally xenografted *rag2*-null myelin wild-type mice for the glial progenitor protein PDGFRα, the astroglial filament GFAP, or oligodendrocytic transferrin. Of note, in this experiment, we immunostained for human PDGFRα rather than NG2 to identify hGPCs, and transferrin rather than CNP to recognize oligodendroglia, so as to permit concurrent staining for BrdU, the fixation conditions for which obscure both NG2/CPSG4 and CNP immunodetection. We validated that our PDGFRα and transferrin immunostaining protocols exclusively recognized NG2- and CNP-expressing cells, respectively, in hGPC-xenografted brains (data not shown). We found that, at both 4 and 8 months, the GPC fraction of all donor-derived cells remained significantly higher in hGPC-engrafted mice than in mice allografted with mGPCs (Fig. 5*B*), consistent with the higher sustained mitotic index of the human donor pool (Fig. 5*A*). Among GPC daughter cells, though, the production of oligodendroglia in myelin wild-type mice was more pronounced by murine than hGPCs at both 4 and 8 months (Fig. 5*C*), suggesting that allografted mouse GPCs are more biased toward rapid oligodendroglial differentiation than their human counterparts. In contrast, hGPCs xenografted into myelin wild-types appeared more biased to astrocytic differentiation, with evident donor-derived astrocytic accumulation between 4 and 8 months (Fig. 5*D*). Accordingly, the mitotic index of donor-derived astroglia was significantly higher in human than in allografted murine GPCs (Fig. 5*E*). Indeed, although human astrocytes typically comprised 5%–10% of BrdU $^{+}$ cells in wild-type recipients, no BrdU $^{+}$ astrocytes were identified in

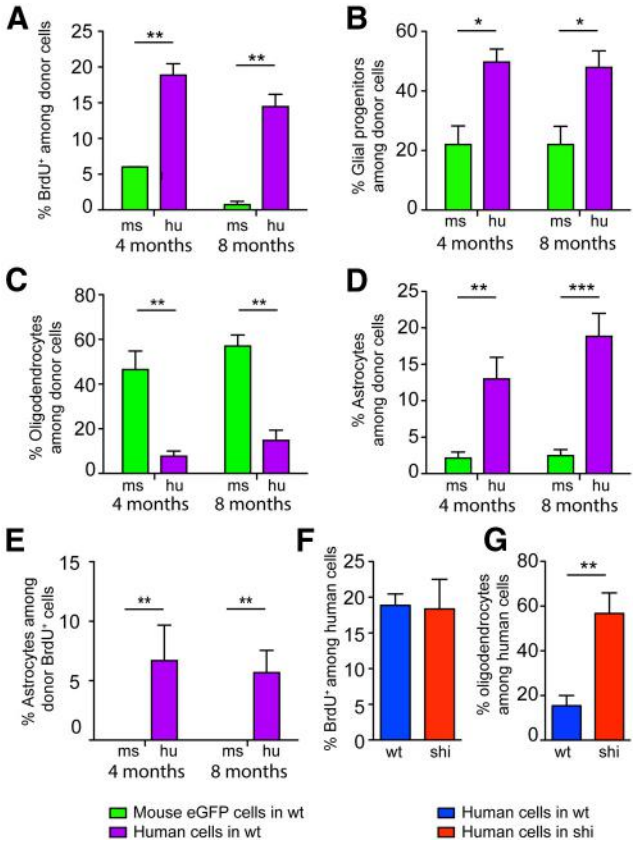


Figure 5. hGPCs expand preferentially relative to mouse GPCs in the murine environment. Neonatal chimeras were established in myelin wild-type *rag2* $^{-/-}$ mice, with either human or allogeneic EGFP-tagged mouse GPCs. These were maintained until 4 or 8 months of age, then injected for 5 d preterminally with BrdU to label the mitotic fraction of resident cells. The mice were then killed and callosal BrdU-tagged cells immunophenotyped. ***A***, At both 4 and 8 months, EGFP $^{+}$ mGPCs (green bars) exhibited substantially lower mitotic indices than the xenografted hGPCs (purple). $^{**}p < 0.0005$ (one-way ANOVA). ***B***, At both 4 and 8 months, higher proportions of human than mouse donor cells could be identified as PDGFRα-defined glial progenitor cells. ***C***, In contrast, allografted mouse GPCs were significantly more likely to differentiate as transferrin-defined oligodendrocytes than were their human counterparts, when injected into matched myelin wild-type recipients. ***D***, Substantially higher proportions of human donor cells differentiated as GFAP-defined astrocytes, compared with allografted mouse GPCs. ***E***, Strikingly, the mitotic index of these astrocytes differed dramatically between mouse and human donors: No mouse GFAP $^{+}$ cells were found to incorporate BrdU at either 4 or 8 months, indicating that, although new human astrocytes were continuously added to these adult brains from resident hGPCs, astrocytic recruitment from murine progenitors appeared to be nil. ***F***, At the 4 month time-point studied, the mitotic index of callosal hGPCs did not differ between *shiverer* and myelin wild-type recipients. ***G***, However, a higher percentage of hGPCs differentiated as transferrin $^{+}$ oligodendrocytes in *shiverer* than in normally myelinated mice, again highlighting the context-dependent differentiation of hGPCs following their widespread, seemingly cell-autonomous migration. $^{*}p < 0.01$ (ANOVA with Bonferroni *t* test). $^{**}p < 0.001$ (ANOVA with Bonferroni *t* test). $^{***}p < 0.0001$ (ANOVA with Bonferroni *t* test).

mGPC-allografted brains (Fig. 5*E*). These data indicate that astrocytic replacement in myelin wild-type glial chimeras derived almost entirely from engrafted human progenitors and thus suggest that hGPC-engrafted mice may experience slow replacement of their astrocytic as well as their progenitor populations. In addition, these findings highlight the persistence of both GPC phenotype and mitotic potential among xenografted human cells as contributing factors for the competitive dominance of hGPCs in the chimeric brain environment.

We next asked whether the mitotic index of human donor GPCs and their derivatives differed whether they were transplanted into wild-type or hypomyelinated recipients. We found no difference in the mitotic index of human donor cells whether introduced to wild-type or *shiverer* brain (Fig. 5*F*) but did note that the hGPCs were significantly more likely to generate oligodendroglia in the *shiverer* callosum, relative to that of myelin wild-types (Fig. 5*G*). No such differences were seen in the callosal production of GPCs or astrocytes as a function of recipient environment (data not shown). These observations again point to the pronounced context dependence of phenotypic differentiation by xenografted hGPCs and are consistent with the preferential instruction of GPCs toward oligodendroglial lineage in the *shiverer* environment.

Human astrocytes exhibit cell-autonomous maturation in chimeric mice

To assess the species-determined features of glial differentiation in these hGPC chimeras, we also assessed the morphological phenotypes of their derived astrocytes, in myelin wild-type recipients. We assessed astrocytic morphologies, and domain architecture in particular, because human astroglia may be readily distinguished *in vivo* from those of mice, spanning considerably larger domain volumes and manifesting significantly greater fiber complexity (Oberheim et al., 2009). We found that, at 8–10 months of age, by which point the majority of all forebrain GPCs in these mice were of human origin, the recipient brains also exhibited large numbers and high relative proportions of human astrocytes, in both gray and white matter. As we had previously noted in an assessment of human astroglial contributions to cognition in glial chimeric mice, the engrafted human glia in murine chimeras appear to develop and mature in a cell-autonomous fashion, in that their diameter, domain size, and morphology all approximate that of astrocytes in the normal adult human brain (Han et al., 2013). In each environment, including both the xenografted mouse brain and native human brain, human astroglia proved significantly more complex and phenotypically heterogeneous than host murine astrocytes (Fig. 6). As we have noted, the replacement of host GPCs by hGPCs leads, over time, to the effective chimerization of the recipient mice with human astrocytes. Because these astrocytes evidently retain human-specific pleomorphism and domain complexity, the astrocytic humanization of these chimeric brains appears to give rise to hominid-specific glial architecture as well as physiology in the murine brain.

Discussion

In this study, we engrafted neonatal mice with hGPCs, so as to evaluate the dispersal, interspecific competitive interactions, and context-dependent fate determination of xenografted hGPCs. We found that a large proportion of glial cells within the recipient mice, often all GPCs and a large proportion of astrocytes, and oligodendrocytes as well, when using hypomyelinated hosts, were ultimately replaced by human donor-derived cells. The extent of this colonization of the mouse brain by human glia appeared so robust that we quantitatively evaluated the absolute numbers, relative proportions, and geographic distributions of human donor cells in the neonatally engrafted recipients. This analysis revealed that hGPCs so dominated and enjoyed such a competitive advantage over their murine counterparts that, by 9 months *in vivo*, virtually all glial progenitors within these mouse brains were typically replaced by hGPCs (Fig. 1).

The higher mitotic index of human donor cells relative to resident mouse GPCs in the host brains suggested a basis for our prior observation that, in *shiverer* homozygotes, an initial dose of

300,000 cells/recipient expanded by at least 40-fold by 12 months, to an average of 12×10^6 human glia in those animals rescued by neonatal progenitor transplantation (Windrem et al., 2008). Our present observations extend this analysis substantially by demonstrating that, in both the *shiverer* homozygotes and myelin wild-type hosts, the selective expansion of the human donor glia appears in part a product of the more sustained proliferation of hGPCs. Because the human donor GPCs are derived from the late second-trimester SVZ, they might be expected to divide for up to 9 months after isolation; our data suggest that they sustain elements of that cell-autonomous program of expansion in the mouse host.

Importantly, *shiverer* and wild-type recipient mice differed in the compositions of their donor-derived phenotypes after neonatal chimerization. Whereas in xenografted adult *shiverers*, virtually all surviving oligodendrocytes were of human donor origin, in wild-type recipients, few donor-derived oligodendrocytes were apparent; rather, these brains had an abundance of hGPCs and astrocytes, the relative proportions of which increased with age. In both recipient environments, the human glial progenitors typically outcompeted their murine counterparts. This process was dynamic in scope and overtly competitive, in that the hGPCs typically expanded outwards from their periventricular and callosal points of introduction, in advancing waves that appeared to repulse resident murine progenitors, which appeared to die concurrent with their replacement, both *in situ* and upon retreat to the cortical surface (Fig. 3). The hGPCs progressively expanded until achieving a relatively uniform distribution, in tissue densities not significantly different from those of the mouse GPCs that they replaced; their assumption of an asymptotic distribution appeared analogous to that reported developmentally by Bergles and colleagues (Hughes et al., 2013). The repulsive signals to which the murine GPCs responded are unknown, as is the molecular basis for the competitive dominance of the hGPCs. Several recent studies have identified differential expression of both MYC- and hedgehog-dependent pathways as contributing to clonal dominance during early ontogeny (Clavería et al., 2013; Amoyel and Bach, 2014; Amoyel et al., 2014), and we are now assessing differential gene expression by mouse and hGPCs *in vivo* in an attempt to define analogous regulators of competition that may distinguish murine and hGPCs. Yet regardless of their mechanistic basis, the competitive interactions between mouse and hGPCs yielded the slow but inexorable glial humanization of these brains, first by hGPCs, and then by their derived astrocytes, as resident mouse astrocytes underwent normal turnover in adulthood, with replacement from the now-humanized progenitor pools (Fig. 3).

The value of such humanized models of *in vivo* brain phenotypes and function is especially clear when one considers that human astrocytes have functional competencies unique to hominids. Human astrocytes are more numerous, larger, and more structurally complex than those of infraprimate mammals, raising the possibility that the functional roles of glia have expanded during evolution (Oberheim et al., 2006, 2009). These evolutionary changes are of particular interest because astrocytes have been shown to play vital roles in information processing within the CNS (Kang et al., 1998; Araque et al., 1999). Astrocytes are required for synaptogenesis and maintenance of synaptic density (Ullian et al., 2001), and a number of specific astrocytic modulators of synaptic plasticity have been identified, including the glypicans (Allen et al., 2012) and TNFα (Stellwagen and Malenka, 2006), among others. Importantly, these ligands may be differentially expressed by human astroglia, thus imparting differential potency to human astroglia in the regulation of synaptic plasticity, relative to infraprimate glia (Oberheim et al., 2009; Han et al., 2013). As a result, the greater structural complexity of human astrocytes relative to those of rodents is accompanied by functional differences: human astrocytes propagate Ca^{2+} wave significantly faster than rodents, and human glial chimeric mice exhibit both enhanced long-term potentiation and facilitated learning in a variety of conditioned response paradigms and cognitive tasks (Han et al., 2013). Together, these observations suggest that the species-specific structural complexity of human astrocytes endows these cells with a fundamentally greater functional importance to synaptic modulation than that of their infraprimate counterparts.

Human astrocytes may differ substantially from their murine counterparts in their origin as well as their function. In our chimeras preterminally tagged with the mitotic marker BrdU, we found that, although new human astrocytes were continuously added to these brains, as recruited from resident engrafted hGPCs, astrocytic production from murine progenitors appeared to be nil; no mouse $GFAP^+$ cells were found to incorporate BrdU at either 4 or 8 months (Fig. 5*E*). This observation may suggest a fundamental distinction in the origin of new astroglia in the brains of adult rodents and humans; although in mice resident astrocytes have been reported as the principal source of new astrocytes in adulthood (Ge et al., 2012), with GPCs serving prin-

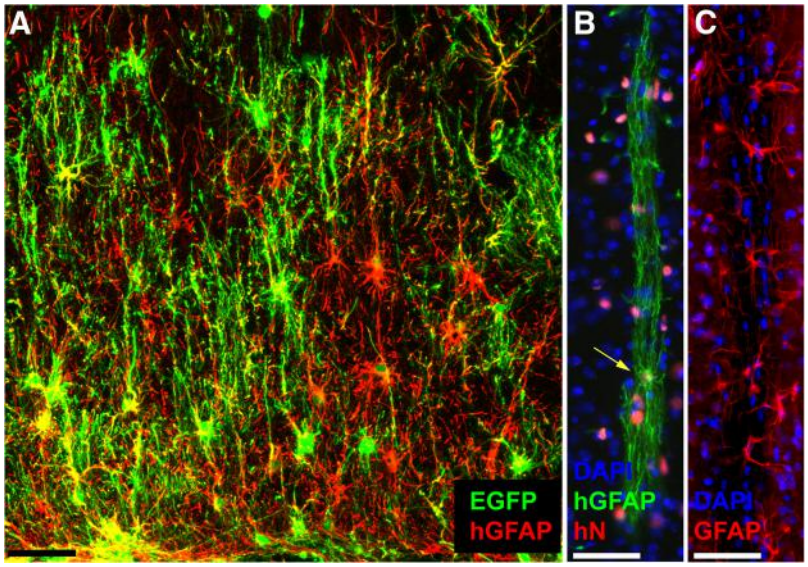


Figure 6. Cell-autonomous differentiation of human astroglia in the chimeric host environment. ***A***, Engraftment of human glial progenitors and astrocytes in the corpus callosum of a 2-year-old myelin wild-type immunodeficient mouse. Human cells were transduced before transplantation with lentiviral EGFP (green) and labeled with anti-human-specific GFAP (red). ***B***, A representative human fibrous astrocyte, resident in a striatal fiber tract in a 1-year-old mouse. Yellow arrow indicates the nucleus (human nuclear antigen, red) of the single astrocyte, whose fibers extend >350 μm within the tract (green, human GFAP). ***C***, Endogenous mouse astrocytes, in a comparable tract through the striatum of an unengrafted mouse: red represents GFAP; blue represents DAPI. Scale bar, 50 μm.

cipally as oligodendrocyte progenitors (Bu et al., 2004; Nishiyama et al., 2009; Kang et al., 2010; Tripathi et al., 2010), human glial progenitors are notably bipotential for both oligodendrocytes and astrocytes in the adult brain (Nunes et al., 2003; Sim et al., 2009; Sim et al., 2011).

Our ability to generate mice in which the bulk of the glial population is of human origin opens the possibility of constructing human glial chimeras in a patient-specific and disease-defined manner, using hGPCs derived from embryonic stem cells and induced pluripotential cells. In particular, the ability to generate astrocytes (Krencik et al., 2011), as well as glial progenitors and oligodendroglia (Wang et al., 2013), from human-induced pluripotential stem cells permits the construction of mice in which we may now assess the relative contributions of human glia to disease pathology *in vivo*. For instance, the derivation of hGPCs from pluripotential stem cells carrying the polyglutamine repeat expansion of Huntington disease (HD), and the establishment of human glial chimeras using those huntingtin mutant hGPCs, may permit us to define the specific contributions of glia to neuropathology in HD, as well as to the clinical phenotype of HD. Similarly, the construction of human glial chimeras using hGPCs derived from induced pluripotential cells generated from patients with hereditary neuropsychiatric conditions may permit us to define and isolate the contribution of glia to these disorders, the phylogenetic appearance of which seems approximately concurrent with the evolution of morphological complexity by human astroglia (Horrobin, 1998; Oberheim et al., 2009). More broadly, such disease-specific induced pluripotential stem cell-derived human glial chimeras may permit us to better define the role of glial dysfunction in a broad swath of nominally neuronal neurodegenerative and neuropsychiatric disorders, in which the relative contribution of glial pathology remains unclear and understudied.

References

Allen NJ, Bennett ML, Foo LC, Wang GX, Chakraborty C, Smith SJ, Barres BA (2012) Astrocyte glypicans 4 and 6 promote formation of excitatory synapses via GluA1 AMPA receptors. Nature 486:410–414. CrossRef Medline

Amoyel M, Bach EA (2014) Cell competition: how to eliminate your neighbours. Development 141:988–1000. CrossRef Medline

Amoyel M, Simons BD, Bach EA (2014) Neutral competition of stem cells is skewed by proliferative changes downstream of Hh and Hpo. EMBO J 33:2295–2313. CrossRef Medline

Araque A, Parpura V, Sanzgiri RP, Haydon PG (1999) Tripartite synapses: glia, the unacknowledged partner. Trends Neurosci 22:208–215. CrossRef Medline

Bu J, Banki A, Wu Q, Nishiyama A (2004) Increased NG2(+) glial cell proliferation and oligodendrocyte generation in the hypomyelinating mutant shiverer. Glia 48:51–63. CrossRef Medline

Clavería C, Giovinazzo G, Sierra R, Torres M (2013) Myc-driven endogenous cell competition in the early mammalian embryo. Nature 500:39–44. CrossRef Medline

Ge WP, Miyawaki A, Gage FH, Jan YN, Jan LY (2012) Local generation of glia is a major astrocyte source in postnatal cortex. Nature 484:376–380. CrossRef Medline

Goldman SA, Schanz S, Windrem MS (2008) Stem cell-based strategies for treating pediatric disorders of myelin. Hum Mol Genet 17:R76–R83. CrossRef Medline

Goldman SA, Nedergaard M, Windrem MS (2012) Glial progenitor cell-based treatment and modeling of neurological disease. Science 338:491–495. CrossRef Medline

Han X, Chen M, Wang F, Windrem M, Wang S, Shanz S, Xu Q, Oberheim NA, Bekar L, Betstadt S, Silva AJ, Takano T, Goldman SA, Nedergaard M (2013) Forebrain engraftment by human glial progenitor cells enhances synaptic plasticity and learning in adult mice. Cell Stem Cell 12:342–353. CrossRef Medline

Horrobin DF (1998) Schizophrenia: the illness that made us human. Med Hypotheses 50:269–288. CrossRef Medline

Hughes EG, Kang SH, Fukaya M, Bergles DE (2013) Oligodendrocyte progenitors balance growth with self-repulsion to achieve homeostasis in the adult brain. Nat Neurosci 16:668–676. CrossRef Medline

Kang J, Jiang L, Goldman SA, Nedergaard M (1998) Astrocyte-mediated potentiation of inhibitory synaptic transmission. Nat Neurosci 1:683–692. CrossRef Medline

Kang SH, Fukaya M, Yang JK, Rothstein JD, Bergles DE (2010) NG2+ CNS glial progenitors remain committed to the oligodendrocyte lineage in postnatal life and following neurodegeneration. Neuron 68:668–681. CrossRef Medline

Krencik R, Weick JP, Liu Y, Zhang ZJ, Zhang SC (2011) Specification of transplantable astroglial subtypes from human pluripotent stem cells. Nat Biotechnol 29:528–534. CrossRef Medline

Nishiyama A, Komitova M, Suzuki R, Zhu X (2009) Polydendrocytes (NG2 cells): multifunctional cells with lineage plasticity. Nat Rev Neurosci 10:9–22. CrossRef Medline

Nunes MC, Roy NS, Keyoung HM, Goodman RR, McKhann G 2nd, Jiang L, Kang J, Nedergaard M, Goldman SA (2003) Identification and isolation of multipotential neural progenitor cells from the subcortical white matter of the adult human brain. Nat Med 9:439–447. CrossRef Medline

Oberheim NA, Takano T, Han X, He W, Lin JH, Wang F, Xu Q, Wyatt JD, Pilcher W, Ojemann JG, Ransom BR, Goldman SA, Nedergaard M (2009) Uniquely hominid features of adult human astrocytes. J Neurosci 29:3276–3287. CrossRef Medline

Oberheim N, Wang X, Goldman S, Nedergaard SA (2006) Astrocytic complexity distinguishes the human brain. Trends Neurosci 29:547–553. CrossRef Medline

Roy N, Wang S, Harrison-Restelli C, Benraiss A, Fraser R, Gravel M, Braun M, Goldman S (1999) Identification, isolation, and promoter-defined separation of mitotic oligodendrocyte progenitor cells from the adult human subcortical white matter. J Neurosci 19:9986–9995. Medline

Roy N, Wang S, Jiang L, Kang J, Benraiss A, Restelli C, Fraser R, Couldwell W, Kawaguchi A, Okano H, Nedergaard M, Goldman S (2000) In vitro neurogenesis by progenitor cells isolated from the adult human hippocampus. Nat Med 6:271–277. CrossRef Medline

Sim FJ, Windrem MS, Goldman SA (2009) Fate determination of adult human glial progenitor cells. Neuron Glia Biol 5:45–55. CrossRef Medline

Sim FJ, McClain CR, Schanz SJ, Protack TL, Windrem MS, Goldman SA (2011) CD140a identifies a population of highly myelinogenic, migration-competent and efficiently engrafting human oligodendrocyte progenitor cells. Nat Biotechnol 29:934–941. CrossRef Medline

Stellwagen D, Malenka RC (2006) Synaptic scaling mediated by glial TNF-alpha. Nature 440:1054–1059. CrossRef Medline

Tripathi RB, Rivers LE, Young KM, Jamen F, Richardson WD (2010) NG2 glia generate new oligodendrocytes but few astrocytes in a murine experimental autoimmune encephalomyelitis model of demyelinating disease. J Neurosci 30:16383–16390. CrossRef Medline

Ullian EM, Sapperstein SK, Christopherson KS, Barres BA (2001) Control of synapse number by glia. Science 291:657–661. CrossRef Medline

Wang S, Bates J, Li X, Schanz S, Chandler-Militello D, Levine C, Maherali N, Studer L, Hochedlinger K, Windrem M, Goldman SA (2013) Human iPSC-derived oligodendrocyte progenitor cells can myelinate and rescue a mouse model of congenital hypomyelination. Cell Stem Cell 12:252–264. CrossRef Medline

West MJ (1998) Stereological methods for estimating the total number of neurons and synapses: issues of precision and bias. Trends Neurosci 22:51–61. CrossRef Medline

Windrem MS, Nunes MC, Rashbaum WK, Schwartz TH, Goodman RA, McKhann G 2nd, Roy NS, Goldman SA (2004) Fetal and adult human oligodendrocyte progenitor cell isolates myelinate the congenitally dysmyelinated brain. Nat Med 10:93–97. CrossRef Medline

Windrem MS, Schanz SJ, Guo M, Tian GF, Washco V, Stanwood N, Rasband M, Roy NS, Nedergaard M, Havton LA, Wang S, Goldman SA (2008) Neonatal chimerization with human glial progenitor cells can both remyelinate and rescue the otherwise lethally hypomyelinated shiverer mouse. Cell Stem Cell 2:553–565. CrossRef Medline

ATTACHMENT B

Schreiweis et al.,

Humanized Foxp2 accelerates learning by enhancing transitions from declarative to procedural performance,

PROCEEDINGS OF THE NATIONAL ACADEMIES OF SCIENCE,
vol. 111 no. 39 pp.14253-14258 (Sept. 30, 2014)

Humanized Foxp2 accelerates learning by enhancing transitions from declarative to procedural performance

Christiane Schreiweis[a,b,1], Ulrich Bornschein[b,1], Eric Burguière[a,c], Cemil Kerimoglu[b,d], Sven Schreiter[b,e], Michael Dannemann[b], Shubhi Goyal[a], Ellis Rea[f], Catherine A. French[g,h], Rathi Puliyadi[h], Matthias Groszer[i], Simon E. Fisher[j,k], Roger Mundry[l], Christine Winter[f], Wulf Hevers[b], Svante Pääbo[b,2], Wolfgang Enard[b,d,2,3], and Ann M. Graybiel[a,2,3]

[a]McGovern Institute for Brain Research and Department of Brain and Cognitive Sciences, Massachusetts Institute of Technology, Cambridge, MA 02139; [b]Department of Evolutionary Genetics, Max Planck Institute for Evolutionary Anthropology, 04103 Leipzig, Germany; [c]Institut National de la Santé et de la Recherche Médicale U 1127, Centre National de Recherche Scientifique Unité Mixte de Recherche 7225, Sorbonne Universités, Université Pierre et Marie Curie, Université Paris 06 Unité Mixte de Recherche S 1127, Institut du Cerveau et de la Moelle épinière, F-75013 Paris, France; [d]Laboratory of Anthropology and Human Genetics, Department of Biology II, Ludwig-Maximilians University Munich, 82152 Martinsried, Germany; [e]DFG Research Center for Regenerative Therapies, Technical University Dresden, 01307 Dresden, Germany; [f]Department of Psychiatry and Psychotherapy, Faculty of Medicine Carl Gustav Carus, Technical University Dresden, 01187 Dresden, Germany; [g]Champalimaud Neuroscience Programme, Champalimaud Centre for the Unknown, 1400-038 Lisbon, Portugal; [h]Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford OX3 7BN, United Kingdom; [i]Institut du Fer à Moulin, Institut National de la Santé et de la Recherche Médicale, Unité Mixte de Recherche S839, Université Pierre et Marie Curie, 75005 Paris, France; [j]Department of Language and Genetics, Max Planck Institute for Psycholinguistics, 6525 XD, Nijmegen, The Netherlands; [k]Donders Institute for Brain, Cognition and Behaviour, Radboud University, 6525 EN, Nijmegen, The Netherlands; and [l]Departments of Developmental and Comparative Psychology and Primatology, Max Planck Institute for Evolutionary Anthropology, 04103 Leipzig, Germany

Contributed by Svante Pääbo, August 6, 2014 (sent for review April 29, 2014)

The acquisition of language and speech is uniquely human, but how genetic changes might have adapted the nervous system to this capacity is not well understood. Two human-specific amino acid substitutions in the transcription factor forkhead box P2 (FOXP2) are outstanding mechanistic candidates, as they could have been positively selected during human evolution and as *FOXP2* is the sole gene to date firmly linked to speech and language development. When these two substitutions are introduced into the endogenous *Foxp2* gene of mice (*Foxp2*^{hum}), cortico-basal ganglia circuits are specifically affected. Here we demonstrate marked effects of this humanization of Foxp2 on learning and striatal neuroplasticity. *Foxp2*$^{hum/hum}$ mice learn stimulus–response associations faster than their WT littermates in situations in which declarative (i.e., place-based) and procedural (i.e., response-based) forms of learning could compete during transitions toward proceduralization of action sequences. Striatal districts known to be differently related to these two modes of learning are affected differently in the *Foxp2*$^{hum/hum}$ mice, as judged by measures of dopamine levels, gene expression patterns, and synaptic plasticity, including an NMDA receptor-dependent form of long-term depression. These findings raise the possibility that the humanized Foxp2 phenotype reflects a different tuning of corticostriatal systems involved in declarative and procedural learning, a capacity potentially contributing to adapting the human brain for speech and language acquisition.

dorsomedial striatum | dorsolateral striatum | T-maze | cross maze | learning strategy

The gene encoding the transcription factor forkhead box P2 (FOXP2) is a promising candidate for investigating the evolutionary basis of human speech and language capabilities. Humans carrying only one functional copy of this transcription factor experience difficulties in learning and performing complex orofacial movements and have receptive and expressive deficits in oral and written language, whereas other cognitive skills are less affected. These speech and language deficits are associated with functional impairments in cortico-basal ganglia and cortico-cerebellar circuits (1). Since the time that the human and chimpanzee lineages separated, approximately 6 Mya, two amino acid substitutions have occurred in FOXP2, a higher rate of change than expected given its conservation in mammals (2, 3). Mice in which the endogenous *Foxp2* gene has been "humanized" for these two amino acid changes (*Foxp2*$^{hum/hum}$ mice) exhibit prominent neurochemical, neurophysiological, and neuroanatomical alterations in the striatum and related cortico-basal ganglia circuits (4, 5). These circuits are known to be essential for acquiring habits and other motor and cognitive behaviors (6), including vocal learning in songbirds (7) and speech and language capabilities in humans (8). However, whether learning behavior depending on these circuits is affected in *Foxp2*$^{hum/hum}$ mice has so far not been investigated.

A key functional distinction has been made between subregions of the striatum that underlie modes of learning also considered to be crucial for speech and language development and performance: declarative learning and procedural learning (9–12). These learning modes were first distinguished in human cognitive studies to differentiate between a conscious form of

Significance

The human form of forkhead box P2 (*FOXP2*) is the leading genetic candidate for human speech and language proficiency. We demonstrate that the introduction of the amino acid changes that occurred during human evolution into murine *Foxp2* (*Foxp2*^{hum}) profoundly affects learning and striatal neuroplasticity. *Foxp2*$^{hum/hum}$ mice learn stimulus–response associations more rapidly than WT mice when declarative (i.e., place-based) and procedural (i.e., response-based) forms of learning could interfere with one another. Dopamine levels, gene expression patterns, and synaptic physiology are oppositely affected in the striatal districts underpinning these learning forms, paralleling the behavioral change. We hypothesize that the human FOXP2 evolution led to differential tuning of corticostriatal systems involved in declarative and procedural learning and thus contributed to adapting the human brain for speech and language acquisition.

Author contributions: C.S., E.B., M.G., C.W., W.H., W.E., and A.M.G. designed research; C.S., U.B., S.S., S.G., E.R., C.A.F., R.P., and W.H. performed research; C.S., U.B., E.B., C.K., M.D., R.M., C.W., W.H., W.E., and A.M.G. analyzed data; C.S., U.B., E.B., S.E.F., R.M., W.H., S.P., W.E., and A.M.G. wrote the paper; and E.B., M.G., S.E.F., R.M., W.H., S.P., and A.M.G. supervised research.

The authors declare no conflict of interest.

Database deposition: Sequences have been deposited in the Gene Expression Omnibus (GEO), (accession no. GSE60659).

[1]C.S. and U.B. contributed equally to this work.

[2]To whom correspondence may be addressed. Email: paabo@eva.mpg.de, enard@bio.lmu.de, or graybiel@mit.edu.

[3]W.E. and A.M.G. contributed equally to this work.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1414542111/-/DCSupplemental.

learning that can be "declared" and nonconscious forms of learning that require repetitive exposure (13). Equivalents for these two forms of learning have been suggested for animals in many pioneering studies, and terminology has been adapted depending on whether the motivational drive (action–outcome vs. stimulus–response; goal-directed vs. habit) or the task objective (place-based vs. response-based) is more central to the learning. In rodents, the two learning systems are often probed by tasks requiring motor learning, a type of learning thought to be mainly procedural, or by navigational maze tasks in which place-based learning is suggested to correspond to declarative learning and response-based learning is representative of procedural learning (13–17).

These systems are thought to interact dynamically to optimize behavior (17–22). Evidence suggests that these interacting systems have the capacity to compensate for each other if key components are pathologically affected (23, 24), but can also compete with each other under normal circumstances (14, 15, 17, 19, 25). In situations in which such competition occurs, learning is lessened but can be facilitated by attenuating one of the two competing learning strategies (19, 25). In a novel context, a fact-oriented, declarative type of learning predominates as the new environment is explored. With extended training, as beneficial behaviors are acquired, the procedural system becomes predominant.

Early suggestions that declarative learning solely depends on the temporal lobe and hippocampus, and procedural learning solely on the striatum and cerebellum, have been replaced by evidence that these functions are distributed. Within the striatum, moreover, strong evidence indicates that the declarative system operates early during learning in circuits engaging the dorsomedial striatum, when action–outcome associations are formed, whereas the eventual automatization or proceduralization of the behavior engages circuits interconnected with the dorsolateral striatum (17, 20–22, 26, 27). In brain imaging studies of humans lacking one functional copy of *FOXP2*, contrasting activation patterns have been reported for regions that are considered to be homologous to the dorsomedial and dorsolateral striatum in rodents (28, 29).

We took advantage of these findings by developing a panel of behavioral learning protocols adapted for mice to determine how humanized *Foxp2* influences these two striatal learning systems.

Results

Motor Skill Learning Is Normal in Humanized Foxp2 Mice. We first evaluated motor skill learning, given that mice lacking one functional allele of murine *Foxp2* are reported to exhibit learning deficits on an accelerating rotarod and a tilted running wheel (30, 31). However, mice homozygous for humanized Foxp2 ($Foxp2^{hum/hum}$) performed at levels equivalent to those of their WT ($Foxp2^{wt/wt}$) controls when tested by these two tasks (n = 9–10 per genotype; Figs. S1 and S2), extending earlier findings based on different protocols (4). Hence, these types of motor skill learning are impaired in heterozygous murine Foxp2 KO mice (31), but they are not detectably affected by humanizing the Foxp2 protein in mice.

Learning Is Enhanced in Humanized Foxp2 Mice When Declarative and Procedural Systems Can Be Active. We next performed a series of navigational maze experiments to probe declarative and procedural learning in the $Foxp2^{hum/hum}$ mice. We began by assessing learning in a context allowing place-based/declarative and response-based/procedural forms of learning. We trained $Foxp2^{hum/hum}$ and $Foxp2^{wt/wt}$ mice on a conditional T-maze task, in which distinctive learning-related activity patterns have been found in the dorsomedial and the dorsolateral striatum (22, 32). The mice were required to associate each of two sensory stimuli—a rough or smooth tactile flooring surface—with a food reward that could be found at either goal-arm of a T-maze. In addition, we surrounded the T-maze with salient spatial cues (Fig. 1*A*).

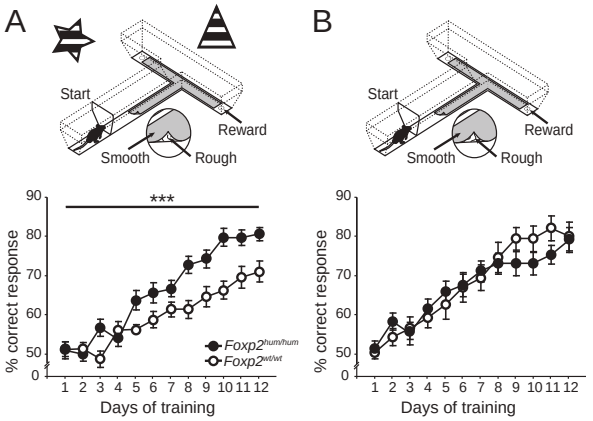


Fig. 1. $Foxp2^{hum/hum}$ mice learn more rapidly than WT littermates in a conditional T-maze paradigm when spatial cues are present. (*Upper*) T-maze task with spatial cues (*A*), promoting place-based/declarative learning; or without spatial cues (*B*), promoting response-based/procedural learning. (*Lower*) Average percent correct responses for $Foxp2^{hum/hum}$ mice (black) and their WT littermates (white) in the two environments. Error bars indicate ± SEM (***$P < 0.001$).

The $Foxp2^{hum/hum}$ mice clearly learned faster than their WT littermates [n = 21–22 per genotype; repeated-measures ANOVA (RMA) days 1–12: $F_{1,41} = 14.94$, $P_{GT} < 0.001$; $F_{7.2,41} = 3.99$, $P_{day*GT} < 0.001$; generalized linear mixed model days 1–12, $z = -3.9$, $P_{day*GT} < 10^{-4}$; Fig. 1*A*, *SI Materials and Methods*, and Table S1]. Moreover, this faster learning in the $Foxp2^{hum/hum}$ mice was specific to the acquisition phase of training. Performance during overtraining, as correct performance was reached and then maintained at greater than 72.5%, did not differ between genotypes (n = 14–15 per genotype; RMA overtraining days 1–10: $F_{1,27} = 0.11$, $P_{GT} = 0.74$; $F_{9,27} = 1.14$, $P_{day*GT} = 0.34$; Fig. S3).

We designed experiments to determine whether this enhancement of learning speed in the $Foxp2^{hum/hum}$ mice reflected enhanced place-based/declarative learning, enhanced response-based/procedural learning, or an altered interaction of these learning systems. An altered interaction, for example, caused by an attenuated declarative system, could enhance performance by accelerating the transition toward the procedural system, an interaction that has been proposed to occur during striatum-dependent learning tasks (17, 18, 21, 22, 27). In the original T-maze surrounded by spatial cues, the mice were provided with at least three learning possibilities. They could associate a sensory stimulus (rough or smooth) with a reward at a constant place (place-based/declarative learning), associate the stimulus with a body turn (procedural/response-based strategy), or shift from a declarative to a procedural strategy during the course of the training. We tested these three alternatives individually.

First, we changed the T-maze task to favor procedural learning by removing extramaze spatial cues (Fig. 1*B*), and we tested acquisition in new, naïve cohorts of mutant and WT mice. In this context, the $Foxp2^{hum/hum}$ and WT mice learned equally well (n = 13–14 per genotype; RMA days 1–12: $F_{1,25} = 0.07$, $P_{GT} = 0.795$; $F_{11,25} = 1.439$, $P_{day*GT} = 0.156$; Fig. 1*B* and Table S2). Analyses of the combined data for the two task paradigms showed that the presence of spatial cues had clearly a different effect on learning in $Foxp2^{hum/hum}$ mice and their WT controls (RMA days 1–12: $F_{7.85,68} = 4.04$, $P_{day*GT*setup} < 0.001$). This difference appears to reflect less efficient learning by WT mice in the presence of spatial cues (Fig. 1). This possibility is in accord with reports of less efficient learning in an environment in which the two learning strategies of declarative/place-based and procedural/response-based learning can interact competitively

(25) and that WT C57BL/6 mice are "essentially place learners" (33–35). By this view, the abundance of spatial cues in the original maze task did not impair the performance of the *Foxp2*$^{hum/hum}$ mice, which might have dealt more effectively with competition between the two available learning strategies.

Given this result, we turned to a cross-maze task often used to discriminate place-based from response-based learning (15, 17, 25). We chose a Tolman variation of the task (16, 36), tailored for our purposes, because the cross-maze variation by Packard and McGaugh (15) has been reported to be difficult for mice (33–35). In this cross-maze paradigm, we were able to test declarative/place-based learning and procedural/response-based learning separately as well as to challenge the interaction between them by testing the ability to change between place-based and response-based learning. The mice started from either of two opposing arms of the maze (north or south), with reward available after a specific response (e.g., right turn; Fig. 2*A*, *Left*) or at a fixed place (e.g., east arm; Fig. 2*A*, *Right*).

Remarkably, we did not observe enhanced learning by the *Foxp2*$^{hum/hum}$ mice in the response-based task or the place-based task. The *Foxp2*$^{hum/hum}$ and WT mice learned both tasks equally rapidly (response-based, $n = 7$–8 per genotype, RMA: $F_{1,13} = 0.43$, $P_{GT} = 0.53$; $F_{4.6,13} = 0.56$, $P_{day*GT} = 0.72$; place-based, $n = 19$–20 per genotype, RMA: $F_{1,37} = 0.45$, $P_{GT} = 0.51$; $F_{6.2,37} = 0.83$, $P_{day*GT} = 0.55$; Fig. S4). Thus, *Foxp2*$^{hum/hum}$ mice did not learn faster when the mice were required to use only place-based learning or only response-based learning to solve the task, despite exhibiting accelerated learning when both strategies could be used.

Prompted by this finding, we tested whether the enhanced performance of the *Foxp2*$^{hum/hum}$ mice resulted from an altered interaction between the two learning systems, attenuating the declarative and favoring the procedural system. We required mice that previously had acquired both tasks without significant difference in performance to shift from place-based learning to response-based learning. We expected to find a difference only during the first days after the task switch, when the two learning systems would likely be in direct competition with each other. To control for general effects on memory or behavioral flexibility, we additionally tested the mice on the opposite direction of transition, measuring learning speeds during the first days after a shift from response-based to place-based learning.

For the transition from place-based to response-based learning, the *Foxp2*$^{hum/hum}$ mice switched significantly more rapidly ($n = 7$–8 per genotype; RMA: $F_{1,13} = 5.68$, $P_{GT} = 0.03$; Fig. 2*B* and Table S3). By contrast, their learning rates did not differ from those of their WT littermates after the opposite, response-to-place transition conditions ($n = 7$–8 per genotype, RMA: $F_{1,13} = 0.19$, $P_{GT} = 0.67$; Fig. 2*C*). These findings suggest that it is specifically the transition from declarative/place-based learning to procedural/response-based learning that is enhanced by the introduction of the humanized form of Foxp2, and not either one of these learning systems alone. The findings further suggest that the competitive interaction between these systems could be lessened in mice with humanized Foxp2, therefore facilitating the transition from declarative to procedural learning that is proposed to occur during striatum-dependent habit learning (18, 20–22).

By contrast, we did not detect differences between *Foxp2*$^{hum/hum}$ mice and their WT siblings in either of these learning systems when they were tested individually. The two genotypes exhibited equivalent procedural/response-based learning as assessed with the accelerating rotarod protocol, the tilted running wheel test, the T-maze protocol in which extramaze cues had been removed, and the procedural/response-based version of the cross-maze task. We also did not observe a difference in the place-based learning of the *Foxp2*$^{hum/hum}$ mice, which we tested in the declarative/place-based version of the cross-maze task. Only when both learning systems could be engaged in parallel and could interact during the early acquisition phase of learning, as in the T-maze task with extramaze cues, did the humanized Foxp2 mice exhibit more efficient learning. By challenging this interaction between the learning systems with the abrupt shift from declarative/place-based to procedural/response-based learning in the cross-maze task, we found that the more rapid learning in the humanized Foxp2 mice could reflect a faster transition from declarative to response learning.

We next tested the possibility that such a change in learning dynamics could reflect differential effects of the Foxp2 humanization on the dorsomedial and dorsolateral striatum, nodes in circuits that differently support these learning forms.

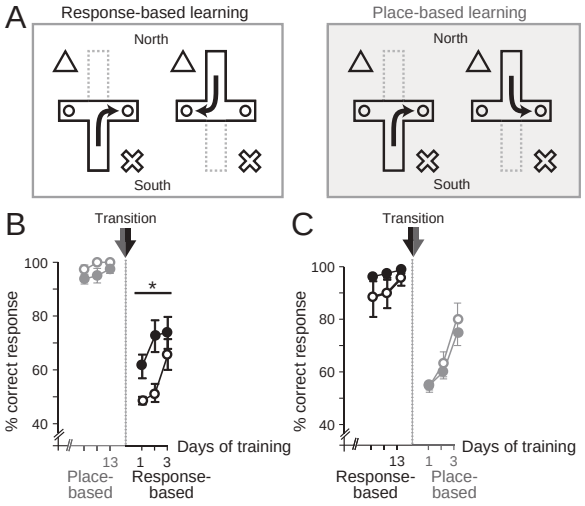


Fig. 2. *Foxp2*$^{hum/hum}$ mice exhibit enhanced ability to make transitions from a declarative to a procedural mode of learning. (*A*) Response-based/procedural (*Left*) and place-based/declarative (*Right*) versions of the cross-maze task. (*B* and *C*) Average percent correct responses (±SEM) for *Foxp2*$^{hum/hum}$ (filled dots) and *Foxp2*$^{wt/wt}$ (open dots) mice successively trained on the two cross-maze task versions and tested on the switch to response-based/procedural (*B*) or place-based/declarative version (*C*) (*$P < 0.05$).

Differential Effects of Humanized Foxp2 on mRNA Expression Profiles in the Dorsomedial and Dorsolateral Striatum. To test the possibility that humanized Foxp2 might influence the dorsomedial and the dorsolateral striatum differently, we isolated striatal samples from each subregion by laser capture microdissection in adult *Foxp2*$^{hum/hum}$ mice and WT littermates ($n = 11$–12 per genotype) and obtained profiles of mRNA expression with >20 million RNA-Sequencing (Seq) reads per sample. We found many differences between the mRNAs in the two regions [5,895 of 25,259 detected genes with a false discovery rate (FDR) < 0.05; $P_{permutations} < 0.001$], but no single gene differed between genotypes (no genes with an FDR < 0.1; $P_{permutations} = 0.17$). This result indicated that the introduction of humanized Foxp2 does not produce massive changes in the expression profile of striatal cells at the level of single genes.

We did detect a significant effect of humanized Foxp2 at the level of functional gene categories, in particular, a down-regulation of genes in the dorsomedial striatum (1,485 of 3,930 categories at an FDR < 0.05; $P_{permutations} = 0.013$; Dataset S1). The most significant category affected was "signaling," and the strongest enrichment was found for "neurotransmitter transporter activity" and many categories involved in synaptic regulatory processes (Fig. S5 and Dataset S1). Effects in the dorsolateral striatum were often smaller and nonsignificant (914 of 3,930 categories at an FDR < 0.05; $P_{permutations} = 0.08$). Thus, we detected differential effects of humanized Foxp2 on genes involved in synaptic regulatory processes in the two striatal regions. These subtle molecular effects could reflect important physiological alterations, if present in a

subset of cells or if produced by differential inputs to the two striatal districts.

Humanized Foxp2 Influences Dopamine Levels Differently in the Dorsomedial and Dorsolateral Striatum. To explore such potential physiological consequences of the Foxp2 humanization, we next analyzed striatal dopamine levels, which are known to be related to learning and to be reduced in striatal samples spanning the dorsomedial and dorsolateral regions in *Foxp2*$^{hum/hum}$ mice (4). Dopamine levels in the dorsomedial striatum of the *Foxp2*$^{hum/hum}$ mice were reduced to 70% of those found in WT control mice (n = 10–22 per genotype; t test, $t_{30} = 3.7$; $P_{GT} = 0.001$), whereas dopamine levels in the dorsolateral striatum were similar in the two genotypes (n = 9–22 per genotype; t test, $t_{29} = 0.7$; $P_{GT} = 0.5$). Thus, humanized Foxp2 influences dopamine levels differently in the sensorimotor and associative regions of the dorsal striatum, reducing them dorsomedially (RMA, $F_{1,29} = 5.73$, $P_{GT*region} = 0.02$; Fig. 3*A*).

Humanized Foxp2 Influences Induction of LTD Differently in the Dorsomedial and Dorsolateral Striatum. To explore potential electrophysiological effects of the Foxp2 humanization, we measured in acute brain slices the induction of dopamine-dependent long-term depression (LTD) after high-frequency stimulation (HFS) in medium spiny neurons (MSNs) located in the dorsolateral and dorsomedial striatum (n = 9–19 cells per genotype and striatal region). In the *Foxp2*$^{hum/hum}$ mice, LTD in the dorsolateral striatum was stronger than that in WT controls (Fig. 3*D*), in accordance with previous results (4, 5). However, in the dorsomedial striatum, LTD tended to be weaker in the *Foxp2*$^{hum/hum}$ mice relative to that in WT controls (Fig. 3*C*), indicating again the presence of a region-specific effect of humanized Foxp2 (n = 9–19; ANOVA, $F_{1,52} = 5.9$, $P_{GT*region} = 0.02$; Fig. 3*B*).

To determine the mechanistic basis of the stronger LTD in the dorsolateral striatum of the *Foxp2*$^{hum/hum}$ mice, we first compared

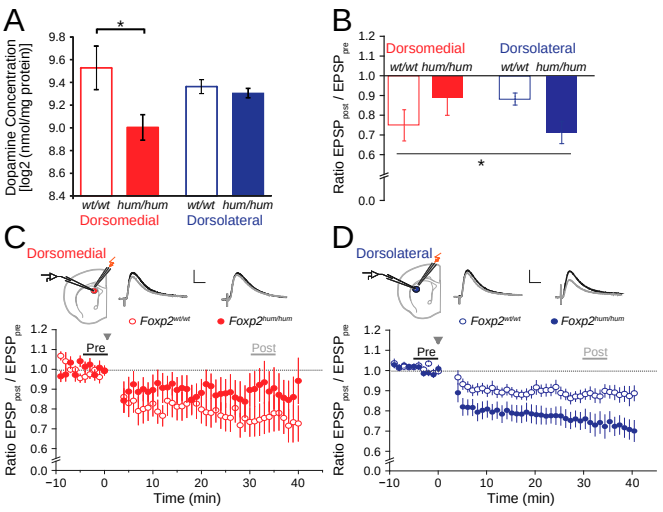


Fig. 3. *Foxp2*$^{hum/hum}$ mice exhibit differential effects of dopamine levels and synaptic plasticity in the dorsomedial and the dorsolateral striatum. (*A*) Average (±SEM) concentrations of dopamine in dorsomedial (red) and dorsolateral (blue) striatal biopsies of *Foxp2*$^{hum/hum}$ mice (*hum/hum*) relative to WT (*wt/wt*) levels (*P < 0.05). (*B*) Averaged excitatory postsynaptic responses (±SEM) in dorsomedial and dorsolateral MSNs in mutant and WT mice 30–40 min after HFS to induce LTD, normalized to baseline levels (*P < 0.05). (*C* and *D*) Recording location, representative traces, and time course of LTD induction (post; mean amplitudes ± SEM), normalized to baseline levels (pre) and after stimulation in the dorsomedial (*C*) and dorsolateral (*D*) striatum. (Scale bars: 2 mV and 10 ms.)

our protocol, involving a modest −70-mV depolarization during induction, vs. the commonly used HFS-LTD protocol in which stronger depolarization to −15 mV (37) favors the activation of voltage-gated calcium channels (38, 39). When we used the strong depolarization, the genotype difference disappeared. We also observed robust LTD in WT mice (n = 7–17 per LTD protocol; ANOVA, $F_{1,22} = 10.1$, $P = 0.004$; Fig. 4 *A* and *B*), and the magnitude of this LTD was similar to that in the *Foxp2*$^{hum/hum}$ mice (n = 7–8 per genotype; ANOVA, $F_{1,13} = 0.28$, $P = 0.6$). This result indicates that LTD is more readily inducible in MSNs of the dorsolateral striatum of the *Foxp2*$^{hum/hum}$ mice and requires less depolarization than LTD in the corresponding region of the WT.

We next tested whether the readily inducible LTD in *Foxp2*$^{hum/hum}$ mice is based on the dopamine D2 receptor (D2R)-dependent striatal mechanism that has been consistently described for LTD in WT mice (38, 40). Applying the D2R antagonist sulpiride to the slice bath eliminated LTD induction in the *Foxp2*$^{hum/hum}$ mice (n = 6–19 per treatment; ANOVA, $F_{1,22} = 5.5$, $P = 0.03$; Fig. 4 *C* and *D*), suggesting that the effect of humanized Foxp2 on striatal LTD depends on D2R-associated mechanisms.

We tested the alternative possibility that the LTD difference could be the result of a confounding effect of long-term potentiation (LTP) present only in WT mice. LTP in striatal MSNs is considered to be mediated by NMDA receptors and is consistently reported to be blocked by APV (38, 41). Therefore, we antagonized NMDA receptors by adding extracellular APV (50 μM) to the bath solution under the modest −70-mV depolarization conditions. The responses in the dorsolateral striatum were not lowered by APV application in the WT mice, excluding the possibility of a confounding LTP effect (n = 5–17; ANOVA, $F_{1,20} = 0.32$, $P = 0.58$; Fig. 4 *C* and *D*). By contrast, in the dorsolateral striatum of the *Foxp2*$^{hum/hum}$ mice, NMDA receptor inhibition abolished the readily inducible, weak-depolarization LTD, so that the response in humanized mice was no longer distinguishable from WT (n = 10–17 per genotype and treatment; ANOVA, $F_{1,25} = 0.42$, $P = 0.52$; Fig. 4 *C* and *D*).

To determine whether this extracellular NMDA receptor blockade in the *Foxp2*$^{hum/hum}$ mice resulted from effects at the presynaptic level or from the actions of postsynaptic receptors on the MSNs themselves, we added the NMDA channel blocker MK801 (1 mM) to the intracellular solution. This treatment blocked the readily inducible LTD in humanized MSNs (n = 5–19 per treatment; $F_{1,22} = 4.3$, $P = 0.04$; Fig. 4*D*), suggesting that, under low-depolarization conditions, postsynaptic NMDA receptor activation accounts for LTD induction in the *Foxp2*$^{hum/hum}$ mice. Our findings thus implicate the humanized form of Foxp2 in enhancing a mechanism of LTD induction in the dorsolateral striatum by means of postsynaptic NMDA receptors. At present, we do not assume a specific increase in NMDA receptors to be responsible for this increased modulation, as the ratio of NMDA to AMPA currents remains unaltered in the *Foxp2*$^{hum/hum}$ mice (Fig. S6*B*).

Discussion

Our findings suggest a striking selectivity in the effects of humanized Foxp2 on behavioral learning dynamics as well as on striatal dopamine levels, gene expression levels, and synaptic plasticity. Based on our experimental findings, we suggest as a working hypothesis that humanized Foxp2 differentially influences the functional contributions of the associative and sensorimotor striatum to learning dynamics (Fig. S7). In this view, the *Foxp2*$^{hum/hum}$ mice exhibited an altered interaction between the declarative and procedural learning strategies, favoring the procedural system when both learning systems were engaged as indicated by their more rapid transition toward procedural behavior in the cue-enriched conditional T-maze task and in the place-to-response switching cross-maze task. This condition

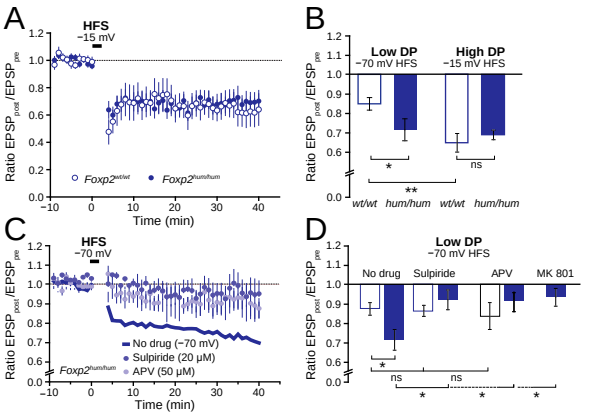


Fig. 4. The enhanced LTD in dorsolateral MSNs of *Foxp2*$^{hum/hum}$ mice is specific for LTD induction under low depolarization (DP) conditions and depends on D2Rs and NMDA channels. (*A*) HFS (gray arrow) with depolarization to −15 mV instead of weaker depolarization of −70 mV (Fig. 3 *B*–*D*) induced comparable LTD in control and mutant mice during the 40 min post-HFS period. (*B*) Changing from HFS with weak depolarization (−70 mV) to high depolarization conditions (−15 mV) enhanced mean LTD levels measured at 30–40 min after the HFS in WT but not in mutant mice. Error bars indicate SEM (**P* < 0.05 and ***P* < 0.005). ns, not significant. (*C*) Readily inducible LTD under low depolarization conditions in dorsolateral MSNs of *Foxp2*$^{hum/hum}$ mice is abolished by the D2R antagonist sulpiride or by external application of the NMDA receptors antagonist APV. Shown are excitatory postsynaptic potential amplitudes (post), normalized to the mean baseline levels (pre), after HFS in the low depolarization condition of −70 mV (gray arrow) in the presence of sulpiride (20 μM) or APV (50 μM). (*D*) In mutant mice, the readily inducible LTD measured 30–40 min after HFS under low depolarization conditions can be reversed to WT levels by blocking D2Rs by sulpiride or NMDA receptors with extracellular APV or intracellular MK801 (1 mM; electrode solution). Recordings in the presence of sulpiride, APV, or MK801 were not different from control recordings obtained without HFS stimulation (n = 5–17; ANOVA, P = 0.32–0.98; Fig. S6*A*). Error bars indicate SEM (**P* < 0.05). ns, not significant.

would contrast with WT conditions, in which the declarative system is thought to dominate and render the naturally occurring transition toward the procedural learning system less than maximally efficient (17, 19, 25).

How this behavioral change in the *Foxp2*$^{hum/hum}$ mice is brought about is not clear. However, the modest effects of humanized Foxp2 on gene expression patterns suggest that generalized molecular or cellular reconfigurations of striatal MSNs are not involved. The region-specific effects of humanized Foxp2 on dopamine content and synaptic plasticity could reflect mechanisms directly related to the behavioral effects, given the differential function of the dorsomedial and dorsolateral striatum in place-based/declarative and response-based/procedural forms of learning. Our electrophysiological recordings indicate a region-specific enhancement of readily inducible LTD in the *Foxp2*$^{hum/hum}$ mice. This form of LTD followed the D2R-dependent mechanism identified for classical strong-depolarization induction protocols (40), but required the activation of NMDA receptors. Such a mechanism has been described in other brain regions (42), but, in the striatum, has been linked mainly, but not exclusively, to the induction of LTP, not LTD (38, 40, 41, 43, 44). Given that the unaltered ratio between NMDA and AMPA currents indicated no increase in NMDA receptors of *Foxp2*$^{hum/hum}$ mice, and that dopamine is critical for striatal synaptic plasticity, one alternative is that an altered dopamine-dependent modulation of NMDA receptors could be responsible for the humanized effect we observed in these mice (45–47).

The contrasting effects in the dorsomedial and dorsolateral striatum of *Foxp2*$^{hum/hum}$ mice are striking given that different regional brain-imaging activation patterns have been reported for what are considered as homologous striatal districts in humans lacking one functional copy of *FOXP2* (28, 29). How these findings relate to the effect of humanized version of Foxp2 in shaping the development of a human brain to enable traits such as language and speech acquisition is unknown. The relation between declarative and procedural learning strategies and language learning is itself unclear (10–12). One possibility raised by our findings is that efficient proceduralization might accelerate probabilistic learning of language features (10) by chunking single speech and language-related actions into sequences, a chunking function that has been suggested to be a core property of the striatum in experimental work (48, 49). If so, such a process could free up declarative capacities by implementing procedural components at earlier time points. Our findings prompt the intriguing speculation that the humanization of this gene imparted a facilitated ability to use procedural forms of learning and therefore to shift more rapidly from declarative to procedural forms of learning, a change that could have been important for the emergence of proficient language and speech.

Materials and Methods

Additional description of study materials and methods is provided in *SI Materials and Methods*.

Animals. A total of 303 *Foxp2*$^{hum/hum}$ mice [5H10 line (4); 1.8–15.2 mo; postnatal day (P)21–P53 for electrophysiological experiments] and WT littermates (160 for behavioral tests, 23 for gene expression assays, 32 for dopamine measurements, and 88 for electrophysiology experiments) were used, and they were balanced for genotype and sex in each experiment. Behavioral procedures were approved by the Committee on Animal Care at the Massachusetts Institute of Technology, and other procedures were in accordance with the United Kingdom Animals (Scientific Procedures) Act of 1986 and guidelines of the Max Planck Institute for Evolutionary Anthropology and federal regulations of Saxony, Germany.

Behavioral Experiments. Rotarod and tilted running wheel experiments were conducted as previously described (31). For the maze experiments, mice were food-restricted and were habituated to apparatus and reward (chocolate milk). They were then trained on a T-maze (40 trials each day) to obtain reward on the correct goal arm as instructed by tactile conditional cues (rough or smooth floor surface) or on a cross maze (10 trials each day) to go to a specific goal (place-based version) or to make a particular turn (response-based version) to receive reward. Statistical analysis was performed by using RMA and generalized linear mixed models (*SI Materials and Methods*).

Laser Capture Microdissection and RNA Sequencing. The dorsomedial and dorsolateral striatum of adult mice was dissected from brain slices by using a laser microscope (P.A.L.M. System; Zeiss). Twenty-five nanograms total RNA were used to construct barcoded mRNA-Seq libraries that were sequenced on a Genome Analyzer IIx platform as described earlier (50). Gene expression analysis was performed by the multifactor model of the R package for differential expression analysis for sequence count data (51). Effects of humanized Foxp2 were summarized by the π-value that multiplies the magnitude and significance of genotype effect (52). This ranking was used for the Wilcoxon rank test implemented in FUNC (https://func.eva.mpg.de/) (53) to identify enriched Gene Ontology categories. Permutations of genotype labels were used to assess global significance ($P_{permutations}$).

Dopamine Content. Tissue samples from 1 mm cryocut slabs of the dorsomedial and the dorsolateral striatum were homogenized, and their protein content was measured. Dopamine was detected at an electrode potential of 0.8 V. Statistical analyses were performed on log2-transformed dopamine amounts per milligram of protein normalized per region, sex, and batch.

In Situ Electrophysiology. Coronal striatal slices (250 μm) were prepared from P21–P53 mice, and responses of MSNs to stimulation of cortical afferents (0.33–0.2 Hz) were measured during periods before (15 min) and after (40 min) a tetanic HFS (4 × 100 Hz, at −70 or at −15 mV) in the presence of the GABA(A) receptor blocker SR95531 (GABAzine) by using a whole-cell patch-clamp setup. We applied one- and two-way ANOVAs to test region- and genotype-specific effects.

ACKNOWLEDGMENTS. We thank H. F. Hall for his expert help with experimental equipment manufacture and setup; H. Jenny, D. Hu, and C. Ye for help with behavioral experiments; J. P. De Bono for assistance with

the running wheel experiments; I. Bliesener and V. Wiebe for technical support; A. McWhinnie for help with graphics; N. Cohen for reading the manuscript; and K. S. Smith and Y. Kubota for help with editing the manuscript. This work was funded by the Nancy Lurie Marks Family Foundation, the Simons Foundation Autism Research Initiative Grant137593, National Institutes of Health Grant R01 MH060379, the Wellcome Trust Grants 075491/Z/04 and 080971, the Fondation pour la Recherche Médicale, and the Max Planck Society.

1. Vargha-Khadem F, Gadian DG, Copp A, Mishkin M (2005) FOXP2 and the neuroanatomy of speech and language. *Nat Rev Neurosci* 6(2):131–138.
2. Enard W, et al. (2002) Molecular evolution of FOXP2, a gene involved in speech and language. *Nature* 418(6900):869–872.
3. Enard W (2011) FOXP2 and the role of cortico-basal ganglia circuits in speech and language evolution. *Curr Opin Neurobiol* 21(3):415–424.
4. Enard W, et al. (2009) A humanized version of Foxp2 affects cortico-basal ganglia circuits in mice. *Cell* 137(5):961–971.
5. Reimers-Kipping S, Hevers W, Pääbo S, Enard W (2011) Humanized Foxp2 specifically affects cortico-basal ganglia circuits. *Neuroscience* 175:75–84.
6. Graybiel AM (2008) Habits, rituals, and the evaluative brain. *Annu Rev Neurosci* 31: 359–387.
7. Bolhuis JJ, Okanoya K, Scharff C (2010) Twitter evolution: Converging mechanisms in birdsong and human speech. *Nat Rev Neurosci* 11(11):747–759.
8. Friederici AD (2006) What's in control of language? *Nat Neurosci* 9(8):991–992.
9. Nicolson RI, Fawcett AJ (2007) Procedural learning difficulties: Reuniting the developmental disorders? *Trends Neurosci* 30(4):135–141.
10. Romberg AR, Saffran JR (2010) Statistical learning and language acquisition. *Wiley Interdiscip Rev Cogn Sci* 1(6):906–914.
11. Teichmann M, Dupoux E, Cesaro P, Bachoud-Lévi AC (2008) The role of the striatum in sentence processing: Evidence from a priming study in early stages of Huntington's disease. *Neuropsychologia* 46(1):174–185.
12. Ullman MT (2004) Contributions of memory circuits to language: the declarative/procedural model. *Cognition* 92(1-2):231–270.
13. Eichenbaum H, Cohen NJ (2001) *From Conditioning to Conscious Recollection. Memory Systems of the Brain* (Oxford Univ Press, New York).
14. Moussa R, Poucet B, Amalric M, Sargolini F (2011) Contributions of dorsal striatal subregions to spatial alternation behavior. *Learn Mem* 18(7):444–451.
15. Packard MG, McGaugh JL (1996) Inactivation of hippocampus or caudate nucleus with lidocaine differentially affects expression of place and response learning. *Neurobiol Learn Mem* 65(1):65–72.
16. Tolman EC, Ritchie BF, Kalish D (1947) Studies in spatial learning; response learning vs. place learning by the non-correction method. *J Exp Psychol* 37(4):285–292.
17. White NM (2009) Some highlights of research on the effects of caudate nucleus lesions over the past 200 years. *Behav Brain Res* 199(1):3–23.
18. Miyachi S, Hikosaka O, Lu X (2002) Differential activation of monkey striatal neurons in the early and late stages of procedural learning. *Exp Brain Res* 146(1):122–126.
19. White NM, McDonald RJ (2002) Multiple parallel memory systems in the brain of the rat. *Neurobiol Learn Mem* 77(2):125–184.
20. Yin HH, Knowlton BJ (2006) The role of the basal ganglia in habit formation. *Nat Rev Neurosci* 7(6):464–476.
21. Yin HH, et al. (2009) Dynamic reorganization of striatal circuits during the acquisition and consolidation of a skill. *Nat Neurosci* 12(3):333–341.
22. Thorn CA, Atallah H, Howe M, Graybiel AM (2010) Differential dynamics of activity changes in dorsolateral and dorsomedial striatal loops during learning. *Neuron* 66(5): 781–795.
23. Dagher A, Owen AM, Boecker H, Brooks DJ (2001) The role of the striatum and hippocampus in planning: A PET activation study in Parkinson's disease. *Brain* 124(Pt 5): 1020–1032.
24. Moody TD, Bookheimer SY, Vanek Z, Knowlton BJ (2004) An implicit learning task activates medial temporal lobe in patients with Parkinson's disease. *Behav Neurosci* 118(2):438–442.
25. Chang Q, Gold PE (2004) Inactivation of dorsolateral striatum impairs acquisition of response learning in cue-deficient, but not cue-available, conditions. *Behav Neurosci* 118(2):383–388.
26. Voorn P, Vanderschuren LJ, Groenewegen HJ, Robbins TW, Pennartz CM (2004) Putting a spin on the dorsal-ventral divide of the striatum. *Trends Neurosci* 27(8): 468–474.
27. Miyachi S, Hikosaka O, Miyashita K, Kárádi Z, Rand MK (1997) Differential roles of monkey striatum in learning of sequential hand movement. *Exp Brain Res* 115(1):1–5.
28. Liégeois F, et al. (2003) Language fMRI abnormalities associated with FOXP2 gene mutation. *Nat Neurosci* 6(11):1230–1237.
29. Vargha-Khadem F, et al. (1998) Neural basis of an inherited speech and language disorder. *Proc Natl Acad Sci USA* 95(21):12695–12700.
30. French CA, et al. (2012) An aetiological Foxp2 mutation causes aberrant striatal activity and alters plasticity during skill learning. *Mol Psychiatry* 17(11):1077–1085.
31. Groszer M, et al. (2008) Impaired synaptic plasticity and motor learning in mice with a point mutation implicated in human speech deficits. *Curr Biol* 18(5):354–362.
32. Thorn CA, Graybiel AM (2014) Differential entrainment and learning-related dynamics of spike and local field potential activity in the sensorimotor and associative striatum. *J Neurosci* 34(8):2845–2859.
33. Middei S, Restivo L, Sgobio C, Passino E, Ammassari-Teule M (2004) Reversible inactivation of hippocampus and dorsolateral striatum in C57BL/6 and DBA/2 inbred mice failed to show interaction between memory systems in these genotypes. *Behav Brain Res* 154(2):527–534.
34. Passino E, Middei S, Restivo L, Bertaina-Anglade V, Ammassari-Teule M (2002) Genetic approach to variability of memory systems: Analysis of place vs. response learning and fos-related expression in hippocampal and striatal areas of C57BL/6 and DBA/2 mice. *Hippocampus* 12(1):63–75.
35. Pittenger C, et al. (2006) Impaired bidirectional synaptic plasticity and procedural memory formation in striatum-specific cAMP response element-binding protein-deficient mice. *J Neurosci* 26(10):2808–2813.
36. Tolman EC, Ritchie BF, Kalish D (1946) Studies in spatial learning; place learning versus response learning. *J Exp Psychol* 36:221–229.
37. Gerdeman GL, Ronesi J, Lovinger DM (2002) Postsynaptic endocannabinoid release is critical to long-term depression in the striatum. *Nat Neurosci* 5(5):446–451.
38. Calabresi P, Maj R, Pisani A, Mercuri NB, Bernardi G (1992) Long-term synaptic depression in the striatum: Physiological and pharmacological characterization. *J Neurosci* 12(11):4224–4233.
39. Choi S, Lovinger DM (1997) Decreased probability of neurotransmitter release underlies striatal long-term depression and postnatal development of corticostriatal synapses. *Proc Natl Acad Sci USA* 94(6):2665–2670.
40. Kreitzer AC, Malenka RC (2005) Dopamine modulation of state-dependent endocannabinoid release and long-term depression in the striatum. *J Neurosci* 25(45): 10537–10545.
41. Partridge JG, Tang KC, Lovinger DM (2000) Regional and postnatal heterogeneity of activity-dependent long-term changes in synaptic efficacy in the dorsal striatum. *J Neurophysiol* 84(3):1422–1429.
42. Jurado S, Biou V, Malenka RC (2010) A calcineurin/AKAP complex is required for NMDA receptor-dependent long-term depression. *Nat Neurosci* 13(9):1053–1055.
43. Pawlak V, Kerr JN (2008) Dopamine receptor activation is required for corticostriatal spike-timing-dependent plasticity. *J Neurosci* 28(10):2435–2446.
44. Spencer JP, Murphy KP (2000) Bi-directional changes in synaptic plasticity induced at corticostriatal synapses in vitro. *Exp Brain Res* 135(4):497–503.
45. Cepeda C, Colwell CS, Itri JN, Chandler SH, Levine MS (1998) Dopaminergic modulation of NMDA-induced whole cell currents in neostriatal neurons in slices: contribution of calcium conductances. *J Neurophysiol* 79(1):82–94.
46. Sarantis K, Antoniou K, Matsokis N, Angelatou F (2012) Exposure to novel environment is characterized by an interaction of D1/NMDA receptors underlined by phosphorylation of the NMDA and AMPA receptor subunits and activation of ERK1/2 signaling, leading to epigenetic changes and gene expression in rat hippocampus. *Neurochem Int* 60(1):55–67.
47. Snyder GL, Fienberg AA, Huganir RL, Greengard P (1998) A dopamine/D1 receptor/protein kinase A/dopamine- and cAMP-regulated phosphoprotein (Mr 32 kDa)/protein phosphatase-1 pathway regulates dephosphorylation of the NMDA receptor. *J Neurosci* 18(24):10297–10303.
48. Graybiel AM (1998) The basal ganglia and chunking of action repertoires. *Neurobiol Learn Mem* 70(1-2):119–136.
49. Jog MS, Kubota Y, Connolly CI, Hillegaart V, Graybiel AM (1999) Building neural representations of habits. *Science* 286(5445):1745–1749.
50. Wunderlich S, et al. (2014) Primate iPS cells as tools for evolutionary analyses. *Stem Cell Res (Amst)* 12(3):622–629.
51. Anders S, Huber W (2010) Differential expression analysis for sequence count data. *Genome Biol* 11(10):R106.
52. Xiao Y, et al. (2014) A novel significance score for gene selection and ranking. *Bioinformatics* 30(6):801–807.
53. Prüfer K, et al. (2007) FUNC: A package for detecting significant associations between gene sets and ontological annotations. *BMC Bioinformatics* 8:41.

ATTACHMENT C

Boyd et al.,

Human-Chimpanzee Differences in a FZD8 Enhancer Alter Cell-Cycle Dynamics in the Developing Neocortex,

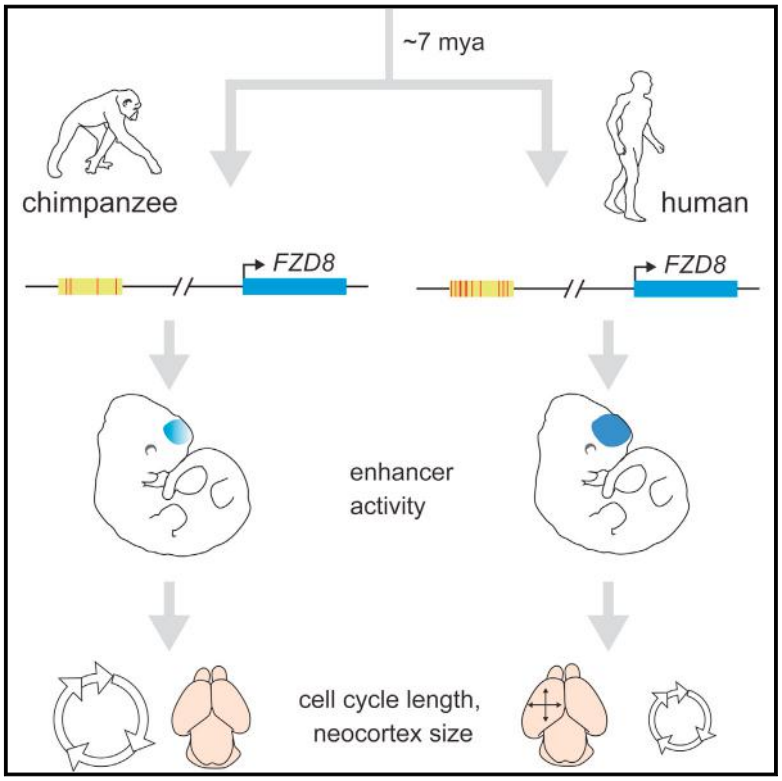
CURRENT BIOLOGY 25, pp.772-770 (March 16, 2014)

Report

Current Biology

Human-Chimpanzee Differences in a *FZD8* Enhancer Alter Cell-Cycle Dynamics in the Developing Neocortex

Graphical Abstract



Authors

J. Lomax Boyd, Stephanie L. Skove, ..., Gregory A. Wray, Debra L. Silver

Correspondence

debra.silver@duke.edu

In Brief

This study reports the discovery of a human-accelerated enhancer of *Fzd8* functioning in brain development. Boyd et al. demonstrate species-specific activity differences and show that the human enhancer promotes a faster progenitor cell cycle and increased neocortical size. Enhancer sequence changes may contribute to unique features of the human brain.

Highlights

- Discovery of a human-accelerated enhancer functioning in the developing neocortex
- Compared to chimpanzee, human *HARE5* drives earlier and more robust brain expression
- The *HARE5* locus physically contacts the core promoter of the WNT receptor, *Fzd8*
- *HARE5*::*Fzd8* mice have an accelerated neural progenitor cell cycle and enlarged brains

Boyd et al., 2015, Current Biology *25*, 772–779
March 16, 2015
http://dx.doi.org/10.1016/j.cub.2015.01.041

Current Biology 25, 772–779, March 16, 2015 ©2015 Elsevier Ltd All rights reserved http://dx.doi.org/10.1016/j.cub.2015.01.041

Report

Human-Chimpanzee Differences in a *FZD8* Enhancer Alter Cell-Cycle Dynamics in the Developing Neocortex

J. Lomax Boyd,[1] Stephanie L. Skove,[1] Jeremy P. Rouanet,[1] Louis-Jan Pilaz,[1] Tristan Bepler,[2] Raluca Gordân,[2,3] Gregory A. Wray,[2,4,5] and Debra L. Silver[1,6,7,8,*]
[1]Department of Molecular Genetics and Microbiology, Duke University Medical Center, Durham, NC 27710, USA
[2]Center for Genomic and Computational Biology, Duke University, Durham, NC 27708, USA
[3]Department of Biostatistics and Bioinformatics, Duke University Medical Center, Durham, NC 27710, USA
[4]Department of Biology, Duke University, Durham, NC 27708, USA
[5]Department of Evolutionary Anthropology, Duke University, Durham, NC 27708, USA
[6]Department of Cell Biology, Duke University Medical Center, Durham, NC 27710, USA
[7]Department of Neurobiology, Duke University Medical Center, Durham, NC 27710, USA
[8]Duke Institute for Brain Sciences, Durham, NC 27710 USA

Summary

The human neocortex differs from that of other great apes in several notable regards, including altered cell cycle, prolonged corticogenesis, and increased size [1–5]. Although these evolutionary changes most likely contributed to the origin of distinctively human cognitive faculties, their genetic basis remains almost entirely unknown. Highly conserved non-coding regions showing rapid sequence changes along the human lineage are candidate loci for the development and evolution of uniquely human traits. Several studies have identified human-accelerated enhancers [6–14], but none have linked an expression difference to a specific organismal trait. Here we report the discovery of a human-accelerated regulatory enhancer (*HARE5*) of *FZD8*, a receptor of the Wnt pathway implicated in brain development and size [15, 16]. Using transgenic mice, we demonstrate dramatic differences in human and chimpanzee *HARE5* activity, with human *HARE5* driving early and robust expression at the onset of corticogenesis. Similar to *HARE5* activity, *FZD8* is expressed in neural progenitors of the developing neocortex [17–19]. Chromosome conformation capture assays reveal that *HARE5* physically and specifically contacts the core *Fzd8* promoter in the mouse embryonic neocortex. To assess the phenotypic consequences of *HARE5* activity, we generated transgenic mice in which *Fzd8* expression is under control of orthologous enhancers (*Pt-HARE5::Fzd8* and *Hs-HARE5::Fzd8*). In comparison to *Pt-HARE5::Fzd8*, *Hs-HARE5::Fzd8* mice showed marked acceleration of neural progenitor cell cycle and increased brain size. Changes in *HARE5* function unique to humans thus alter the cell-cycle dynamics of a critical population of stem cells during corticogenesis and may underlie some distinctive anatomical features of the human brain.

*Correspondence: debra.silver@duke.edu

Results

Identification of Human-Accelerated Enhancer Loci in the Developing Neocortex

The dramatic expansion of the neocortex during hominoid evolution is proposed to underlie the emergence of our uniquely human cognitive abilities [20–22], although strong genetic correlations between these traits have remained elusive [23]. The evolution of human cortical features, such as enlarged brain size, has been attributed to cellular changes including neuron number and neural progenitor cell cycle [1–5, 15]. However, the genetic basis for these traits, which so markedly distinguish humans from other primates, remains poorly understood. Mutations within regulatory elements have been proposed to play a significant role in the evolution of human-specific traits [24, 25]. Recent genomic studies support this notion and have collectively identified highly conserved non-coding regions that are rapidly evolving along the human lineage [6–10]. Of note, these human-accelerated non-coding loci are frequently located nearby genes implicated in brain development and function [11, 26, 27]. Together, these studies suggest that the evolution of human neocortical traits may have occurred through modification of *cis*-regulatory enhancers involved in brain development. Yet to date, just a handful of human-accelerated regions have been shown to function as forebrain enhancers [11–13], and none have been shown to impact neocortical expansion. Here we sought to discover human-accelerated regulatory loci important for corticogenesis in order to gain insights into the genetic basis for the evolution of uniquely human brain features.

We identified *HARE5* from an in silico screen for rapidly evolving human non-coding regions predicted to function as developmental enhancers in the mammalian neocortex (Figures S1A and S1B, Table S1, and the Supplemental Experimental Procedures) [6–8, 28, 29]. Using a standard mouse transient transgenic assay [11, 14], we found that *HARE5* reporter activity was robust in the lateral neocortex and dorso-lateral midbrain (15/15 embryos) (Figures 1A and S1C). *HARE5* was prioritized due to this enhancer activity and its chromosomal location adjacent to Frizzled 8 (*FZD8*), a receptor for the Wnt signaling pathway that is implicated in neocortical development (Figure 1B) [15–18, 30, 31]. The *Homo sapiens* (*Hs*) *HARE5* ortholog contains 16 changes compared to *Pan troglodytes* (*Pt*). Based on outgroup comparison, ten mutations were fixed on the human branch and six on the chimpanzee branch since the latest common ancestor (Figure 1B). A phylogenetic analysis of the 1.2 Kb *HARE5* locus across several great-ape species revealed a longer branch for the *Hs* ortholog compared to that of *Pt* (Figure 1C). This is consistent with the original signature of positive selection detected in the human relative to chimpanzee lineage [7]. Analysis of predicted transcription factor binding sites across the *HARE5* locus revealed differences, particularly at human-derived mutations, for key transcription factors relevant to corticogenesis (see Table S2) [32]. Together, these results support the prediction that *Hs-HARE5* acquired unique enhancer activity since diverging from the common chimpanzee lineage.

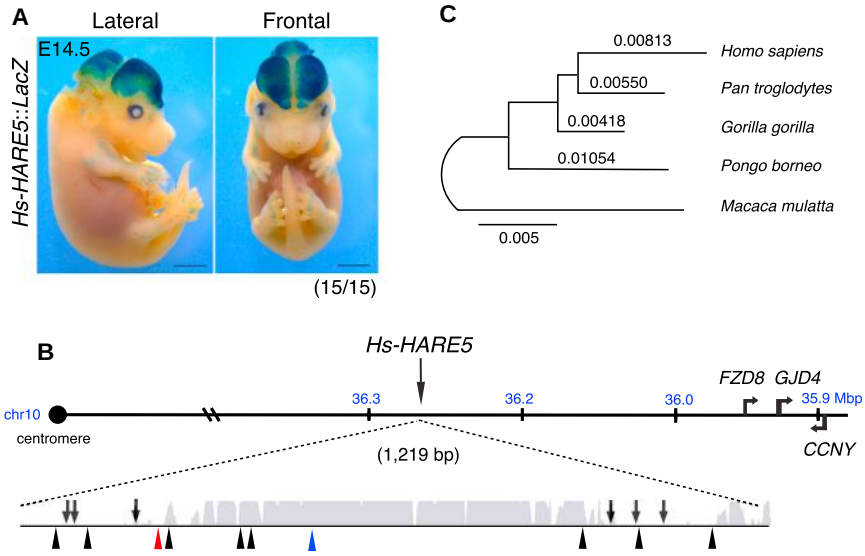


Figure 1. Identification of *Hs-HARE5* as a Human-Accelerated Neocortical Enhancer

(A) Representative E14.5 *Hs-HARE5*::*LacZ* embryo stained for β-galactosidase (LacZ) activity. Scale bars, 2 mm.

(B) Schematic of *Hs-HARE5* locus on human chromosome 10 (hg19). The 1,219-bp-long *HARE5* genomic locus with enhancer activity includes the original 619-bp human-accelerated sequence and flanking 5′ and 3′ sequences. Represented below is a PhastCons conservation track for the *HARE5* locus, shown with the region of high conservation (gray). Also shown are lineage-specific mutations for chimpanzee (six; arrows, above line) and human (ten; arrowheads, bottom), including one Denisovan (red) and one currently identified human polymorphism (blue).

(C) Maximum-likelihood phylogenetic tree for the *HARE5* orthologous locus from five anthropoid primates.

See also Figure S1 and Table S1.

Distinct Enhancer Activity of Human and Chimpanzee *HARE5* in the Developing Neocortex

We postulated that human and chimpanzee *HARE5* might differentially regulate gene expression during corticogenesis. To test this, we generated independent stable mouse transgenic lines (*Pt-HARE5::LacZ* and *Hs-HARE5::LacZ*). Corticogenesis initiates at embryonic day 9.5 (E9.5) and continues to E18.5 [2]. At E9.5, both *Pt-HARE5* and *Hs-HARE5* enhancer activity were undetectable (Figures 2A–2C). However, within a half day of development at E10.0, *Hs-HARE5* activity was rapidly and robustly upregulated in the lateral telencephalon (Figures 2E and 2F). In contrast, *Pt-HARE5* activity in the E10.0 telencephalon was markedly weaker and was limited to more lateral regions (Figures 2D and 2F). This spatial difference in enhancer activity was sustained at E10.5, as evidenced by both whole-mount embryos and coronal brain sections (Figures 2G–2I and S2A–S2D). By E11.5, species-specific differences in *HARE5*-driven LacZ activity were still evident, although they were far less dramatic (Figures 2J–2L). These results indicate that *HARE5* orthologs drive expression in the developing lateral telencephalon. However, relative to chimpanzee, the human enhancer has considerably earlier and robust activity during corticogenesis.

Having established spatial and temporal differences in chimpanzee and human *HARE5* enhancer activity, we next sought a more sensitive and dynamic readout of *HARE5* transcriptional activity. The LacZ protein is stable for at least 48 hr, whereas destabilized fluorescent proteins with PEST motifs are only stable for 2 hr post-translation [33]. We generated new stable transgenic mouse lines, *Pt-HARE5*::*tdTomato-PEST* and *Hs-HARE5*::*EGFP-PEST*, and compared native fluorescence in embryos co-expressing the reporters (Figures 2M and 2N). Both orthologs drove enhancer activity in the E11.0 neocortex; however, *Hs-HARE*::*EGFP* was considerably brighter than *Pt-HARE5*::*tdTomato*, despite tdTomato having intrinsically brighter fluorescent emission than EGFP (Figures 2N–2T) [33]. This reporter difference was sustained at E12.5, though the chimpanzee enhancer remained active (Figures 2U–2AA and S2E–S2H). We quantified enhancer activity by qRT-PCR measurement of reporter transcript levels in E12.5 neocortices. *Hs-HARE5*::*EGFP* embryos showed 10- to 30-fold higher transcript levels than *Pt-HARE5*::*tdTomato* (Figure 2BB). Hence, multiple independent reporter lines (LacZ and fluorescent) demonstrate that, compared to chimpanzee *HARE5*, human *HARE5* drives dramatically higher enhancer activity in the telencephalon.

In the E10.5 telencephalon, the predominant neural progenitor populations are neuroepithelial cells, and by E12.5 these are replaced by radial glia (termed neural stem cells) [2]. At E10.5, both enhancers were active in the majority (about 75%) of Pax6-positive neuroepithelial cells and in some TuJ1-positive neurons (Figures S3I–S3U). At E12.5, reporter activity was highest in the ventricular zone (VZ) (Figure S2E–S2H), where radial glial cells reside. Thus, both human and chimpanzee *HARE5* enhancers are active in neural progenitors of the developing neocortex.

Chromosome Conformation Capture Detects *HARE5-Fzd8* Interactions

Having established *HARE5* activity within the lateral telencephalon, we next sought to identify the likely target gene. The most proximal gene, *Hs-FZD8*, is located 307,758 bp downstream from *HARE5* and was an obvious candidate due to its expression in the developing human and mouse neocortex [17–19, 30, 31]. LacZ reporter activity and *Fzd8* in situ hybridization showed similar expression patterns in E10.5 and E11.5 whole-mount embryos and neocortical sections (Figure S3; http://developingmouse.brain-map.org and http://www.emouseatlas.org) [31]. We used chromosome conformation capture (3C) assays [34] to test for physical association between endogenous mouse (*Mm*) *HARE5* and the core *Fzd8* promoter within E12.5 mouse neocortices (Figure 3A). In neocortices, we observed a strong peak of interaction between *Mm-HARE5* and the proximal *Fzd8* promoter compared to flanking loci (Figure 3B). In contrast, no interactions were evident between *Mm-HARE5* and *Fzd8* in age-matched liver, which lacks detectable *HARE5* activity and *Fzd8* expression. These data indicate that *HARE5* physically and specifically associates with the core *Fzd8* promoter in the developing mouse neocortex. Given the *cis*-regulatory activity of *HARE5* orthologs, we propose that *HARE5* functions as a distal-acting enhancer of *FZD8* during early human neocortical development.

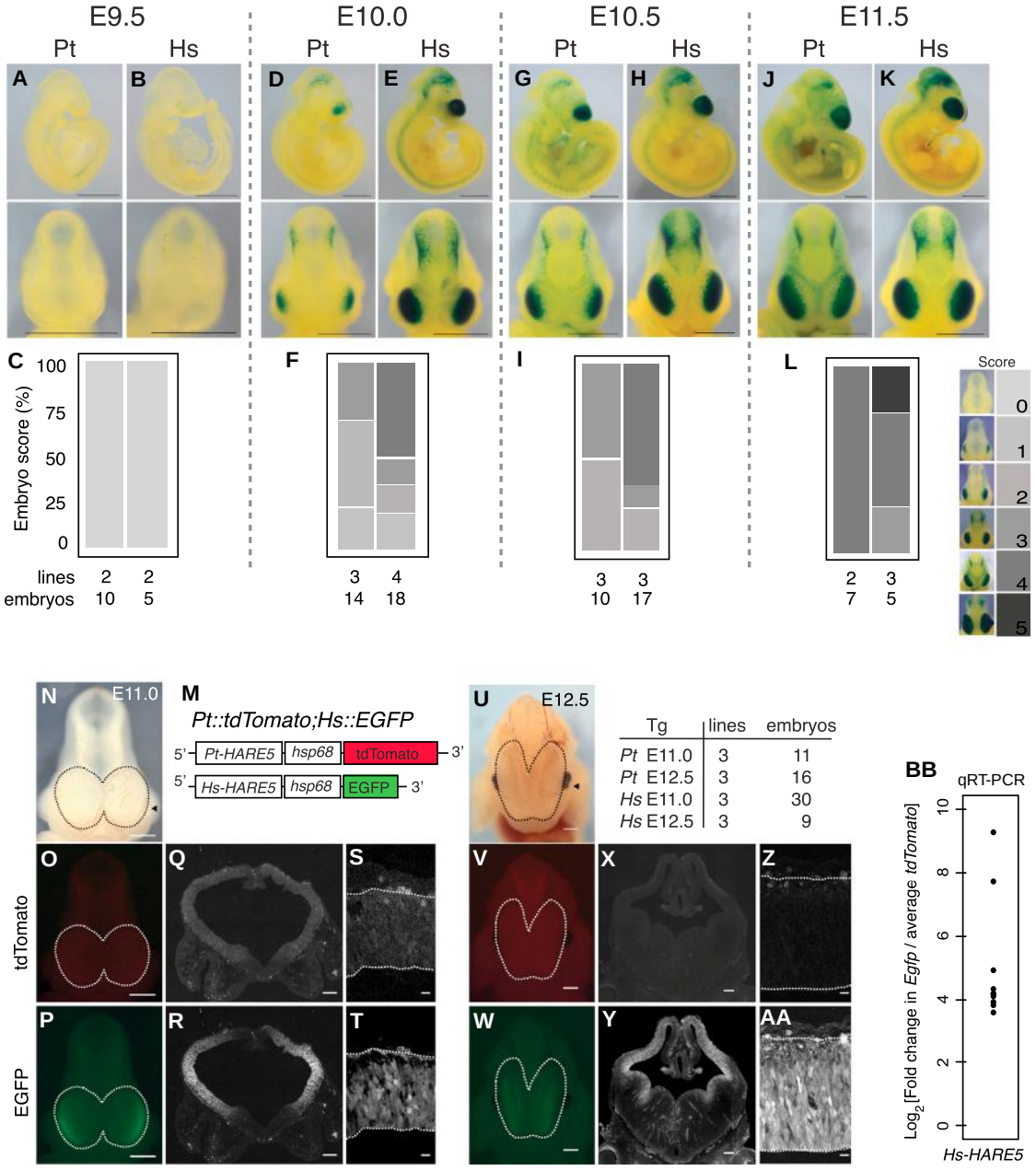


Figure 2. *Hs-HARE5* Activity Drives Robust, Early Enhancer Activity Relative to *Pt-HARE5* during Corticogenesis

(A–L) Developmental time series of *Pt-HARE5::LacZ* (A, D, G, and J) and *Hs-HARE5::LacZ* (B, E, H, and K) reporter activity from stable transgenic lines. Representative images of LacZ stained embryos from lateral (top) and anterior (bottom) views are shown. Enhancer activity was qualitatively scored in the telencephalon, using the indicated scoring schema shown on the right, on a scale from no reporter activity (score 0) to full telencephalic activity (score 5) (C, F, I, and L). The number of embryos and independent transgenic lines analyzed for each stage is listed below. Embryos were scored blindly and independently by at least three individuals.

(M) Schematic of destabilized reporter constructs drawn to scale.

(N–AA) Representative embryos from dual reporter transgenic *Pt-HARE5::tdTomato*; *Hs-HARE5::EGFP* E11.0 (N–T) and E12.5 (U–AA) embryos detected by brightfield (N and U), and endogenous fluorescence for tdTomato (O, Q, S, V, X, and Z) and EGFP (P, R, T, W, Y, and AA) channels. Dotted lines demarcate dorsal neocortices of whole-mount embryos (N–P and U–W).

(Q, R, X, and Y) Coronal sections from mid-cortex (plane indicated by arrowheads in N and U) in tdTomato (Q and X) and EGFP (R and Y) channels.

(S, T, Z, and AA) High-magnification images of the lateral telencephalon for tdTomato (S and Z) and EGFP (T and AA). The number of embryos and lines for each analysis is listed beside (U). Endogenous fluorescence images were captured using identical exposure conditions.

(BB) Graph depicting log fold changes for qRT-PCR from E12.5 neocortices. Each data point is the average fold change for an individual *Hs-HARE5::EGFP* embryo relative to the aggregated average for all *Pt-HARE5::tdTomato* embryos. mRNA input levels were normalized to *Gapdh*. n = 4 technical replicates per embryo; n = 9 embryos from three transgenic lines from each genotype.

Scale bars represent 1 mm (A–K), 500 μm (N–P and U–W), 150 μm (Q, R, X, and Y), and 25 μm (S, T, Z, and AA). See also Figure S2 and Table S2.

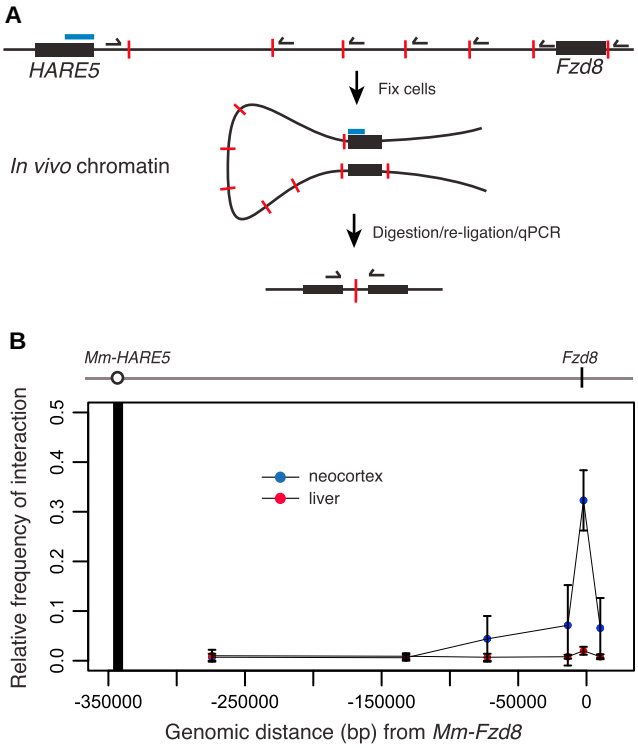


Figure 3. 3C Analysis Showing that *HARE5* Physically Contacts the *Fzd8* Promoter

(A) Schematic of 3C protocol showing *HARE5* and *Fzd8* loci (black bars), with the indicated TaqMan probe (blue bar), test primers (black half arrows), and HindIII restriction sites (red lines).

(B) 3C assay of E12.5 mouse neocortices (blue dots) and liver control tissue (red dots). The dark vertical line indicates location of TaqMan probe and constant primer anchored within the *Mm-HARE5* locus. The 0 position indicates ATG of *Fzd8* coding sequence. The graph depicts the relative frequency of interactions between *Mm-HARE5* and six genomic locations. Error bars indicate the SD.

See also Figure S3.

Human HARE5 Accelerates Neural Progenitor Cell Cycle and Impacts Neocortical Size

We next assessed the functional consequences of chimpanzee and human *HARE5* activities during corticogenesis. We generated new independent transgenic mouse lines in which *Hs-HARE5* or *Pt-HARE5* drove expression of a MYC-tagged mouse *Fzd8* coding sequence (*Pt-HARE5*::*Fzd8* and *Hs-HARE5*::*Fzd8*; Figure 4A). Expression of MYC in embryonic neocortices was confirmed by western blot analysis (Figure S4A). We postulated that *Fzd8* expression driven by the *HARE5* enhancer would impact the cell-cycle state of neural progenitors based upon the following rationale. First, both *Hs-HARE5* and *Pt-HARE5* drive expression in neural progenitors. Second, modulation of *Fzd8* levels impacts the neural progenitor cell cycle in the retina [18]. Third, overexpression of stabilized β-catenin, a Wnt signaling component downstream of Frizzled, induces an expanded and gyrencephalic brain and slows cell-cycle exit of neural stem cells in mice [15]. Fourth, cell-cycle length is critical for corticogenesis and is postulated as a likely mechanism for the evolutionary expansion of the primate neocortex [35, 36].

We measured the cell-cycle state of progenitors at E12.5, predicting that species-specific differences in *HARE5* activity would be evident within two days of onset of enhancer activity. At this stage, radial glial progenitors primarily undergo symmetric divisions to expand laterally, but a subset of these divide asymmetrically to produce excitatory neurons [2]. Quantification of G2/M phases using phospho-histone H3 (PH3) staining revealed a significant 1.3-fold increase in the proportion of total PH3-positive cells in *Hs-HARE5*::*Fzd8* brains relative to both *Pt-HARE5*::*Fzd8* and non-transgenic wild-type (WT) littermates (Figures 4B–4E). We also observed a trend toward more Pax6-positive radial glia in *Hs-HARE5*::*Fzd8* brains, with a significant increase relative to the WT (Figure S4B). These snapshot measurements indicate that at E12.5, *Hs-HARE5*-driven expression of *Fzd8* alters the proliferating population. More G2/M-positive progenitors may indicate a faster overall cell cycle with similar G2/M phases or, alternatively, an identical cell cycle with longer G2/M.

To help discriminate between these possibilities, we quantified cell-cycle duration at E12.5. We used a paradigm of 2 hr BrdU exposure and 30 min EdU exposure coupled with Ki67 staining, as previously described [37] (Figure 4F). Both WT and *Pt-HARE5*::*Fzd8* progenitors cycled for about 12 hr, as previously reported for this age [37, 38]. In contrast, *Hs-HARE5*-driven *Fzd8* expression significantly accelerated both the total cell cycle (to approximately 9.2 hr) and S phase, by 25% (Figures 4G–4J and Table S3). These cell-cycle differences correspond to a 23% shorter G1/G2/M duration (Tc-Ts) of *Hs-HARE5*::*Fzd8* progenitors compared to *Pt-HARE5*::*Fzd8* ($p = 0.003$). Thus, this functional analysis reveals that relative to both the WT and *Pt-HARE5*::*Fzd8*, human *HARE5*-directed expression of *Fzd8* accelerates neural progenitor cell cycle.

Increased proliferation of neural progenitors is frequently associated with changes in brain size. Therefore, we measured the cortical dimensions of transgenic E18.5 brains. Compared to *Pt-HARE5*::*Fzd8* and WTs, the dorsal area of *Hs-HARE5*::*Fzd8* cortices was significantly larger by 12% (Figures 4K–4O). Across five additional measurements, *Hs-HARE5*::*Fzd8* cortices were consistently larger than both *Pt-HARE5*::*Fzd8* and WTs (Figures S4F–S4H). As a larger cortical area could be due to increased cortical thickness or tangential length, we quantified these dimensions in sagittal and coronal sections (Figures 4P–4S). *Hs-HARE5*::*Fzd8* brains were thinner than *Pt-HARE5*::*Fzd8* and WT brains, although differences were only significant in comparison to the WT (Figure S4I). In contrast, compared to both *Pt-HARE5*::*Fzd8* and WTs, *Hs-HARE5*::*Fzd8* brains showed significantly longer tangential distance along the cortical VZ (Figure 4S). As seen in other mutants with longer tangential growth, *Hs-HARE5*::*Fzd8* brains also showed enlarged ventricles. The increased tangential length phenotype is often associated with greater progenitor proliferation and larger cortical size, as evidenced in mouse embryonic brains mis-expressing β-catenin or FGF2 [15, 39]. These data indicate that tangential expansion is a likely contributing factor for the increased cortical area.

We predicted that faster progenitor proliferation would ultimately be associated with more neurons. To test this, we quantified the densities of FoxP1-positive neurons (mid-layers III–V), born between E13.5 and E16.5, and FoxP2 neurons (deep-layer VI), born around E12.5 (Figures 4T–4AA), within radial columns of E18.5 brains [40, 41]. Compared to chimpanzee, *Hs-HARE5*::*Fzd8* brains showed a significant 14% increase in the density of FoxP1 neurons, but no difference in FoxP2 neurons, nor any notable apoptosis. Thus, *Hs-HARE5*::*Fzd8* brains contain a higher density of neurons that are produced beginning around E13.5. Together, these data indicate that compared to *Pt-HARE5*, *Hs-HARE5* promotes

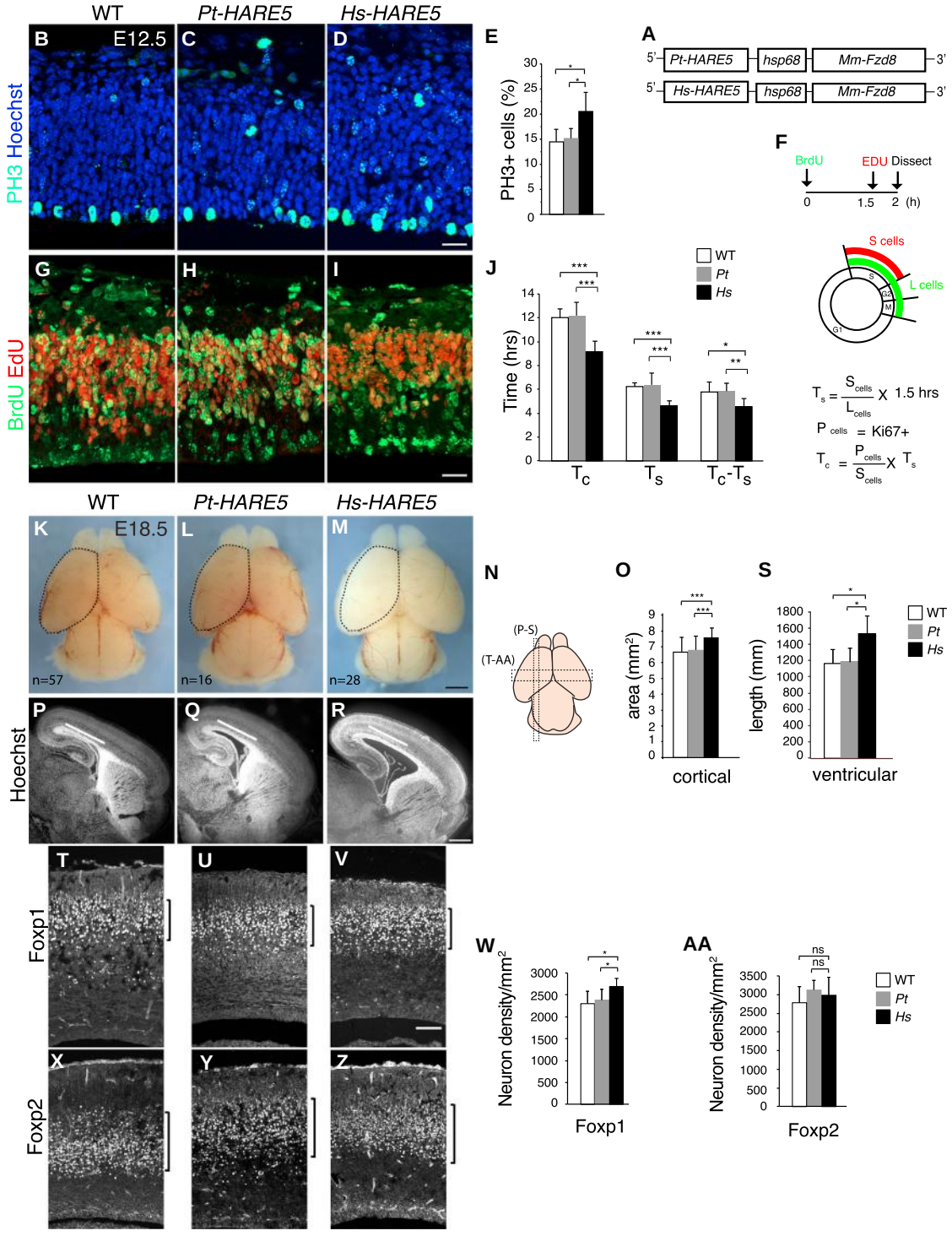


Figure 4. *Hs-HARE5*-Driven Expression of *Fzd8* Accelerates the Cell Cycle of Neural Progenitors and Increases Neuron Number and Neocortical Size

(A) Schematic of *Pt-HARE5::Fzd8* and *Hs-HARE5::Fzd8* constructs.

(B–I) Images of coronal sections from E12.5 WT littermate (B and G), *Pt-HARE::Fzd8* (C and H), and *Hs-HARE5::Fzd8* (D and I) transgenic cortices. Sections were stained for PH3 (green) and Hoechst (blue) (B–D) or BrdU (green) and EdU (red) (G–I). A graph of WT (white), *Pt-HARE::Fzd8* (gray), and *Hs-HARE5::Fzd8* (black) depicting percentage of all cells that are PH3-positive is shown in (E). The paradigm for analysis of cell-cycle length using double pulse of BrdU and EdU is shown in (F). Nucleotide analogs were injected at the indicated time points, and overall cell-cycle length (Tc) and S phase length (Ts) were calculated as shown.

(J) Graph of WT (white), *Pt-HARE::Fzd8* (gray), and *Hs-HARE5::Fzd8* (black) cell-cycle lengths of cycling progenitors.

(K–M) Whole-mount E18.5 brains from the indicated genotypes (n, number of brains examined). A dotted line was drawn on the WT cortex in (K) to indicate dorsal cortical area and was then superimposed on transgenic cortices in (L) and (M).

(N) Schematic cartoon representation of E18.5 brain with indicated regions of analyses for sagittal sections (P–S) and coronal sections (T–AA).

(legend continued on next page)

faster progenitor cell cycle, which is ultimately associated with increased Foxp1 excitatory neuron density, and overall larger cortical size.

Discussion

The neocortex expanded spectacularly during human evolution, giving rise to distinctively human anatomical and cognitive capabilities [1, 2, 20–22]. Yet to date, just a handful of genetic loci have been associated with human-specific brain traits [3, 5, 25], and none have been shown to functionally impact corticogenesis in an evolutionarily divergent fashion. In this study, we report the discovery of the first human-accelerated enhancer that functions in brain development. We demonstrate dramatic temporal and spatial differences in activity of human and chimpanzee enhancers of *FZD8* during early corticogenesis and show that these differences impact neural progenitor cell cycle and brain size. Our study suggests the intriguing hypothesis that evolutionary changes in *HARE5* sequence and activity contributed to the origin of unique features of the human brain.

The evolutionarily divergent activities of *HARE5* support a model proposed 16 years ago by Pasko Rakic: that species differences in progenitor proliferation may contribute to distinctions in brain size between humans and non-human primates [36]. The proposed radial unit hypothesis predicts that the number and proliferative capacity of progenitor cells drives the evolution of brain cytoarchitecture and explains species differences in neocortical size and structure. Indeed, both empirical and predicted measurements of the neural progenitor cell cycle reveal stark differences between humans, non-human primates, and mice [1, 36, 42]. In non-human primates, distinct G1 phase durations are associated with unique brain cytoarchitecture [35]. Moreover, genetic evidence strongly supports a causal link between neural stem cell proliferation and human brain size [43].

How might a faster cell cycle impact human brain size? We speculate that in the context of extended human corticogenesis and gestation, *HARE5* increases progenitor proliferation, which expands the progenitor pool during early corticogenesis. Increased progenitor expansion would ultimately produce more neurons and a larger neocortex. This could involve altering progenitor cell-cycle exit and/or the division state of progenitors from neurogenic to proliferative. In E14.5 mice, proliferating and neurogenic neural progenitors have distinct S phase durations [44]. Experimental shortening of the G1 phase in mice promotes proliferative divisions in lieu of neurogenic divisions, impacting neuron production [45, 46]. Our study implicates shorter G1 as a potential mechanism, as the Tc-Ts fraction was shorter in human transgenic brains. Follow-up studies of the *Hs-HARE5::Fzd8* mouse will clarify the detailed relationship between altered cell cycle and brain size and elucidate whether modifications in structural and behavioral traits exist.

We have shown that a key target gene of *HARE5* activity in the neocortex is *FZD8*, which encodes a Wnt receptor. Given the neurogenesis roles of β-catenin and Lef/Tcf, it is likely that *FZD8* acts via canonical Wnt signaling [16]. *FZD8* expression in the neonatal human brain is highest in cortical areas at 9 weeks post-conception (http://brainspan.org) [19], when neural stem cells are rapidly expanding during early corticogenesis [2], but is markedly lower in non-cortical areas. The *FZD8* expression pattern correlates strongly with the neural stem cell markers *SOX2* and *PAX6* ($r > 0.90$) [19, 47]. Hence, the pattern of *HARE5* activity and *FZD8* expression is consistent with a functional relationship in neural stem cell regulation in humans. Although chimpanzee expression data are not available, developing rhesus macaque (*Macaca mulatta*) neocortical data are (http://www.blueprintnhpatlas.org). Relative to ten common transcripts of human and macaque developing neocortices, *FZD8* was more abundant in humans. As RNA expression data become available [48], it may become possible to more directly compare *FZD8* levels in human and non-human primates.

In addition to its requirement for early mouse corticogenesis, Wnt signaling is implicated in human brain traits. In 2002, Chenn et al. showed that expression of stabilized β-catenin induced a larger, gyrencephalic phenotype reminiscent of the human brain [15]. However, evidence for the involvement of this pathway in human brain evolution has remained elusive until now. Our identification of *HARE5* highlights the transcriptional regulation of Wnt signaling components as a new avenue to explore for understanding the evolutionary origin of human-specific anatomical and cognitive traits. With the ability to identify regulatory elements active during development [49], we are now poised for the discovery of additional loci and pathways whose modification provided the underpinnings for the evolution of the human brain.

Supplemental Information

Supplemental Information includes Supplemental Experimental Procedures, four figures, and four tables and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2015.01.041.

Author Contributions

J.L.B., G.A.W., and D.L.S. conceived the study and wrote the paper. J.L.B., S.L.S., J.P.R., L.-J.P., T.B., R.G., and D.L.S. performed and analyzed experiments.

Acknowledgments

We thank Dr. Len Pennacchio, Dr. Jérôme Collignon, Dr. Jeremy Nathans, and Dr. Yanshu Wang for sharing reagents; Meilang Flowers and Cheryl

(O) Graph of WT (white), *Pt-HARE::Fzd8* (gray), and *Hs-HARE5::Fzd8* (black) dorsal cortical area measurements. Note that a 12% increase was seen in *Hs-HARE5::Fzd8* cortical area.

(P–R) Sagittal E18.5 sections from brains of indicated genotypes. A line drawn on the WT cortex in (P) indicates ventricular length and was superimposed on transgenic cortices in (Q) and (R). Note no evidence of cortical gyrification was seen.

(S) Graph depicting ventricular length for indicated genotypes.

(T–V and X–Z) Coronal E18.5 sections from neocortices of indicated genotypes and stained for Foxp1 (T–V) and Foxp2 (X–Z). Note no significant apoptosis was observed.

(W and AA) Graphs depicting densities of Foxp1 (W) and Foxp2 (AA) neurons in radial columns of neocortical sections.

The following were analyzed for each genotype: for (B)–(E), n = 5 embryos each from three transgenic lines; for (F)–(J), five to seven embryos each from two to three transgenic lines; for (K)–(O), 16–57 embryos each from two to three transgenic lines; for (P)–(S), four to five embryos each (two to five sections per embryo) from two to three transgenic lines; and for (T)–(AA), five to six embryos each (two to four sections per embryo) from two to three transgenic lines. All analyses were done blind to genotype. Error bars indicate the SD. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. Scale bars represent 25 μm (B–I), 1 mm (K–M), 500 μm (P–R), and 100 μm (T–Z). See also Figure S4 and Table S3.

Bock (Duke Transgenic Mouse Facility) for generating mouse transgenics; Autumn Rorrer for assistance with mouse husbandry; Dr. Hiro Matsunami for reading the manuscript; members of the D.L.S. and G.A.W. labs and Fan Wang, Erich Jarvis, and Dave McClay for helpful discussions; Han-Yu Shih for advice on 3C analysis; Emily Miller for assistance with western blotting; and D.L.S. lab members for assistance in blind scoring of phenotypes. Funding was provided by a Research Incubator grant from the Duke Institute for Brain Sciences (to D.L.S. and G.A.W.), R01NS083897 (to D.L.S.), and NSF HOMIND BCS-08-27552 (to G.A.W.).

Received: May 16, 2014
Revised: August 28, 2014
Accepted: January 16, 2015
Published: February 19, 2015

References

1. Geschwind, D.H., and Rakic, P. (2013). Cortical evolution: judge the brain by its cover. Neuron *80*, 633–647.
2. Lui, J.H., Hansen, D.V., and Kriegstein, A.R. (2011). Development and evolution of the human neocortex. Cell *146*, 18–36.
3. Enard, W., Gehre, S., Hammerschmidt, K., Hölter, S.M., Blass, T., Somel, M., Brückner, M.K., Schreiweis, C., Winter, C., Sohr, R., et al. (2009). A humanized version of Foxp2 affects cortico-basal ganglia circuits in mice. Cell *137*, 961–971.
4. Herculano-Houzel, S. (2012). The remarkable, yet not extraordinary, human brain as a scaled-up primate brain and its associated cost. Proc. Natl. Acad. Sci. USA *109* (1), 10661–10668.
5. Dennis, M.Y., Nuttle, X., Sudmant, P.H., Antonacci, F., Graves, T.A., Nefedov, M., Rosenfeld, J.A., Sajjadian, S., Malig, M., Kotkiewicz, H., et al. (2012). Evolution of human-specific neural SRGAP2 genes by incomplete segmental duplication. Cell *149*, 912–922.
6. Prabhakar, S., Noonan, J.P., Pääbo, S., and Rubin, E.M. (2006). Accelerated evolution of conserved noncoding sequences in humans. Science *314*, 786.
7. Bird, C.P., Stranger, B.E., Liu, M., Thomas, D.J., Ingle, C.E., Beazley, C., Miller, W., Hurles, M.E., and Dermitzakis, E.T. (2007). Fast-evolving noncoding sequences in the human genome. Genome Biol. *8*, R118.
8. Lindblad-Toh, K., Garber, M., Zuk, O., Lin, M.F., Parker, B.J., Washietl, S., Kheradpour, P., Ernst, J., Jordan, G., Mauceli, E., et al.; Broad Institute Sequencing Platform and Whole Genome Assembly Team; Baylor College of Medicine Human Genome Sequencing Center Sequencing Team; Genome Institute at Washington University (2011). A high-resolution map of human evolutionary constraint using 29 mammals. Nature *478*, 476–482.
9. Bush, E.C., and Lahn, B.T. (2008). A genome-wide screen for noncoding elements important in primate evolution. BMC Evol. Biol. *8*, 17.
10. Pollard, K.S., Salama, S.R., King, B., Kern, A.D., Dreszer, T., Katzman, S., Siepel, A., Pedersen, J.S., Bejerano, G., Baertsch, R., et al. (2006). Forces shaping the fastest evolving regions in the human genome. PLoS Genet. *2*, e168.
11. Capra, J.A., Erwin, G.D., McKinsey, G., Rubenstein, J.L.R., and Pollard, K.S. (2013). Many human accelerated regions are developmental enhancers. Philos. Trans. R. Soc. Lond. B Biol. Sci. *368*, 20130025.
12. Kamm, G.B., López-Leal, R., Lorenzo, J.R., and Franchini, L.F. (2013). A fast-evolving human NPAS3 enhancer gained reporter expression in the developing forebrain of transgenic mice. Philos. Trans. R. Soc. Lond. B Biol. Sci. *368*, 20130019.
13. Oksenberg, N., Stevison, L., Wall, J.D., and Ahituv, N. (2013). Function and regulation of AUTS2, a gene implicated in autism and human evolution. PLoS Genet. *9*, e1003221.
14. Prabhakar, S., Visel, A., Akiyama, J.A., Shoukry, M., Lewis, K.D., Holt, A., Plajzer-Frick, I., Morrison, H., Fitzpatrick, D.R., Afzal, V., et al. (2008). Human-specific gain of function in a developmental enhancer. Science *321*, 1346–1350.
15. Chenn, A., and Walsh, C.A. (2002). Regulation of cerebral cortical size by control of cell cycle exit in neural precursors. Science *297*, 365–369.
16. Freese, J.L., Pino, D., and Pleasure, S.J. (2010). Wnt signaling in development and disease. Neurobiol. Dis. *38*, 148–153.
17. Fischer, T., Guimera, J., Wurst, W., and Prakash, N. (2007). Distinct but redundant expression of the Frizzled Wnt receptor genes at signaling centers of the developing mouse brain. Neuroscience *147*, 693–711.
18. Liu, C., Bakeri, H., Li, T., and Swaroop, A. (2012). Regulation of retinal progenitor expansion by Frizzled receptors: implications for microphthalmia and retinal coloboma. Hum. Mol. Genet. *21*, 1848–1860.
19. Miller, J.A., Ding, S.-L., Sunkin, S.M., Smith, K.A., Ng, L., Szafer, A., Ebbert, A., Riley, Z.L., Royall, J.J., Aiona, K., et al. (2014). Transcriptional landscape of the prenatal human brain. Nature *508*, 199–206.
20. Berwick, R.C., Friederici, A.D., Chomsky, N., and Bolhuis, J.J. (2013). Evolution, brain, and the nature of language. Trends Cogn. Sci. *17*, 89–98.
21. Whiten, A. (2011). The scope of culture in chimpanzees, humans and ancestral apes. Philos. Trans. R. Soc. Lond. B Biol. Sci. *366*, 997–1007.
22. Shettleworth, S.J. (2012). Modularity, comparative cognition and human uniqueness. Philos. Trans. R. Soc. Lond. B Biol. Sci. *367*, 2794–2802.
23. Schoenemann, P.T. (2006). Evolution of the size and functional areas of the human brain. Annu. Rev. Anthropol. *35*, 379–406.
24. King, M.C., and Wilson, A.C. (1975). Evolution at two levels in humans and chimpanzees. Science *188*, 107–116.
25. Bae, B.-I., Tietjen, I., Atabay, K.D., Evrony, G.D., Johnson, M.B., Asare, E., Wang, P.P., Murayama, A.Y., Im, K., Lisgo, S.N., et al. (2014). Evolutionarily dynamic alternative splicing of GPR56 regulates regional cerebral cortical patterning. Science *343*, 764–768.
26. Haygood, R., Babbitt, C.C., Fedrigo, O., and Wray, G.A. (2010). Contrasts between adaptive coding and noncoding changes during human evolution. Proc. Natl. Acad. Sci. USA *107*, 7853–7857.
27. Johnson, M.B., Kawasawa, Y.I., Mason, C.E., Krsnik, Z., Coppola, G., Bogdanović, D., Geschwind, D.H., Mane, S.M., State, M.W., and Sestan, N. (2009). Functional and evolutionary insights into human brain development through global transcriptome analysis. Neuron *62*, 494–509.
28. Creyghton, M.P., Cheng, A.W., Welstead, G.G., Kooistra, T., Carey, B.W., Steine, E.J., Hanna, J., Lodato, M.A., Frampton, G.M., Sharp, P.A., et al. (2010). Histone H3K27ac separates active from poised enhancers and predicts developmental state. Proc. Natl. Acad. Sci. USA *107*, 21931–21936.
29. Visel, A., Blow, M.J., Li, Z., Zhang, T., Akiyama, J.A., Holt, A., Plajzer-Frick, I., Shoukry, M., Wright, C., Chen, F., et al. (2009). ChIP-seq accurately predicts tissue-specific activity of enhancers. Nature *457*, 854–858.
30. Kim, A.S., Lowenstein, D.H., and Pleasure, S.J. (2001). Wnt receptors and Wnt inhibitors are expressed in gradients in the developing telencephalon. Mech. Dev. *103*, 167–172.
31. Summerhurst, K., Stark, M., Sharpe, J., Davidson, D., and Murphy, P. (2008). 3D representation of Wnt and Frizzled gene expression patterns in the mouse embryo at embryonic day 11.5 (Ts19). Gene Expr. Patterns *8*, 331–348.
32. Berger, M.F., Philippakis, A.A., Qureshi, A.M., He, F.S., Estep, P.W., 3rd, and Bulyk, M.L. (2006). Compact, universal DNA microarrays to comprehensively determine transcription-factor binding site specificities. Nat. Biotechnol. *24*, 1429–1435.
33. Shaner, N.C., Steinbach, P.A., and Tsien, R.Y. (2005). A guide to choosing fluorescent proteins. Nat. Methods *2*, 905–909.
34. Hagège, H., Klous, P., Braem, C., Splinter, E., Dekker, J., Cathala, G., de Laat, W., and Forné, T. (2007). Quantitative analysis of chromosome conformation capture assays (3C-qPCR). Nat. Protoc. *2*, 1722–1733.
35. Lukaszewicz, A., Savatier, P., Cortay, V., Giroud, P., Huissoud, C., Berland, M., Kennedy, H., and Dehay, C. (2005). G1 phase regulation, area-specific cell cycle control, and cytoarchitectonics in the primate cortex. Neuron *47*, 353–364.
36. Rakic, P. (1988). Specification of cerebral cortical areas. Science *241*, 170–176.
37. Martynoga, B., Morrison, H., Price, D.J., and Mason, J.O. (2005). Foxg1 is required for specification of ventral telencephalon and region-specific regulation of dorsal telencephalic precursor proliferation and apoptosis. Dev. Biol. *283*, 113–127.
38. Takahashi, T., Nowakowski, R.S., and Caviness, V.S., Jr. (1995). The cell cycle of the pseudostratified ventricular epithelium of the embryonic murine cerebral wall. J. Neurosci. *15*, 6046–6057.
39. Rash, B.G., Tomasi, S., Lim, H.D., Suh, C.Y., and Vaccarino, F.M. (2013). Cortical gyrification induced by fibroblast growth factor 2 in the mouse brain. J. Neurosci. *33*, 10802–10814.
40. Ferland, R.J., Cherry, T.J., Preware, P.O., Morrisey, E.E., and Walsh, C.A. (2003). Characterization of Foxp2 and Foxp1 mRNA and protein in the developing and mature brain. J. Comp. Neurol. *460*, 266–279.

41. Greig, L.C., Woodworth, M.B., Galazo, M.J., Padmanabhan, H., and Macklis, J.D. (2013). Molecular logic of neocortical projection neuron specification, development and diversity. Nat. Rev. Neurosci. *14*, 755–769.
42. Kornack, D.R., and Rakic, P. (1998). Changes in cell-cycle kinetics during the development and evolution of primate neocortex. Proc. Natl. Acad. Sci. USA *95*, 1242–1246.
43. Mirzaa, G.M., Parry, D.A., Fry, A.E., Giamanco, K.A., Schwartzentruber, J., Vanstone, M., Logan, C.V., Roberts, N., Johnson, C.A., Singh, S., et al.; FORGE Canada Consortium (2014). De novo CCND2 mutations leading to stabilization of cyclin D2 cause megalencephaly-polymicrogyria-polydactyly-hydrocephalus syndrome. Nat. Genet. *46*, 510–515.
44. Arai, Y., Pulvers, J.N., Haffner, C., Schilling, B., Nüsslein, I., Calegari, F., and Huttner, W.B. (2011). Neural stem and progenitor cells shorten S-phase on commitment to neuron production. Nat. Commun. *2*, 154.
45. Pilaz, L.-J., Patti, D., Marcy, G., Ollier, E., Pfister, S., Douglas, R.J., Betizeau, M., Gautier, E., Cortay, V., Doerflinger, N., et al. (2009). Forced G1-phase reduction alters mode of division, neuron number, and laminar phenotype in the cerebral cortex. Proc. Natl. Acad. Sci. USA *106*, 21924–21929.
46. Lange, C., Huttner, W.B., and Calegari, F. (2009). Cdk4/cyclinD1 overexpression in neural stem cells shortens G1, delays neurogenesis, and promotes the generation and expansion of basal progenitors. Cell Stem Cell *5*, 320–331.
47. Lui, J.H., Nowakowski, T.J., Pollen, A.A., Javaherian, A., Kriegstein, A.R., and Oldham, M.C. (2014). Radial glia require PDGFD-PDGFRβ signalling in human but not mouse neocortex. Nature *515*, 264–268.
48. Pollen, A.A., Nowakowski, T.J., Shuga, J., Wang, X., Leyrat, A.A., Lui, J.H., Li, N., Szpankowski, L., Fowler, B., Chen, P., et al. (2014). Low-coverage single-cell mRNA sequencing reveals cellular heterogeneity and activated signaling pathways in developing cerebral cortex. Nat. Biotechnol. *32*, 1053–1058.
49. Visel, A., Taher, L., Girgis, H., May, D., Golonzhka, O., Hoch, R.V., McKinsey, G.L., Pattabiraman, K., Silberberg, S.N., Blow, M.J., et al. (2013). A high-resolution enhancer atlas of the developing telencephalon. Cell *152*, 895–908.

ATTACHMENT D

Coghlan,

The smart mouse with the half-human brain,

NEWSCIENTIST (Dec. 1, 2014)

The smart mouse with the half-human brain

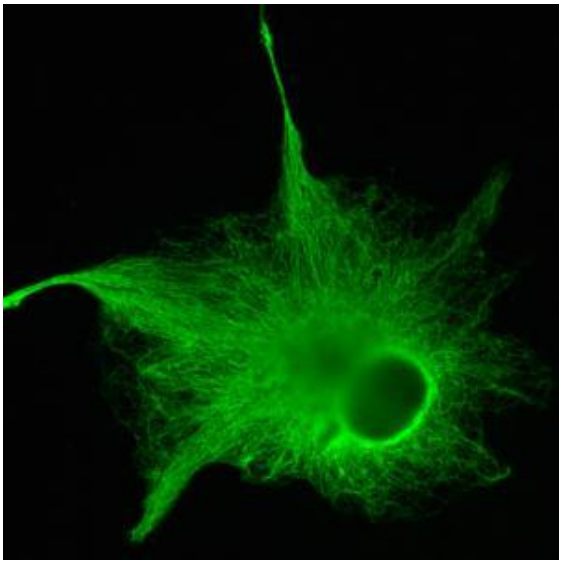
12:45 01 December 2014 by **Andy Coghlan**
For similar stories, visit the **The Human Brain** Topic Guide

What would Stuart Little make of it? Mice have been created whose brains are half human. As a result, the animals are smarter than their siblings.

The idea is not to mimic fiction, but to advance our understanding of human brain diseases by studying them in whole mouse brains rather than in dishes.

The altered mice still have mouse neurons – the "thinking" cells that make up around half of all their brain cells. But practically all the glial cells in their brains, the ones that support the neurons, are human.

"It's still a mouse brain, not a human brain," says Steve Goldman of the University of Rochester Medical Center in New York. "But all the non-neuronal cells are human."



Astrocyte nerve cells make a wealth of connections *(Image: Riccardi Cassiani Ingoni/SPL)*

Rapid takeover

Goldman's team extracted immature glial cells from donated human fetuses. They injected them into mouse pups where they developed into astrocytes, a star-shaped type of glial cell.

Within a year, the mouse glial cells had been completely usurped by the human interlopers. The 300,000 human cells each mouse received multiplied until they numbered 12 million, displacing the native cells.

"We could see the human cells taking over the whole space," says Goldman. "It seemed like the mouse counterparts were fleeing to the margins."

Astrocytes are vital for conscious thought, because they help to strengthen the connections between neurons, called synapses. Their tendrils (see image) are involved in coordinating the transmission of electrical signals across synapses.

Human astrocytes are 10 to 20 times the size of mouse astrocytes and carry 100 times as many tendrils. This means they can coordinate all the neural signals in an area far more adeptly than mouse astrocytes can. "It's like ramping up the power of your computer," says Goldman.

Intelligence leap

A battery of standard tests for mouse memory and cognition showed that the mice with human astrocytes are much smarter than their mousy peers.

In one test that measures ability to remember a sound associated with a mild electric shock, for example, the humanised mice froze for four times as long as other mice when they heard the sound, suggesting their memory was about four times better. "These were whopping effects," says Goldman. "We can say they were statistically and significantly smarter than control mice."

Goldman first reported last year that mice with human glial cells are smarter. But the human cells his team injected then were mature so they simply integrated into the mouse brain tissue and stayed put.

This time, he injected the precursors of these cells, glial progenitor cells, which were able to divide and multiply. That, he says, explains how they were able to take over the mouse brains so completely, stopping only when they reached the physical limits of the space.

Species cross

"It would be interesting to find out whether the human astrocytes function the same way in the mice as they do in humans," says Fred Gage, a stem cell researcher at the Salk Institute in La Jolla, California. "It would show whether the host modifies the fate of cells, or whether the cells retain the same features in mice as they do in humans," he says.

"That the cells work at all in a different species is amazing, and poses the question of which properties are being driven by the cell itself and which by the new environment," says Wolfgang Enard of Ludwig-Maximilians University Munich in Germany, who has shown that mice are better at learning if they have the human *Foxp2* gene, which has been linked with human language development.

In a parallel experiment, Goldman injected immature human glial cells into mouse pups that were poor at making myelin, the protein that insulates nerves. Once inside the mouse brain, many of the human glial cells matured into oligodendrocytes, brain cells that specialise in making the insulating material, suggesting that the cells somehow detected and compensated for the defect.

This could be useful for treating diseases in which the myelin sheath is damaged, such as multiple sclerosis, says Goldman, and he has already applied for permission to treat MS patients with the glial progenitor cells, and hopes to start a trial in 12 to 15 months.

Still a mouse

To explore further how the human astrocytes affect intelligence, memory and learning, Goldman is already grafting the cells into rats, which are more intelligent than mice. "We've done the first grafts, and are mapping distributions of the cells," he says.

Although this may sound like the work of science fiction – think *Deep Blue Sea*, where researchers searching for an Alzheimer's cure accidently create super-smart sharks, or Algernon, the lab mouse who has surgery to enhance his intelligence, or even the pigoons, Margaret Atwood's pigs with human stem cells – and human thoughts – Goldman is quick to dismiss any idea that the added cells somehow make the mice more human.

"This does not provide the animals with additional capabilities that could in any way be ascribed or perceived as specifically human," he says. "Rather, the human cells are simply improving the efficiency of the mouse's own neural networks. It's still a mouse."

However, the team decided not to try putting human cells into monkeys. "We briefly considered it but decided not to because of all the potential ethical issues," Goldman says.

Enard agrees that it could be difficult to decide which animals to put human brain cells into. "If you make animals more human-like, where do you stop?" he says.

Journal reference: *Journal of Neuroscience*, DOI: 10.1523/JNEUROSCI.1510-14.2014

MORE FROM NEW SCIENTIST

Is this ET? Mystery of strange radio bursts from space

English speakers, you stink at identifying smells

Make the NHS a well-being service not sickness service

Wine and glue tape ideal for post-surgery patch-ups

Back to article

ATTACHMENT E

Animals containing human material,

THE ACADEMY OF MEDICAL SCIENCES (July 2011)

The **Academy** *of* **Medical Sciences**

Animals containing human material

July 2011

The Academy of Medical Sciences

The Academy of Medical Sciences promotes advances in medical science and campaigns to ensure these are converted into healthcare benefits for society. Our Fellows are the UK's leading medical scientists from hospitals and general practice, academia, industry and the public service. The Academy seeks to play a pivotal role in determining the future of medical science in the UK, and the benefits that society will enjoy in years to come. We champion the UK's strengths in medical science, promote careers and capacity building, encourage the implementation of new ideas and solutions – often through novel partnerships – and help to remove barriers to progress.

Image credit: Neil Leslie

ISBN No: 978-1-903401-32-3

The **Academy** *of*
Medical Sciences

Animals containing human material

July 2011

Acknowledgements and disclaimer
The Academy of Medical Sciences is most grateful to Professor Martin Bobrow CBE FRS FMedSci and to the members of the working group for undertaking this study. We thank the Academy's Officers, Council members and staff, the external review group, study observers, as well as our Fellows and all those who have contributed through the call for evidence or by correspondence. We are grateful to the consortium led by Ipsos MORI for conducting the public dialogue programme, to the participants for their contributions and to Laura Grant Associates for evaluating the programme. We thank the study secretariat led by Dr Laura Boothman.

The Academy is grateful to the Department for Business, Innovation and Skills' Sciencewise Expert Resource Centre, the Department of Health, Medical Research Council, and Wellcome Trust for their financial contribution to the study.

This report is published by the Academy of Medical Sciences and has been endorsed by its Officers and Council. Contributions by the working group were made purely in an advisory capacity. The members of the working group participated in an individual capacity and not as representatives of, or on behalf of, their affiliated hospitals, universities, organisations or associations. Their participation should not be taken as endorsement by these bodies.

All web references were accessed in July 2011.

Contents

Summary **5**

1 Introduction **11**

2 Research involving inter-species mixtures **15**

3 Future science and implications **31**

4 Welfare and safety aspects of ACHM **59**

5 Ethical and social concerns **69**

6 Legal and regulatory considerations **83**

7 International perspective **99**

8 Conclusions and recommendations **109**

Annex I Report preparation **115**

Annex II Consultation and evidence gathering **121**

Annex III Overview of dialogue methodology and evaluation **125**

Annex IV Glossary of terms and abbreviations **129**

Summary

This report considers research that involves the introduction of human DNA sequence into animals, or the mixing of human and animal cells or tissues, to create entities we refer to as 'animals containing human material' (ACHM). Such approaches are long-established, and thousands of different ACHM have been used in biomedical research, yet they have received relatively little public discussion. Technical and scientific advances (such as those in stem cell science) are rapidly increasing the sophistication of ACHM and expanding their utility. This report considers the new opportunities in research and medicine, and the ethical and regulatory issues that emerge.

ACHM are used to study human biological functions or disease that cannot be accurately modelled in cell cultures or through computer simulation; where experiments using humans are infeasible or considered unethical; and where modification of an animal's body makes it more closely represent that of the human. Their use enables more accurate conclusions to be reached about the functions of DNA sequence, aspects of biology and the nature of disease. ACHM are widely used in finding new ways of diagnosing and treating disease, and in the development and even production of therapeutics.

We describe many examples, including: mice genetically altered to acquire susceptibility to diseases which do not normally affect them such as human immunodeficiency virus (HIV) and hepatitis; chimæric mice, engrafted with pieces of human tumour, which have for several decades been an invaluable system in cancer research, and in which radiotherapy and anti-cancer drugs have been tested; monoclonal antibody anti-cancer therapies which have been developed using mice with their immune system 'humanised' by replacement of mouse by human genes; and goats which produce a human substance used to treat a blood clotting disorder. Across the spectrum of ACHM use, the modification of animals to make them more similar to humans, in specific biological or disease characteristics, may improve the utility of the research results and outcomes.

The use of animals in research generally has received intense public discussion, and remains unacceptable in principle to some people. We did not revisit that wider discussion, but started from the current legislative position that animal research is permissible (and acceptable to the majority of the UK population) provided that it is carried out for good reason, where there are no feasible alternatives, and under strict regulation. We then considered what new ethical and regulatory issues might arise that would be specific to the creation and use of ACHM.

At the outset of our study, we commissioned a consortium led by Ipsos MORI to facilitate a public dialogue on ACHM. The findings showed a high degree of public acceptance of ACHM research provided it is well regulated, and justified by the potential gain in understanding or treating medical conditions. Areas of particular sensitivity were identified; however, in general, the dialogue participants did not regard ACHM research as being significantly different from other research involving animals.

Many ACHM models, such as transgenic rodents each containing one (or a few) human genes, and animals with human tissue grafts, have a long history of research use without major ethical or regulatory difficulties. However, technologies are advancing rapidly; more extensive sections of DNA can be manipulated, and methods using human stem cells to replace parts of tissue, or even whole organs, are becoming increasingly refined. By enabling progressively more extensive, and precise, substitution of human material in animals, these approaches may soon enable us to modify animals to an extent that might challenge social, ethical, or regulatory boundaries. Based on the evidence we received, the published literature, our public dialogue, and our own discussions, we identified areas which might merit special consideration, including:

- Extensive modification of the brain of an animal, by implantation of human-derived cells, which might result in altered cognitive capacity approaching human 'consciousness' or 'sentience' or 'human-like' behavioural capabilities.
- Situations where functional human gametes (eggs, sperm) might develop from precursor cell-types in an animal; and where fertilisation between either human (or human-derived) gametes and animal gametes might then occur.
- Cellular or genetic modifications which could result in animals with aspects of human-like appearance (skin type, limb or facial structure) or characteristics, such as speech.

Current scientific knowledge often does not permit precise prediction of the effects that modification of an animal's organs might produce. However, we anticipate some important reasons for possibly undertaking such research in the future. We therefore recommend additional expert scrutiny and regulation of experiments in these sensitive areas.

As researchers seek to create more effective research models and to evaluate potentially important medical interventions, there is a need to ensure a comprehensive system for the regulation of ACHM that protects animal welfare, maintains the highest standards of safety and ethics, and keeps the issues of public acceptability of research to the forefront. Before making recommendations on the regulatory system itself, we considered how each of these aspects applies specifically to ACHM.

We concluded that research involving ACHM does not have a generally increased potential for causing animal suffering, in comparison to other licensed research involving animals, and that the development and use of ACHM could indeed contribute to refining and improving the effectiveness of experiments involving animals. Research involving ACHM should be subject to scrutiny, licensing and advancement from an animal welfare perspective, in the same manner as other animal studies.

We considered whether the creation of ACHM might pose particular safety issues, for example through the close combination of human and animal tissue allowing opportunities for viral reactivation, as well as the potential consequences of accidental or deliberate release of ACHM from containment. We concluded that risks are very low, but not zero, and that scientists, research institutions and regulators should remain alert to these risks and take appropriate precautions.

To consider the distinctive ethical issues raised by ACHM, we drew from broader ethical perspectives: concerns about animal welfare and human dignity, and considerations arising from our stewardship responsibility towards animals. We considered how the portrayal of animal–human entities in literature and culture influences societal values.
While recognising that, as with any research, positive outcomes cannot be predicted, and timescales from research to application may be long, we concluded that, in our view, research involving ACHM can in general be justified by the prospect of facilitating novel insights into human biology, and treatments for serious human disorders.

The principal legislation relevant to the research use of ACHM in the UK is the Animals (Scientific Procedures) Act (1986) (ASPA), which is enforced by the Home Office through a system of licensing and inspection. The Department of Health, Human Fertilisation and Embryology Authority, Human Tissue Authority, the UK Stem Cell Bank and other bodies also regulate aspects of the use of ACHM. In all, the regulatory framework is complex, it involves several different Government departments and agencies, it was not developed specifically in reference to ACHM, and the interface between the different regulators has received little consideration.

The recommendations of this report should ensure that valuable and justifiable research involving ACHM can proceed within a robust, proportionate regulatory system, which is capable of responding to developing scientific knowledge and social attitudes, and which avoids undue bureaucracy and duplication of regulation.

We recommend that ACHM research should be classified in three categories, which would determine the level of regulatory scrutiny required prior to authorisation:

1. The great majority of ACHM experiments pose no novel issues and should continue to be regulated through the same procedures as other research involving animals.
2. A limited number of types of ACHM research should be permitted subject to additional specialist scrutiny by a national expert body. We outline a graded approach that should be considered for research in this category.
3. A very narrow range of ACHM experiments should not currently be undertaken, because they raise very strong ethical concerns and lack sufficient scientific justification.

While indicating the types of experiment that we would currently place within these categories, we emphasise that this classification would necessarily change over time, in response to new scientific understanding, and evolving social attitudes. The regulatory system should be capable of adapting to such changes.

Assessment of research in the second and third categories will require specialist knowledge, and decisions to license such research may be socially sensitive; moreover the number of experiments is likely to be relatively small. Consequently we recommend that the Home Office put in place a single, national expert body with a duty to advise on the use of ACHM, taking social, ethical and scientific considerations into account. This body would regularly review the system of categorisation; advise on the licensing approach to be taken for experiments in the second category; maintain consideration of areas where concerns may arise; and develop guidance for Government and for researchers. We recommend that the national expert body should be multidisciplinary, transparent, and open to public scrutiny. It should engage actively and regularly with the public, the scientific community and with other regulators to maintain a broad coordinated framework for regulating research involving ACHM.

There are clear advantages; in terms of consistency of practice, operational efficiency, and the best use of specialist expertise; that research involving ACHM is considered by the same body that advises Government on other aspects of animal research. Therefore, the national expert body we recommend should be integral to the wider system for the regulation of animal research.

In implementing the European Directive 2010/63/EU by 2012, the Home Office will consult on the requirement to establish a UK 'national committee for the protection of animals use for scientific purposes'. We have placed emphasis on the value of ACHM being considered alongside other animal research, and suggest that every effort is made to ensure that the 'national committee' mandated by the Directive has within its remit and competence, the function of the 'national expert body for ACHM' that we recommend.

We have described the complexity of the current regulatory system as it relates to ACHM, and the involvement of several Government departments and regulatory agencies. There are areas in which the close alignment of various regulators will be essential in securing comprehensive and functionally efficient governance of ACHM. The most striking example is research involving human admixed embryos, which is tightly regulated by the Human Fertilisation and Embryology Authority (HFEA) under the Human Fertilisation and Embryology Act (HFE Act). It is a matter

of expert judgement to distinguish between embryos that are 'predominantly human' and so come under the HFE Act, and embryos that are considered to be narrowly on the other side of the boundary and so 'predominantly animal', and outwith the terms of the HFE Act. These latter embryos are not currently regulated during early gestation (although their mothers are regulated under ASPA). Since such cases will fall at the boundaries of the two regulators, we recommended that the Department of Health and Home Office (and their expert advisory bodies) work closely together to ensure that there are no regulatory gaps, overlaps, or inconsistencies, between their respective regulatory systems. It is essential that a smooth operational interface be established to ensure the timely and appropriate assessment of such research.

As with much biomedical research, ACHM research frequently involves international collaboration. We have noted a paucity of international guidance relating specifically to ACHM. We recommend raising international awareness of ACHM, promoting international consistency in research practice, and the development of international standards and guidance. This is an area in which the UK can lead.

Public dialogue findings

A majority of participants in the public dialogue accepted and were ultimately supportive of research using ACHM, on the condition that such research is conducted to improve human health or to combat disease. Three areas of particular sensitivity to participants were identified: ACHM research involving the brain, reproductive tissues or aspects of human-like appearance. Participants also expressed broader concerns, including those relating to the welfare of the animals involved, safety aspects of research involving ACHM and its regulation.

Categorisation of ACHM

We propose that experiments involving ACHM could be usefully classified into three categories:

Category 1

The great majority of ACHM experiments, which do not present issues beyond those of the general use of animals in research, should be subject to the same oversight and regulation under ASPA as other animal research.

Category 2

A limited number of types of ACHM research (outlined below) should be permissible, subject to additional specialist scrutiny by the national expert body we propose[1]. Although we would expect this list to evolve over time as knowledge advances, the major types of research that we would currently include in this category are:

- Substantial modification of an animal's brain that may make the brain function potentially more 'human-like', particularly in large animals.
- Experiments that may lead to the generation or propagation of functional human germ cells in animals.
- Experiments that could be expected to significantly alter the appearance or behaviour of animals, affecting those characteristics that are perceived to contribute most to distinguishing our species from our close evolutionary relatives.
- Experiments involving the addition of human genes or cells to non-human primates (NHPs). We recognise that research on NHPs is appropriate, and in some types of research probably essential if it is to lead to clinical benefit, but such research should remain under a high degree of regulatory scrutiny.

Category 3

A very narrow range of experiments should not, for now, be licensed because they either lack compelling scientific justification or raise very strong ethical concerns. The list of such experiments should be kept under regular review by the proposed national expert body, but should at present include:

- Allowing the development of an embryo, formed by pre-implantation mixing of NHP and human embryonic or pluripotent stem cells, beyond 14 days of development or the first signs of primitive streak development (whichever occurs first); unless there is persuasive evidence that the fate of the implanted (human) cells will not lead to 'sensitive' phenotypic changes in the developing fetus.[1,2,3]
- Transplantation of sufficient human-derived neural cells into an NHP as to make it possible, in the judgement of the national expert body, that there could be substantial functional modification of the NHP brain, such as to engender 'human-like' behaviour. Assessing the likely phenotypic effect of such experiments will be informed by prior work on other species (possibly including stem cell transfer between NHPs) or by data on the effects of 'graded' transplantation of human cells into NHPs.
- Breeding of animals that have, or may develop, human derived germ cells in their gonads, where this could lead to the production of human embryos or true hybrid embryos within an animal.[4]

1 Such experiments should be approached with caution. Strong scientific justification should be provided to the national expert body, who should closely consider the ethical and any safety issues in addition to the potential value of the research. Authorisation may require studies to adopt an incremental (graduated) approach. Proposed studies should be assessed on a case-by-case basis, at least until experience allows the formulation of guidelines

2 This applies whether the embryo is implanted within an animal uterus or maintained as an intact embryo in vitro. Equivalent statutory restrictions are applicable to human and human admixed embryos under the HFE Act (see 6.2.2).

3 This supplements the 14 day provision applied to human admixed embryos under the HFE Act, so that mixed embryos, which are judged to not quite meet the criteria for being 'predominantly human', should nevertheless be regulated on the basis of the likely phenotypic effect on the embryos created. Currently, any mixed origin embryo judged to be 'predominantly human' is regulated by HFEA and cannot be kept beyond the 14 day stage, whereas an embryo judged to be predominantly animal is unregulated until the mid-point of gestation (likely to be increased to two-thirds on implementation of the European Directive 2010/63/EU) and can in principle be kept indefinitely. As to whether or not an admixed embryo is predominantly 'human' is an expert judgement, including an assessment of likely phenotype, but neither the precise eventual composition of an individual embryo nor the phenotypic effect of the admixture will be easily predictable in the current state of knowledge.

4 Placement of human embryos into animals is prohibited by the HFE Act, which seems likely to be interpreted to include placement of human embryos into animals modified to contain human uterine tissue.

Recommendations

1. We recommend that the Home Office ensures that a national expert body with a duty to advise on the use of ACHM in research is put in place.
2. We recommend that this national expert body should:
   - 2.1 Be multidisciplinary, involving people with knowledge of ethics, the humanities, social sciences, law and the biological sciences as well as people without specific expertise in these fields, and be able to co-opt additional expertise when relevant.[5]
   - 2.2 Be transparent, making its proceedings, deliberations, reasoning, conclusions and recommendations available for public scrutiny.
   - 2.3 Be outward facing so that interested persons are aware of its function and feel able to input into its work programme.
   - 2.4 Be actively involved in public engagement and consultation; and maintain regular forward-looking dialogue with the scientific community.
   - 2.5 Have the power to develop guidelines to promote consistency and transparency in the regulatory process.
3. We recommend that the Home Office ensures that the body that meets the requirement of the 'national committee for the protection of animals used for scientific purposes' in the UK has within its remit and competence the function of the national expert body for ACHM.
4. We recommend that, for those classes of ACHM where it is relevant, a risk assessment should be undertaken and appropriate containment levels specified. The risk assessment is the responsibility of investigators, research institutions and regulators, and should where relevant take the advice of an independent virologist.
5. We recommend that the Home Office and the Department of Health work closely together to ensure that there are no regulatory gaps, overlaps or inconsistencies, between the two regulatory systems. We consider it essential that the Home Office and the Human Fertilisation and Embryology Authority (HFEA) (or, as appropriate, the Department of Health) work together to develop and maintain a smooth, functionally integrated operational interface, at the boundaries of their areas of responsibility. This should be supported by clear guidance to the research community, to ensure the timely and appropriate adjudication of innovative scientific projects without undue bureaucracy. Such an interface may well involve the expert advisory bodies in the two systems, as well as officials acting for the agencies concerned.
6. We recommend raising international awareness of ACHM, promoting international consistency in research practice involving their use, and exploring the development of international standards or guidance. This might be achieved through international collaboration among regulators, policy-makers, national and international bioethics bodies and medical research councils, or initiatives within the research community. This is an area in which the UK should provide leadership.

5 Given the special issues associated with experiments on NHPs, we recommend that the national expert body should include either in its membership or as an advisor, an independent scientist with experience in NHP research who should be present to advise the group when such issues are discussed.

1 Introduction

Animals containing human genetic or cellular material are widely used in laboratories worldwide. There is a long and successful history of their role in advancing our understanding of human and animal physiology and disease, and increasingly in the development of new treatments. Of the thousands of examples of animals containing human material (which we refer to as 'animals containing human material' (ACHM)) developed since the 1960s, the great majority are mice each containing a single human gene, used to study gene function and disease.

The scientific techniques used to transfer genetic or cellular material from one entity to another are becoming increasingly sophisticated. Far greater quantities of genetic sequence can be manipulated, and stem cell technologies have enabled significant percentages of an animal's tissues or organs to be replaced with equivalents derived from human tissues. These techniques are applicable to fields of research as diverse as neuroscience, reproductive biology, cancer research, immunology and many more.

In 2007, the Academy convened a working group to examine the use of embryos combining human and animal material in medical research. To support the revision of UK legislation that was underway at that time, the study was concerned with human embryos incorporating animal material, and focused on one type of these now known as '*human* admixed embryos'.[6] However, the study's report, '*Inter-species embryos*', also mentioned research involving the converse situation i.e. the use of embryonic or adult animals containing human material.[7] The report drew attention to the need to review the regulatory environment in this area in light of the rapidly developing science, and to engage the public in discussion of these issues.

Whilst the UK Human Fertilisation and Embryology Act (2008) (the HFE Act) provided a contemporary legislative framework for research involving human embryos, it was noted that the 'animal end of the spectrum of human–animal mixture' had received relatively little consideration. Having recognised the possibility that this area of science could present future regulatory and ethical challenges in the UK and beyond, and the relatively little public attention that it had received, the Academy committed to undertake further work in this area to inform future public debate.

1.1 Scope and terms of reference

The Academy's study on the use of ACHM in biomedical research was launched in Autumn 2009. The scope of the study was to: examine the scientific, social, ethical, safety and regulatory aspects of research involving non-human embryos and animals containing human material. The study's terms of reference were to:

- Agree definitions for animals, and animal embryos, containing human genetic or cellular material.
- Describe the current use of animals containing human material in medical research, and to anticipate future research directions and challenges for this work.
- Assess future applications of research involving animals containing human material – including potential requirements for preclinical (animal) studies of candidate human stem cell therapies.
- Address safety concerns surrounding the generation and use of animals containing human material in research, and to consider welfare issues which apply specifically to animals containing human material.

6 Academy of Medical Sciences (2007). *Inter-species embryos.* http://www.acmedsci.ac.uk/p48prid51.html
7 Academy of Medcal Sciences (2007). *Non-human embryos and animals incorporating human material.* In *Inter-species embryos.* http://www.acmedsci.ac.uk/p48prid51.html

- Explore societal and ethical aspects of medical research involving the creation of animals that include significant amounts of human material, and to develop a constructive public dialogue in this area.
- Explore the current and future regulation of the use of animals and embryos containing human material for research purposes, including primary legislation, regulations and guidelines.
- Draw conclusions and make recommendations for action.

To avoid replication of previous work and debates, several wider areas were excluded from the study scope. These are not addressed in any depth:

- Scientific or ethical issues relating to the general use of animals in research. While recognising the debate in this area, and the need to be constantly aware of the importance of minimising the impact of research on experimental animals, this report concerns ACHM, which are a small proportion of animals used in medical research. We therefore start by accepting as given, all legislative and other controls that currently regulate animal experimentation in the UK, and restrict our consideration to specific issues of animal welfare arising from the inter-species nature of ACHM research.
- The use of human admixed embryos in research. These and other closely related issues were subject to full public debate throughout the passage of the HFE Act (2008).
- Broader issues relating to genetic modification in a wider sense and not involving human material, such as the genetic modification of animals, or plants, for agricultural purposes.

1.2 Conduct of the study

The study was conducted by a working group chaired by Professor Martin Bobrow CBE FRS FMedSci, which included expertise in biomedical science, philosophy, ethics, social science and law. Observers from Government and research funding bodies joined working group meetings but not discussion of the study's conclusions and recommendations. (See Annex I for a list of working group members and observers.)

The Academy issued an open call for evidence in November 2009 to which submissions were received from a wide range of organisations and individuals. Additional consultation was achieved through oral evidence sessions and correspondence between the working group and additional experts (Annex II details contributors to the study).

The strength of public opinion around the creation of mixed human–animal entities was evident throughout parliamentary debates around the HFE Act (2008), and in associated media coverage. The Academy's '*Inter-species embryos*' report recognised the importance of public values and judgements in informing the development of law and policy in these areas, but also warned of a gulf between current and future scientific practices, and public awareness of them. A programme of public dialogue was therefore commissioned to inform the current study (see Annex III for the dialogue methodology). Its findings were published in full in 2010 and are also incorporated into this report (see blue boxes).[8] An independent evaluation of the dialogue process has also been published.[9]

8 Ipsos MORI (2010). *Exploring the boundaries: report on a public dialogue into animals containing human material*. http://www.acmedsci.ac.uk/index.php?pid=209

9 Laura Grant Associates (2010). *Exploring the boundaries: a dialogue on animals containing human material. Evaluation report*. http://www.acmedsci.ac.uk/index.php?pid=240

The report was reviewed by a group appointed by the Academy's Council (see Annex I) and has been approved by the Academy's Council.

We thank all those who contributed to this study. We are grateful the Department for Business, Innovation and Skills' Sciencewise Expert Resource Centre, the Department of Health, Medical Research Council, and Wellcome Trust for their financial contribution to the study.

1.3 Overview and terminology

Chapter 2 describes the types of ACHM and briefly illustrates how and why they are used in biomedical research. In Chapter 3 we consider methodological areas in which developments relevant to the creation of ACHM are apparent, and areas in which future research may approach social, ethical or regulatory boundaries. Specific welfare and safety considerations related to ACHM use are discussed in Chapter 4. Social and ethical considerations are described in Chapter 5.

Chapter 6 provides an overview of the regulatory framework governing ACHM use in the UK; a wider international perspective is then outlined in Chapter 7. Chapter 8 sets out our conclusions and recommendations.

Common terminology has as far as possible been used for simplicity, and a glossary of terms is given in Annex IV. Though in correct scientific taxonomy, humans are both primates and animals, in this text 'animal' (rather than 'non-human animal') is used to refer to animals of all species in the animal kingdom *except* humans, whereas humans are referred to as either 'human', or 'man'. Primate species *except* humans are referred to as 'non-human primates', abbreviated as 'NHPs'.

A lay summary of this report is available separately.[10]

10 The lay summary is available at www.acmedsci.ac.uk/publications

2 Research involving inter-species mixtures

2.1 Overview

A broad range of inter-species entities, including both animal–animal and animal–human mixtures, are created and used in biomedical research. This report focuses on animal–human mixtures which involve the incorporation of human genetic or cellular material into animals. We refer to these as 'animals containing human material' (ACHM).

2.1.1 Why are ACHM used in medical research?

Experiments involving ACHM are undertaken for several overlapping reasons:

- Understanding human body function, or malfunction in disease, often requires *in vivo* study carried out in humans or, where that is morally or practically infeasible, in animals. This is because substitutes such as cell culture or computer simulation often do not satisfactorily mimic the complex three-dimensional structures that typify human tissues and organs, or their change over time.
- DNA sequence data from many species is increasingly available, but often the only way to determine the function of a specific piece of DNA is to observe its effect in a living animal. For example, this can reveal whether the function of the DNA in man is the same as in other species, or if it affects development, or causes disease.
- In many cases research is driven by a desire to improve our ability to diagnose and treat disease. Animals containing human DNA or cells provide important methods to study human disease more effectively, to test potential solutions and sometimes to develop or produce therapeutics.[11]

Of course, scientists like everyone else, are also motivated by wider factors (e.g. a desire to understand how things work, career advancement) and this applies to ACHM research in the same way as it does to other areas of science. The outcome of their work may be just as important, irrespective of their motives.

Animals used in the laboratory are sufficiently good models of aspects of human biology that their use can often generate useful information. However, the differences between species mean that experimental findings in animals always need careful consideration before extrapolation to man. Modifying animals to make them more similar to humans, in specific biological or disease characteristics, may improve the utility of results from such experiments. We recognise that, as for other types of animal research, the creation and use of ACHM has the potential to cause pain, suffering or harm to the animals involved. Consideration of these matters is the basis of UK regulation of animal research, which serves to minimise these concerns (see 4.1 and 5.5).

2.1.2 What species of animals are used?

A wide range of animals are used as recipients of human material in research. Mice are the most frequently used due to their small size, short generation time and well-understood biology and genetics; the development of rodents with biology more like that of humans is an important aspect of inter-species research. Some species are used because of their inherent similarity to humans (e.g. the size and physiology of organs such as the heart in pigs; the organisation of the NHP brain), others because aspects of their biology facilitate the techniques used (e.g. human DNA can be easily inserted into the eggs of frogs).[12]

It is difficult to estimate the number of ACHM used in UK research as these data are not systematically collected. But, although ACHM are only a proportion of the animals used in research, their development and use can support animal research welfare principles by contributing to the improvement of research approaches (see 4.1).

11 It is usually a regulatory requirement to test drugs and other therapies in animals before they can be used in humans, to assess both safety and efficacy. Because ACHM are likely to provide more relevant data than normal animals, it is possible that in future fewer animals may need to be used. The use of ACHM may also in some situations replace the use of NHPs.

12 For a broader discussion of the use of animals in research see Nuffield Council on Bioethics (2005). *The ethics of research involving animals*. Section 2, 83–184.

2.1.3 Types of research involving ACHM

ACHM are used in both investigational research (to understand underlying biology) and translational research (to find treatments and diagnostics), although the distinction between these is not clear cut. We consider the research uses of ACHM in two broad groups:

- **Investigating health and disease.** By substituting part of an animal's genetic material or tissues with a human equivalent, animals can be made to more closely replicate aspects of human biology, or to become susceptible to human diseases. These 'animal models' are used in investigational studies to understand human biological processes in health and in disease.
- **Developing and testing therapeutic products.** Animals are increasingly used both to produce humanised substances (e.g. proteins and antibodies) for use as therapeutic agents, and to test drugs and other therapies (including human-derived products such as stem cell therapies).

There are many different research avenues, and thousands of studies, in these overlapping fields. In section 2.3 we give illustrative examples of work across these areas, to give a flavour of the research which is being undertaken. These examples are intended to inform readers about the range and nature of work we are discussing, and not to imply that ACHM research is uniformly successful or that other research avenues are less valuable.

Box 2.1 What do we mean by a 'species'?

To discuss inter-species (between different species) mixtures it is helpful to consider the meaning of the term 'species'. At a simple level, the distinction between animals of different species is intuitively obvious; a cat is easily recognised as different from a dog, and we instinctively think of animals from separate species as different 'kinds'. However, all animals are evolutionarily related, with a clear gradient of relationships from distant (e.g. beetles and fish) to close relations (e.g. gorillas and chimpanzees). Some species are so closely related that they can interbreed, although the resulting offspring are generally sterile: for example a horse and donkey can breed to produce a mule.

A common biological definition of 'species' is *'a group of organisms capable of interbreeding and producing fertile offspring'*. However, this definition has some limitations, e.g. where breeding is not attempted owing to geographical separation, we do not know whether mating would produce fertile offspring.

Since the late 1980s scientists have explored species differences by comparing DNA sequence similarity – which can be quantified at a molecular level. DNA sequences of closely related species are more similar than those of distantly related species, and this principle has enabled the evolutionary relationships between different species to be clarified (an approach known as molecular phylogenetics). Studies are also now underway to identify regions of DNA that are species-specific, including those unique to humans and our ancestors (human-lineage specific sequences: see 3.2).

There must be sequences of DNA that contain the critical variations which set different species apart by determining their unique spectra of physical characteristics and their ability to interbreed, but most of these are still unknown. Species boundaries cannot be adequately defined as percentage variation between DNA sequences, or by the inclusion of currently known specific DNA sequences, and therefore currently continue to depend on distinctions between visible characteristics and the ability to interbreed. Indeed, DNA of closely related species is very similar – and much research involving inter-species mixtures is only possible because sections of DNA moved between even distantly related species can remain functional.

2.2 Types of ACHM

ACHM are a range of 'inter-species' entities in which the animal component predominates over the human (for definitions see Box 2.1 and Annex IV).[13] We consider three types of ACHM: genetically altered animals (including transgenics), chimæras and hybrids.

2.2.1 Genetically altered animals

There are two principal ways in which human DNA sequence can be incorporated into an animal's genome:

1. A section of human DNA sequence can be inserted into the genome of an animal cell. Cells carrying the inserted (human) gene sequence, or animals developed from them, are often referred to as 'transgenic'. This approach is possible in several animal species, using a range of techniques (see Box 2.3).
2. The genome of an animal can be modified so that it has, in part, the same DNA sequence as that found in the human. This can be achieved using 'gene-targeting' techniques, which are well-established in mice and in development for use in other species (including rat and some NHPs) (see Box 2.3). Specific DNA sequences can also be deleted to mimic aspects of the human genome, such as when genes or regulatory regions have been lost during human evolution (see Box 2.2). In such cases the animal's genome can be considered to have been humanised because it is altered to resemble the human, even though no human DNA sequence has been added. The use of such animals in research should therefore be governed by the same principles as ACHM.

These approaches create an animal with a genetic sequence that, in a specific part, resembles that of the human (the animal's DNA is humanised or made 'human-like'). For simplicity we refer to animals created by these methods as 'genetically altered'.

Genetic alterations can range from changes to one or two DNA base pairs (see *FOXP2*, 3.6.2), up to the exchange of extensive regions of animal DNA for human equivalents (see a-globin locus, 3.2), or the addition of an entire human chromosome (see Down's mouse, 3.2). Where 'human' DNA is used to create ACHM, it is very rarely taken directly from a person. DNA may be derived from cultured human cell lines, grown as recombinant DNA in bacteria, or artificially synthesised to produce the exact sequence found in humans.

Usually, almost every cell of a genetically altered animal contains the same DNA.[14] Where genetic alterations are present in the reproductive (germ) cells of the animal, they can be transmitted to offspring. Methods have also been developed to introduce genes into particular somatic tissues (e.g. the lung or eye) of animals. In this case, modifications are not introduced into animals' reproductive cells, and would not be transmitted. These techniques are the basis of 'gene therapy' approaches to treating disease (see 2.3.2).

Sections 2.3.1 and 2.3.2 illustrate research uses of animals humanised by genetic alteration.

2.2.2 Chimæras

Chimæras are formed by mixing together whole cells originating from different organisms. The new organism that results is made up of a 'patchwork' of cells from the two different sources. Each cell of a chimæra contains genes from only one of the organisms from which it is made.[15,16,17] In contrast to transgenics, DNA from different origins is not mixed within individual cells. The 'mixture' of cells found in tissues of a chimæra is not transmitted to future generations.

13 For a discussion of entities in which the *human* element is predominant see Academy of Medical Sciences (2007). *Inter-species embryos.* http://www.acmedsci.ac.uk/p48prid51.html

14 With the exception of some unusual cell types e.g. red blood cells that lack DNA, and germ cells after they have undergone meiotic recombination, where the DNA sequence is shuffled.

15 With the exception of certain cell types that naturally undergo cell fusion such as specific cells in the placenta (syncytial trophoblast), and skeletal and cardiac muscle cells.

16 For an example of inter-species fusion involving muscle cells see Gentile A, *et al.* (2011). *Human epicardium-derived cells fuse with high efficiency with skeletal myotubes and differentiate toward the skeletal muscle phenotype: a comparison study with stromal and endothelial cells*. Mol Biol Cell **22**, 581–92.

17 There are also reports of rare cell fusion events, which complicate the interpretation of results of investigation of stem cell potential in chimæras, see Ying QL, *et al*. (2002). *Changing potency by spontaneous fusion*. Nature **416**, 545–8.

Chimæras can occur naturally, including in man. For example, cells from a developing fetus can colonise the mother, maternal cells can colonise a developing fetus, two pre-implantation embryos can combine, and in rare instances, cells can be transferred between siblings during twin pregnancy.[18]

The extent to which cells from different origins become integrated into the body of a chimæra depends on several factors including:

- The kind of cells involved (e.g. cells from the early embryo with broad developmental potential (the potential to develop into many kinds of tissue) may integrate widely; stem cells derived from an adult tissue such as liver or brain with narrower potential may integrate less widely).
- The relative numbers of cells of the two species.
- The developmental stage of the recipient animal (e.g. embryonic, fetal, newborn or adult). Earlier mixing is more likely to lead to widespread integration of the different species' cells, in many organs and tissues (although this also depends on the potential of the donor cells and on species compatibility: for example, slowly dividing human cells may not contribute widely to a rapidly growing animal embryo).

For the purposes of our discussions, we consider two types of chimæra:

- **Primary chimæras** are formed by mixing together two early embryos, or an early embryo with isolated embryonic cell types obtained from a different embryo or cultured stem cell line. The resulting chimæra has cells of different origins, in many tissues.
- **Secondary chimæras** are formed experimentally by transplanting (or grafting) cells or tissues into animals at later stages of development, including late fetal stages, post-natal or even adult animals.[19] The donor cells are only present in a few tissues.[20] The recipient animal is often chosen to be immune-deficient, or immune-suppressed.[21] However, especially with recent developments in imaging techniques, it is possible to introduce cells into an embryo *in utero* (or *in ovo*) and to study the results in live-born animals. This can be done before the development of the host's immune system, such that the grafted cells are recognised as 'self' and not rejected.

In making primary chimæras, various methods can be used to bias the contribution of 'donor' versus 'host' embryo cells. For example, if one pre-implantation embryo is more advanced than the other, the smaller cells of the former preferentially contribute to the inner cell mass (ICM; developing embryo proper) of the resulting blastocyst, whereas the larger cells of the latter tend to give rise to extra-embryonic tissues of the placenta. If chimæras are being made with pluripotent stem cells (embryonic stem (ES) or induced pluripotent stem (iPS) cells; for further information on stem cells see 3.3) combined with cleavage stage embryos, the former will preferentially end up in the ICM. A more rigorous way to alter the contribution of cells from two different sources ('donor' and 'host') to an embryo is to use a method termed 'tetraploid complementation' (see 6.2.2). Some stem cell types, including ES or iPS cells, (at least of the mouse) readily contribute to the embryo proper (the developing body of the organism) but not to extra-embryonic tissues (e.g. placental tissues). In contrast, embryo cells made to have double the normal number of chromosomes ('tetraploid cells') are able to produce extra-embryonic tissues, but contribute poorly to the embryo proper, especially in a chimæra where they are in competition with normal cells. By combining tetraploid host embryos with pluripotent stem cells, the latter can give rise to the entire fetus and thus to the live-born animal while the host embryo cells become confined to the placental tissues. This is an example of cell selection. More sophisticated examples of such approaches using genetic methods can replace a whole organ with cells from another species (see examples in 2.2.3).

18 Boklage CE (2006).*Embryogenesis of chimæras, twins and anterior midline asymmetries.* Hum Reprod **21,** 79–91.
19 There is no distinct boundary between primary and secondary chimæras.
20 The mixture of tissues in a secondary chimæra cannot be transmitted to its offspring.
21 The term 'xenotransplantation' is commonly used to refer to animal-to-human xenotransplantation.

Human cells used to create chimæras can be taken with appropriate consent directly from early embryos (e.g. surplus from IVF treatments), aborted fetuses, or a live-born person (e.g. human liver cells, or a cancer biopsy) or from cultured human cell lines. Sections 2.3.3 and 2.3.4 illustrate the uses of animal–human chimæras in research.

2.2.3 Hybrids

Animals formed by the fertilisation of an egg of one species by the sperm of a different species are called 'true hybrids'.[22] Each cell of the hybrid embryo, and the resulting animal if development occurs, has a complete set of genes from each parent. A small number of true hybrid animals occur in nature, as a consequence of mating between closely related animal species. The offspring are usually infertile (e.g. a mule is the sterile hybrid of horse and donkey). It is now possible to attempt techniques of assisted reproduction, such as intra-cytoplasmic sperm injection (ICSI), using eggs from one species and sperm from another. However, we are not aware of the production of viable offspring between animal species, other than those that are very closely related, in this way.[23]

The use of true hybrid *animals* formed from the combination of human and animal gametes is not currently envisaged in medical research. The fertilisation of animal eggs (hamster or mouse) by human sperm was previously used in sperm fertility testing.[24] It continues to be used in studies of reproductive biology, and has enabled, for example, identification of the roles of ion channels and enzymes found in human sperm in the process of egg activation, and the relationship between factors such as the sperm head shape and successful egg activation.[25,26] This information has been claimed to improve the selection of sperm for clinical use in assisted reproductive techniques.[27]

Although the creation of true hybrids using human cells is permitted in the UK, it is illegal to keep or use the hybrid embryos *in vitro* beyond very early developmental stages, or to implant them into a uterus (of a woman or animal) (see Box 6.5). Such entities would in any case be very unlikely to survive into later stages of development (except perhaps between very closely related species) because of the multiple biochemical and molecular incompatibilities between different species.

In contrast to hybrid *animals,* inter-specific *cell hybrids,* created by the fusion of cells from two different species (e.g. human cells fused with mouse cells) are widely used in research. Fusions are usually made between somatic cells rather than germ cells, and the cell hybrids do not develop into animals. They can, however, be made to grow for long periods of time in cell culture. On fusion, each hybrid cell contains a full set of chromosomes from each species; however, chromosomes are shed during cell culture, resulting in cell lines in which chromosomes from one species often predominate. Thousands of hybrid cell lines have been used over the past 30 years to explore fundamental issues in biology. Many human genes were mapped in the 1970s using this kind of cell hybrid, as a prelude to the human genome project.[28]

22 *True hybrids* are one of five types of *human admixed embryos* described in the UK's Human Fertilisation and Embryology Act (see Box 6.4). For further discussion of their use in research see Academy of Medical Sciences (2007). *Inter-species embryos.* http://www.acmedsci.ac.uk/p48prid51.html

23 Cross-species reproductive cloning methods involve the production of 'cytoplasmic hybrids', with nuclear DNA from one species and cytoplasm (containing mitochondrial DNA) from another. Such techniques have been investigated as a method of 'preserving' endangered species. For example, successful cloning of closely related sub-species has been achieved in the cat and wolf. However, a recent attempt to clone the panda using rabbit eggs was unsuccessful. See Lanza RP, *et al*. (2000). *Cloning of an endangered species (Bos gaurus) using interspecies nuclear transfer.* Cloning **2,** 79–90; Gomez MC, *et al.* (2008). *Nuclear transfer of sand cat cells into enucleated domestic cat oocytes is affected by cryopreservation of donor cells.* Cloning Stem Cells **10**, 469–83; Oh HJ, *et al*. (2008). *Cloning endangered gray wolves (Canis lupus) from somatic cells collected postmortem*. Theriogenology **70,** 638–47; Chen DY, *et al*. (2002). *Interspecies implantation and mitochondria fate of panda–rabbit cloned embryos*. Biol Reprod **67,** 637–42.

24 The 'hamster zona-free ovum test' initially proved to be a promising new test of fertilisation potential but was not found to be of significant clinical use compared with routine semen analysis. See Yanagimachi H, *et al*. (1976). *The use of zona-free animal ova as a test-system for the assessment of the fertilizing capacity of human spermatozoa*. Biology of Reproduction **15** (4), 471–76; Aitken RJ (1985). *Diagnostic value of the zona-free hamster oocyte penetration test and sperm movement characteristics in oligozoospermia*. Int J Androl **8,** 348–56.

25 Li CY, *et al.* (2010). *CFTR is essential for sperm fertilizing capacity and is correlated with sperm quality in humans*. Hum Reprod **25**, 317–27.

26 Heytens E, *et al*. (2009). *Reduced amounts and abnormal forms of phospholipase C zeta (PLCzeta) in spermatozoa from infertile men.* Hum Reprod **24**, 2417–28.

27 Ito C, *et al*. (2009). *Oocyte activation ability correlates with head flatness and presence of perinuclear theca substance in human and mouse sperm*. Hum Reprod **24**, 2588–95.

28 By creating a range of cell lines with differing human chromosome content, and comparing the chromosome content with the gene expression and function of different cell lines, specific genes could be mapped to specific chromosomes. See Griffiths AJ, *et al.* (2000). *Mapping human genes by using human–rodent somatic cell hybrids*. In: An Introduction to Genetic Analysis. Freeman WH, New York.

Box 2.2 Genes and their function

What is a gene?

Most genes encode proteins that are the molecules that comprise much of our cells and tissues. DNA coding for one protein is seldom found in a single stretch of DNA sequence, but is split into sections (exons) along the DNA molecule. By splicing different parts a single gene together, cells can sometimes make several related proteins from a single section of genetic template. The regulatory elements that function as switches to control gene expression are located adjacent to the protein coding region, or sometimes at considerable distances 'upstream' or 'downstream' and/or within the intervals (called 'introns') between the protein coding parts of genes.

How do genes 'work'?

In simple terms, a length of DNA known as a gene is 'read' (transcribed), by an enzyme in the cell nucleus, creating a matching chemical message (messenger RNA (mRNA)) which passes into the cell body and is translated into the protein encoded by the gene. DNA in many different organisms is remarkably similar, so that some genes can be made to 'work' in this way even when moved between very different organisms. For example, human gene sequences (such as the *cdc2* gene, see 2.3.1) can be read by yeast cells, producing human gene products that can function in conjunction with other yeast cell components. Some genes do not code for proteins, but for active RNA molecules, many of which are involved in regulating genes.

What is a 'disease gene'?

While people often speak loosely of a 'gene for' a disease, genes actually code for functional proteins, and disease is a consequence of an error ('mutation') within the gene or its regulating regions, which means the corresponding protein does not function properly. For example, a 'gene for haemophilia' actually codes for a protein that is needed in blood clotting; patients with the damaged gene lack the functional protein, and the resultant failure of normal clotting is called haemophilia.

What is a 'human' gene?

What do we mean when we use the terms 'human gene' or 'mouse gene'? We are referring to the DNA sequence of a gene found in humans or mice. However, DNA can be made synthetically from its chemical parts, and it is possible to create pieces of DNA identical to the genes found in a human or mouse, that have never been part of a living animal. The 'artificial life form' created in 2010 is an extreme illustration of this; a copy of the full genome of the bacterium *Mycoplasma mycoides* was artificially synthesised and inserted into a cell of another bacterium, producing an organism able to grow and self-replicate under the direction of artificial DNA alone.[29]

The DNA sequence of a particular gene is often very similar in different species. For example the DNA sequence of the *PAX6* gene, which codes for a protein in eye and brain development, is almost identical in human and mouse; the protein coded by the gene has the same amino-acid sequence in both species. There are also large regions, up to 1000 nucleotides long, of *PAX6* regulatory DNA that are completely identical in humans and mice.[30]

What we really mean by a 'human' gene is a section of DNA performing a particular function, which carries the few distinctive bits of sequence (which may only be a few percent of its total length) and which differ between humans and other species. However, there are probably some genes (and perhaps more regulatory regions) that are unique to humans. We can determine their importance and relevance to human evolution by asking how they work in transgenic animals.

29 Gibson DG, *et al.* (2010). *Creation of a bacterial cell controlled by a chemically synthesized genome*. Science **329**, 52-6.
30 van Heyningen V & Williamson KA (2002). *PAX6 in sensory development*. Hum Mol Genet **11**, 1161-7.

2.3 How are ACHM used in research?

2.3.1 Genetically altered animals in investigating health and disease

The DNA sequence of many species is sufficiently similar for sections from one species to retain their function when incorporated into cells of a different species. In a classic experiment, human DNA was inserted into mutant yeast cells defective in a gene (*cdc2*) known to be crucially important in regulating yeast cell division. Remarkably, some pieces of human DNA were able to compensate for the defective yeast gene, allowing the mutant cells to divide normally. Researchers thus identified the human *cdc2* gene, which is so similar that it could compensate for the defective yeast gene.[31] These experiments were important in demonstrating that some genes responsible for controlling basic cell functions like cell division are highly conserved (meaning they have retained the same structure and function throughout evolution). The process of cell division is fundamental to understanding cancer, and variants of the *cdc2* gene are associated with some forms of human cancer. (See Box 2.4 for uses of genetically altered cells.)

It is now almost routine to incorporate human DNA into animal eggs or embryos; the resulting genetically altered animals are used ubiquitously in research to investigate the function of human genes and the proteins they encode. For example, the melanocortin receptor (MC1R) regulates pigmentation in mammals and is necessary for the production of dark melanin pigment in skin and hair. Humans with certain MC1R variants have red hair, pale ultraviolet-sensitive skin and are at increased risk of skin cancer. Mice expressing these human MC1R variants have yellow coats, and have been used to study the activation of MC1R receptors, and to identify the cell signalling pathways through which they work.[32]

Where the genetic basis of a disease in humans is known or suspected, the particular variant of the human gene associated with the disease can be incorporated into an animal to study the disease (see Box 2.3). We received many submissions describing the use of mice expressing human genes to study conditions as varied as migraine, anxiety disorders, osteoporosis, diabetes, heart disease and cancer.[33] However, the use of a wider range of species was also evident, including fruit flies expressing human ion channels used to study neurodegenerative disorders, and pigs expressing human polypeptide receptors in diabetes research.[34,35]

31 Lee MG, *et al*. (1987). *Complementation used to clone a human homologue of the fission yeast cell cycle control gene cdc2.* Nature **327** (6117), 31–5.
32 Jackson IJ, *et al*. (2007). *Humanized MC1R transgenic mice reveal human specific receptor function*. Hum Mol Genet **16**, 2341–8.
33 Eikermann-Haerter K, *et al*. (2009). *Androgenic suppression of spreading depression in familial hemiplegic migraine type 1 mutant mice*. Ann Neurol **66**, 564–8; Jennings KA, *et al*. (2006). *Increased expression of the 5-HT transporter confers a low-anxiety phenotype linked to decreased 5-HT transmission*. J Neurosci **26**, 8955–64; Daley E, *et al*. (2010). *Variable bone fragility associated with an Amish COL1A2 variant and a knock-in mouse model*. J Bone Miner Res **25**, 247–61; King M, *et al*. (2008). *Humanized mice for the study of type 1 diabetes and beta cell function*. Ann N Y Acad Sci **1150**, 46–53; Su Q, *et al*. (2008). *A DNA transposon-based approach to validate oncogenic mutations in the mouse*. Proc Natl Acad Sci USA **105**, 19904–9.
34 Moffat KG (2008). *Drosophila genetics for the analysis of neurobiological disease*. SEB Exp Biol Ser **60**, 9–24.
35 Renner S, *et al*. (2010). *Glucose intolerance and reduced proliferation of pancreatic ß-cells in transgenic pigs with impaired glucose-dependent insulinotropic polypeptide function*. Diabetes **59**, 1228–38.

Box 2.3 Examples of research methods used to make genetically altered animals

1. Transgenesis can be achieved in a wide range of species, using methods including:

- **DNA microinjection.** Copies of a segment of (e.g. human) DNA are directly injected into the nucleus of a fertilised animal egg, which is gestated in a surrogate female.[36] The genomes of the offspring are analysed, and animals in which the injected DNA has integrated are bred for use. DNA insertion occurs at random, and often in multiple copies. Genes within the introduced DNA can be expressed in a manner that is expected, or they can show ectopic (out of place) expression depending on the site of integration. In a minority of cases the integration event can disrupt the activity of an endogenous gene.[37]
- **Retrovirus-mediated gene transfer.** A modified carrier virus (or 'vector') is used to insert a transgene into the cells of a developing embryo, which is gestated in a surrogate female. The resulting offspring are often genetic 'mosaics', developed from a mixture of cells with one or more copies of the inserted sequence at different places in their genomes. Animals where the germ cells have the required integrated DNA are bred to create transgenic animal strains. Recent studies indicate that it may be possible to generate transgenic NHPs in this way.[38]

2. Gene-targeting methods include:

- **Homologous recombination in embryonic stem (ES) cells** is used to engineer precise changes in the mouse genome.[39] ES cells are genetically modified *in vitro,* e.g. to add, remove or exchange a specific genetic sequence at a specific location in the genome. Individual cells can be selected that following rare DNA recombination events, have the intended changes to their DNA.[40,41] These cells are injected into early stage mouse embryos to make chimæras. Mice with germ cells developed from the altered ES cells are bred, to create a line of genetically altered mice.

These methods in the mouse have become very sophisticated. Similar techniques are being developed in other species (see 3.2). In theory it ought to be possible make chimæras with NHP ES cells (which have very similar properties to human ES cells, distinct from those of the mouse) and NHP embryos, though this has not yet been attempted to our knowledge.[42] It is not clear whether human pluripotent cells can contribute to pre-implantation human embryos to make chimæras.[43] (Additional methods of transgenesis and gene targeting see [44])

3. Somatic cell 'gene therapy'.Techniques have been developed to integrate transgenes into particular somatic tissues (such as immune cells, the lung or retina). These methods often use modified viruses as 'vectors' to carry sections of DNA into the cells of adult animals or humans, rather than embryos. These methods generally involve gene addition rather than replacement, with the purpose of restoring the function of an abnormal gene.

36 Gestation in a surrogate is used for research involving mammals; the embryos of other genetically altered species, including chick, frog and fish can develop by themselves.

37 For an overview see Gama Sosa MA, *et al*. (2010). *Animal transgenesis: an overview.* Brain Struct Funct **214**, 91–109.

38 Niu Y, *et al*. (2010). *Transgenic rhesus monkeys produced by gene transfer into early-cleavage-stage embryos using a simian immunodeficiency virus-based vector*. Proc Natl Acad Sci USA **107**, 17663–7.

39 The types of change can include deletions, insertions, or replacement of one DNA sequence with another. These methods rely on the use of DNA sequences, at the ends of the donor DNA that are homologous to (match) the target site in the ES cell genome.

40 While DNA usually integrates at random in mammalian cells, even rare homologous recombination events can be found by screening large numbers of ES cells.

41 Gordon JW, *et al*. (1980). *Genetic transformation of mouse embryos by microinjection of purified DNA*. Proc Natl Acad Sci USA **77**, 7380–4.

42 Wianny F, *et al*. (2011). *Embryonic stem cells in non-human primates: An overview of neural differentiation potential*. Differentiation **81**, 142–52.

43 Although the HFE Act (2008) would allow these experiments to be initiated, it would be illegal to keep such entities intact *in vitro* for more than 14 days or to implant them (see Box 6.5).

44 **a. Sperm-mediated gene transfer.** Can also be used to create transgenics. A sequence of DNA is introduced into the head of a sperm, which is then used for fertilisation. This approach has been used in species including frog, mouse, rat and pig.
b. Genetic alteration of somatic cells combined with nuclear transfer. In species for which ES cells are unavailable (e.g. sheep) gene targeting can be conducted by combining the use of somatic cells (e.g. fibroblasts) genetically modified in culture, with nuclear transfer cloning techniques. See Denning C, *et al*. (2001). *Gene targeting in primary fetal fibroblasts from sheep and pig*. Cloning Stem Cells **3**, 221–31.
c. Zinc-finger nuclease (ZFN) methods. These methods can be used on cells in culture, or after DNA microinjection into fertilised eggs. In principle this method can be used to introduce human DNA into any animal species and in a targeted fashion. See Whyte JJ, *et al.* (2011). *Gene targeting with zinc finger nucleases to produce cloned eGFP knockout pigs*. Mol Reprod Dev **78**, 2.
d. Genetic modification of spermatogonial stem cells. Male germ-line (spermatogonial) stem cells can be genetically modified and transplanted into the testicular tissue of an infertile male animal where they give rise to modified sperm cells. This approach has been developed in the mouse. See Takehashi M, *et al*. (2010). *Generation of genetically modified animals using spermatogonial stem cells*. Dev Growth Differ **52,** 303–10.

Box 2.4 Transgenic and genetically altered cells

Individual animal cells, or cell lines, into which human genes are inserted (or 'transfected') are widely used in investigational research and drug development.

Expression of human DNA in frog eggs has been used to understand the function of some human transporter proteins (molecules that move substances into and out of cells). One of the first demonstrations of the chloride channel function of the cystic fibrosis gene was achieved using this approach.[45] More recently, suggestions arose of an association between variants of the human gene *SLC2A9* with high uric acid levels in gout. Human *SLC2A9* was initially thought to encode a protein used only to transport sugars; however, its expression in frog eggs revealed a new role for the transporter in carrying uric acid, and suggested a rationale for the links between human *SLC2A9* gene variants and gout.[46]

Transfected cells lines expressing human genes are also used in the pharmaceutical industry in screening to identify novel drug molecules, and to express human proteins (marketed products include human erthyropoetin for use in anaemia, and blood clotting factors for use in haemophilia, produced in Chinese hamster ovary cells).[47]

(See also 2.2.3 for the uses of inter-specific cell hybrids.)

Huntington's disease (HD) is a genetic neurodegenerative condition, in which nerve cells in some parts of the brain accumulate granular protein and subsequently die. Animal models of HD have been created in flies, zebrafish, mice and sheep by incorporating the mutant form of the human Huntingtin gene, which causes HD in man, into the animals' genomes.[48,49] A rhesus macaque transgenic model of the disease was also reported in 2008, although the mutant human Huntingtin gene did not transmit to offspring.[50]

Studies using cell cultures and these animal models indicated that the abnormal granular protein product of the mutant Huntingtin gene, which is toxic to brain cells, could be cleared by a process called autophagy. Drugs that induce autophagy were identified, and found to enhance the removal of the protein and thus decrease its toxicity. The consistent effect of this strategy in the animal models of HD suggested that a drug might similarly modify the accumulation of the toxic protein granules in human brain cells. Safety testing of one these drugs is now underway, as a precursor to clinical trials in patients.[51] Autophagy has also been implicated in other diseases including Parkinson's, Alzheimer's, and forms of cancer – some of the evidence for this association comes from comparable studies in transgenic mice expressing the human proteins mutated in these diseases.

The study of Duchenne muscular dystrophy (DMD), a condition that causes progressive muscle wasting in boys leading to death in early adulthood, has been facilitated by genetically altered animals expressing human gene variants. A mouse was first discovered that carried a dystrophin gene mutation similar to that causing DMD in humans.[52] Although the mouse had some biochemical and physical features of DMD, it lacked the characteristic

45 Bear CE, *et al*. (1991). *Cl- channel activity in Xenopus oocytes expressing the cystic fibrosis gene*. J Biol Chem **266**, 19142-5.
46 Vitart V, *et al*. (2008). *SLC2A9 is a newly identified urate transporter influencing serum urate concentration, urate excretion and gout*. Nat Genet **40**, 437-42.
47 See the European Medicines Agency http://www.ema.europa.eu/; EMEA/H/C/000726 epoetin alfa for the treatment of anaemia; EU/3/09/655: Human recombinant octocog alfa for the treatment of haemophilia A.
48 Williams A, *et al*. (2008). *Novel targets for Huntington's disease in an mTOR-independent autophagy pathway*. Nat Chem Biol **4**, 295–305.
49 Jacobsen JC, *et al*. (2010). *An ovine transgenic Huntington's disease model*. Hum Mol Genet **19**, 1873–82.
50 Yang SH, *et al*. (2008). *Towards a transgenic model of Huntington's disease in a non-human primate*. Nature **453**, 921–4.
51 Rose C, *et al*. (2010). *Rilmenidine attenuates toxicity of polyglutamine expansions in a mouse model of Huntington's disease*. Hum Mol Genet **19**, 2144–53.
52 Bulfield G, *et al*. (1984). *X chromosome-linked muscular dystrophy (mdx) in the mouse.* Proc Natl Acad Sci USA **81**, 1189–92.

severe early onset, and so did not fully mimic human DMD. A second gene, utrophin, was later identified and found to have a very similar function to the dystrophin gene. Although the utrophin gene is inactivated early in embryonic life in humans, mice can partially re-activate this gene in adulthood, compensate for an absence of dystrophin, and ameliorate the effects of DMD. Mice genetically altered to lack the function of both genes show severe disease and more closely model human DMD. Research using mouse models has since led to the development of several putative DMD treatments, including an approach which partially corrects the genetic defect in many cases of DMD, now in clinical trial.[53]

A strain of dog with a spontaneous dystrophin mutation has also been used in DMD research.[54] Large animal models are not always needed in disease research, and pre-clinical research in such species including dogs is not necessarily a pre-requisite for drug development. However, conditions such as heart disease and cognitive dysfunction may require large animal models because of the significant biological differences between man and mouse; humanised animal models may in future be of use in the development of therapies for such diseases.

While many human diseases (e.g. HD, DMD) are caused by mutations in protein coding regions of DNA, disease-causing mutations also occur in DNA regulatory regions (which do not encode protein but regulate gene expression). Regulatory regions are often located at a considerable distance from the genes they control, and the creation of accurate animal disease models involving mutations in these regions therefore requires the transfer of extensive sections of DNA (see the modification of α-globin gene locus used to model the blood disorder α-thalassaemia in the mouse in 3.2). The study of human gene regulatory regions in transgenic animals (mice, chick, embryos, frogs and fish), combined with detailed sequence comparisons, has also led to basic understanding of how these function normally, or are defective in genetic disease, and how they and the gene regulatory mechanisms have evolved.[55,56,57] We anticipate that it will become increasingly possible to accurately manipulate large sections of human DNA.

2.3.2 Genetically altered animals used in developing and testing therapeutics

Animals containing human genetic sequence can be developed to produce humanised substances (e.g. proteins and antibodies) for use as 'biological therapeutics' in people with deficiency of a particular substance, or in other forms of novel treatment.[58]

In an approach sometimes called 'pharming', transgenic animals have been created which carry a human gene, and secrete the associated human protein e.g. as a component of their milk. The protein is extracted, purified and used for treatment. Such 'therapeutic proteins' have been produced in sheep, goats, cattle, and rabbits; chickens have been developed which produce human proteins in their egg white.[59] In 2009, *ATryn*, a human anti-thrombin protein made by transgenic goats was licensed for use during surgery in patients with a congenital blood clotting disorder.[60] Similar products in development include human α-1 antitrypsin for emphysema treatment, and blood clotting factors for haemophilia treatment. In these approaches the genetically altered animals are, in effect, used to manufacture often large amounts of fully functional proteins, which cannot be produced in cell lines.

53 Kinali M, *et al*. (2009). *Local restoration of dystrophin expression with the morpholino oligomer AVI-4658 in Duchenne muscular dystrophy: a single-blind, placebo-controlled, dose-escalation, proof-of-concept study*. Lancet Neurol **8**, 918–28.
54 To date these have not been used extensively in therapeutic drug development.
55 For an example of an early transgenic experiment see Koopman P, *et al*. (1989). *Widespread expression of human alpha 1-antitrypsin in transgenic mice revealed by in situ hybridization*. Genes Dev **3**, 16–25.
56 For an example of a recent paper involving a systematic study of regulatory sequences see Schmidt D, *et al*. (2010). *Five-vertebrate ChIP-seq reveals the evolutionary dynamics of transcription factor binding*. Science **328**, 1036–40.
57 For an example of a recent work considering loss of regulatory sequences in human evolution see McLean CY, *et al*. (2011). *Human-specific loss of regulatory DNA and the evolution of human-specific traits*. Nature **471**, 216–9.
58 Biological therapies are treatments for diseases that involve the use of biological materials or biological response modifiers, such as genes, cells, tissues, organs, serum, vaccines, antibodies or humoral agents. In contrast, pharmacological or chemical therapies are those which use small drug molecules.
59 Written evidence from the Biotechnology and Biological Sciences Research Council (BBSRC), and see for example Lillico SG, *et al*. (2007). *Oviduct-specific expression of two therapeutic proteins in transgenic hens*. Proc Natl Acad Sci USA **104**, 1771–6.
60 The European Public Assessment Report (EPAR), produced by the European Medicines Agency for ATryn is available at http://www.ema.europa.eu/humandocs/Humans/EPAR/atryn/atryn.htm

Humanised antibodies produced in animals are increasingly used as biological therapeutics. Animals produce a huge range of different antibodies which underpin the immune recognition and rejection of 'foreign' proteins ('the adaptive immune response'). Each antibody interacts highly specifically with a particular protein. This ability has been used to develop 'therapeutic antibodies' in which an antibody can act directly as a 'biological drug' by blocking some cellular function or killing the cell type targeted (e.g. cancer cells); or can be coupled to a drug which the antibody delivers to a specific target. This field is fast-growing; in mid-2009, there were close to 50 approved therapeutic antibodies on the market, and over 150 applications for new antibody products under consideration in the USA.[61] Antibodies are large, complex proteins, which are difficult to produce synthetically, but they can be obtained from animals or certain cell lines. However, animal antibodies injected into humans would be recognised as 'foreign protein' and eliminated by the human immune system. Recently, mice with 'humanised immune systems' have been engineered to produce antibodies that are not rejected by the human body, and so can be used in therapy.[62] This has been achieved using mice with antibody genes replaced by human equivalents (e.g. XenoMouse, see also 3.2).[63] In response to immunisation the mouse humanised immune systems respond by producing humanised antibodies, which can be selected and manufactured in cell lines. The human antibody Panitumumab, licensed for colorectal cancer treatment, was developed in this way. It targets a growth factor receptor, and inhibits tumour growth and vascularisation.[64]

The concept of 'gene replacement therapy' was first discussed in the early 1970s, but safe, effective procedures have proved difficult to develop. Gene therapy is based on the concept of inserting a functional copy of a gene into tissues where the gene is dysfunctional or absent (see Box 2.2). The aim is to perform human–human gene transfer; however, animal models are necessary to develop and refine the required reagents and techniques.

Leber congenital amaurosis (LCA) is a set of genetic eye diseases which often lead to complete blindness. One form of LCA is caused by a mutation in the *RPE65* gene, which encodes a protein needed for the recycling of visual pigment in the eye's light-sensing cells. Gene therapy aims to carry functional copies of the *RPE65* gene into the retina using a modified viral carrier introduced into the eye.[65] These methods have been developed in transgenic mice with a defective *RPE65* gene and in the Briard dog which naturally lacks the *RPE65* gene.[66] Both the mouse and dog models have early, severe visual impairment similar to that in human LCA; however, the dog eye is more similar to the human eye in size and structure. The effectiveness of this therapy in these animals enabled the approach to be taken forward into clinical trials; initial results suggest that it can be effective in humans, though further refinement will be required to produce a licensed treatment.[67,68] This approach may in future also turn out to be applicable to other eye diseases. There are particular sensitivities in using 'companion' animals such as dogs and cats for experimental purposes, but there are some unusual situations where they have clear advantages (either because of some aspect of

61 Nelson AL, *et al*. (2010). *Development trends for human monoclonal antibody therapeutics*. Nat Rev Drug Discov **9**, 767–74.
62 Kyowa Hakko Kirin California, Inc. have developed the TransChromo Mouse (TC Mouse™) that is capable of producing a variety of fully human monoclonal antibodies. They are also developing the TransChromo Cow (TC Cow™) for the production of polyclonal antibodies. See: http://kyowa-kirin-ca.com/tc_pubs.cfm
63 Jakobovits A, *et al*. (2007). *From XenoMouse technology to panitumumab, the first fully human antibody product from transgenic mice*. Nat Biotechnol **25**, 1134–43; Written evidence from the NC3Rs.
64 Giusti RM, *et al*. (2007). *FDA drug approval summary: panitumumab (Vectibix).* Oncologist **12**, 577–83.
65 Acland GM, *et al*. (2001). *Gene therapy restores vision in a canine model of childhood blindness*. Nat Genet **28**, 92–5. See also http://www.ucl.ac.uk/ioo/research/patients/clinical_trials.html
66 Bemelmans AP, *et al*. (2006). *Lentiviral gene transfer of RPE65 rescues survival and function of cones in a mouse model of Leber congenital amaurosis.* PLoS Med **3**, e347.
67 Bainbridge JW, *et al*. (2008). *Effect of gene therapy on visual function in Leber's congenital amaurosis*. N Engl J Med **358**, 2231–9.
68 Maguire AM, *et al*. (2008). *Safety and efficacy of gene transfer for Leber's congenital amaurosis*. N Engl J Med **358**, 2240–8.

their normal function or, as here, because of the presence of a naturally occurring disease which closely resembles a human disorder) as to outweigh this aversion. Animal models are also contributing to attempts to develop gene therapies for conditions including spinal muscular atrophy and β-thalassemia.[69,70]

Owing to a shortage of human donor organs, tissue from animals, particularly pigs, has for many years been investigated as a source of tissue for transplant, although safety concerns hampered the development of the field. Another major barrier to the xenotransplantation of organs from pigs to humans is the 'hyperacute immune response' in which the recipient's immune system destroys the lining of blood vessels in the engrafted tissue. Such rejection occurs in part because an antigen (alpha-Gal), which is not made by humans, is expressed on the surface of pig cells. Attempts are under way to develop pigs which do not express alpha-Gal.[71] An alternative approach is the development of transgenic pigs expressing critical human proteins which inhibit the human immune response, and whose organs are therefore less likely to be rejected. Evidence from pre-clinical studies has indicated the potential of this approach, for example hearts from transgenic pigs have been found to function following transplant into NHPs treated with immunosuppressive drugs.[72]

Transgenic mice may, in future, be used in drug-toxicity testing and in testing biological products such as live vaccines. These are avenues in which the use of humanised animals may reduce, or ultimately replace, the use of larger animal species. However, the development of such methods can take several decades, not only for the necessary scientific development, but in subsequently gaining acceptance from regulatory agencies.[73]

2.3.3 Chimæras in investigating health and disease

Primary chimæras

Chimæras are formed by combining whole cells from different origins (see 2.2.2). Primary chimæras, created by mixing together early embryos, or embryos and cells, have been used in the study of developmental biology for several decades. Embryonic cells (including ES cells, see Box 3.3) that are identifiably marked, are isolated from specific regions or at different embryonic stages, combined with normal embryos, and traced throughout subsequent development, revealing the origins of the different types of cells, organs and tissues in the developing animal.[74] Such research was fundamental to understanding early vertebrate development.[75] Usually such chimæras are constructed using embryonic cells from the same species, although a variety of inter-specific combinations have been tried. The latter usually only work at early embryonic stages when the two species are very close in evolutionary terms, otherwise incompatibilities, for example in growth rates or cell adhesion, lead to abnormalities and to early embryo failure. The recent availability of human ES and iPS cells (see Box 3.3) opens the way for an expanding amount of work of this sort, though we are aware of relatively few studies involving the introduction of human ES pluripotent cells into animal embryos. In 3.2 we consider situations in which the introduction of human stem cells into animals might require particularly careful consideration.

Secondary chimæras

Although human biology and disease pathology can often be studied directly in volunteers or patients, this approach is sometimes infeasible or unethical. Secondary chimæras, made by transplanting human cells or tissues into adult animals (see 2.2.2) are therefore used to:

69 Chiara F, *et al*. (2010). *Systemic Delivery of scAAV9 Expressing SMN Prolongs Survival in a Model of Spinal Muscular Atrophy*. Science Translational Medicine **2**, 35ra42.
70 Sadelain M (2006). *Recent advances in globin gene transfer for the treatment of beta-thalassemia and sickle cell anemia*. Curr Opin Hematol **13**, 142–8.
71 Cooper DK, *et al*. (2007). *Alpha1,3-galactosyltransferase gene-knockout pigs for xenotransplantation: where do we go from here?* Transplantation **84**, 1–7.
72 Ekser B, *et al*. (2009). *Xenotransplantation of solid organs in the pig-to-primate model*. Transpl Immunol **21**, 87–92. See also 4.2.4.
73 Since 2004, the European Medicines Agency have recognised a role for a some specific transgenic mice carrying human genes in the carcinogenicity testing of new drugs. See *Addendum to ICH S6: preclinical safety evaluation of biotechnology-derived pharmaceuticals* at http://www.ema.europa.eu/
74 These are sometimes known as cell 'potential' and 'lineage' experiments.
75 Le Douarin N & McLaren A (1984). *Chimæras in Developmental Biology.* Harcourt Brace Jovanovich, London.

- Maintain human cells and tissues, enabling their study *in vivo* (e.g. cancer biopsies).
- Model human organs or organ systems, by substituting an animal's cells or tissues with human equivalents. These approaches use human cell types which replicate and colonise in the recipient (e.g. human blood stem cells used to humanise the immune system of mice).
- Study infectious diseases which are normally human-specific (e.g. HIV) by introducing human cells which confer disease-susceptibility to the animal.

Engraftment of human cells into animals is complicated by the recipient's immune system, which often rejects foreign tissue. Immuno-compromised mice, which lack the ability to mount an adaptive immune response, and can therefore accept xenografts, have greatly facilitated such research.[76]

Studies, particularly in mice, have played a fundamental role in research over the past 50 years to understand the complex processes underpinning cancer. In these studies, excised pieces of human cancers, cancer cells or human cancer cell lines, are grafted into immune-deficient animals. These models have enabled investigation of the mechanisms of cancer tumour initiation and spread and facilitated the development of therapies including chemo- and radiotherapy.

For example, a recent use of cancer xenograft models has been to investigate the roles of certain cancer cell types in leukaemia (blood cancer). Studies in mice engrafted with human blood stem cells or leukaemic cells led to the identification of 'self-renewing' or 'cancer stem' cells. Evidence indicates that these cells can be responsible for the creation or relapse of tumours, and that they are resistant to chemotherapy and radiation therapy. The significance of these cells was a major conceptual change in the field, which is now being investigated in carcinomas (solid tumour types). Primary xenograft models (using tissue taken directly from patients) are becomingly increasingly used in preclinical drug development as they can show closer similarity to human cancer, including a better representation of cancer pathways and variation in therapeutic response, than earlier cell culture methods. Biopsied human cells can also be genetically modified before implantation, to investigate the specific mechanisms involved in particular cancers. These same models can be used to test therapeutics *in vivo*.[77]

Type 1 diabetes results from destruction of the insulin-producing β-cells in parts of the pancreas called islets, by the person's own immune system. Mice implanted with human islets have been used to study this condition. Recently, combined models have been made by engrafting human blood stem cells into immune-deficient mice (these cells colonise and humanise the mouse immune system) and subsequently implanting human islets. This approach is being used to refine techniques for transplanting islets between humans in the clinic. A long-term research goal is to develop treatments to restore human β-cells in diabetics (e.g. using stem cell therapy). The combined mouse model can be used in the development of these treatments, to study how human β-cells, derived from stem cells, colonise and function in human islets in the presence of a humanised immune system.[78]

A humanised mouse model has been used to study *Salmonella enterica serovar Typhi*, the bacterium that causes typhoid and usually only infects humans. Mice lacking their own lymphatic system, but engrafted with human leukocytes (a form of white blood cell), were found to be susceptible to the bacterial infection and after inoculation displayed symptoms

76 Immune-deficient mice are widely used in medical research. Their lack of immune response means that they do not reject foreign tissue and can be used to 'incubate' cells or tissue from mice or other species, typically as grafts under the skin on the back. The first mice to be used in this way were the 'nude mice' in which a mutation in the *FOXN1* gene results in the lack of the thymus organ (and so immune deficiency) together with a hairless appearance.

77 Dick JE (2008). *Stem cell concepts renew cancer research*. Blood **112**, 4793–807.

78 Brehm MA, *et al*. (2010). *Human immune system development and rejection of human islet allografts in spontaneously diabetic NOD-Rag1null IL2rgammanull Ins2Akita mice*. Diabetes **59**, 2265–70.

similar to the human disease. The mice have been used to study the mechanisms of typhoid disease progression (and to correlate these to the four stages of untreated typhoid in humans), and to investigate therapeutic strategies.[79]

The Epstein-Barr virus (EBV) is associated with lymphatic system cancers (lymphomas); the same virus, in adolescence, causes glandular fever. EBV is a human-specific pathogen; however, 'BLT' mice, humanised by transplantation of human fetal blood stem cells, liver and thymus tissues are susceptible to the virus.[80] Studies in these mice using modified viruses have clarified the way that EBV establishes lytic (cell killing) rather than latent (delayed) infection. Findings indicate that the outcome of EBV infection can be moderated by immune system responses, and that the lytic functions of EBV are important in lymphoma formation.[81]

Mammalian liver is capable of restoring its own damaged cells because liver cells (hepatocytes) have the ability re-enter the cell cycle and replicate. Isolated human hepatocytes can be introduced into surgically reduced, or genetically compromised livers of immune-deficient mice which they colonise, resulting in organs made up of cells of both species, which partially resemble human liver. Up to 95% of mouse liver cells can be replaced by human hepatocytes in this way.[82] Mice with such humanised livers are used to study liver diseases including hepatitis B and C (viruses that usually only infect humans and chimpanzees), and to test antiviral drugs.[83] Mice with humanised livers of this kind should also be useful for drug toxicity testing, as they should predict the metabolism of drugs by the human liver more effectively than tests on 'ordinary' mice.

Chimæric mice with humanised immune systems have been important in studying many aspects of HIV infection. For example 'BLT mice'[84] have been used to investigate how HIV infection causes depletion of a form of white blood cell important in the immune response ('T cells', which express a protein called CD4), leaving patients vulnerable to other infections. Studies in these mice have provided evidence that HIV causes this effect by directly infecting CD4-expressing cells, rather than by acting on other cell types. Humanised mouse models have also been used in studies to determine the mechanism of viral spread within the female reproductive tract, and to investigate putative prophylactics.[85]

2.3.4 Chimæras in developing and testing therapeutics

Stem cell treatments are a form of biological therapy (see footnote 55) ultimately intended to treat human patients with human stem cells. However, chimæric animal models are used to develop and to establish the methodologies involved.

Parkinson's disease (PD) is a degenerative disorder of the central nervous system, which involves the loss of multiple populations of nerve cells. Since the early 1980s human fetal tissues have been experimentally transplanted into the brain of patients to replace these lost neurons. These clinical studies have been supported by research in an NHP model, in which grafts of human fetal cells were shown to reverse Parkinsonian-like movement deficits induced by treatment with a neurotoxin. Although early clinical studies showed benefit, subsequent studies indicated a more variable outcome with some patients also experiencing adverse effects caused possibly by the abnormal innervation of the brain by different populations

79 Firoz Mian M, *et al*. (2010). *Humanized mice are susceptible to Salmonella typhi infection*. Cell Mol Immunol **8**, 83–7.
80 'BLT' is an abbreviation for blood, liver and thymus.
81 Ma SD, *et al.* (2011). *A new model of Epstein-Barr virus infection reveals an important role for early lytic viral protein expression in the development of lymphomas*. J Virol **85**, 165–77.
82 Bissig KD, *et al*. (2010). *Human liver chimeric mice provide a model for hepatitis B and C virus infection and treatment*. J Clin Invest **120**, 924–30.
83 Lupberger J, *et al.* (2011). *EGFR and EphA2 are host factors for hepatitis C virus entry and possible targets for antiviral therapy*. Nat Med **17**, 589–95.
84 Immune-deficient mice in which the immune system is humanised through implantation of human bone marrow stem cells, and the tissues of the fetal thymus and liver: see footnote 76.
85 Olesen R, *et al.* (2011). *Immune reconstitution of the female reproductive tract of humanized BLT mice and their susceptibility to human immunodeficiency virus infection*. J Reprod Immunol **88**, 195–203.

of cells within the graft (see 3.3.5). However, the preclinical studies in NHP plus the clinical studies have provided important proof-of-concept for the stem cell therapy and clinical trials that are currently envisaged, and so have been pivotal in the development of this field. Studies aimed at refining treatments involving both NHP and rodent models of PD are underway, and include the improvement of the preparation of the tissue and the way it is implanted in the brain.[86]

Rats engrafted with stem cells have been used to study the potential for repairing damage to the brain caused by stroke. A rat model of stroke has been developed in which the middle cerebral (brain) artery is transiently blocked, causing a loss of blood supply to brain tissue, as occurs in the commonest form of human stroke. The rats subsequently have human stem cells engrafted into the brain. Human neural stem cells derived from a human fetal tissue sample and grown *in vitro*, mesodermal or haematopoietic stem cells derived from bone marrow, or cord blood have been tested.

Typically, a few hundred thousand cells are injected, so that less than 0.001% of the rat's cells are replaced by the human cells. Evidence suggests that some stem cells become integrated in the rat brain, but this may not be necessary to achieve therapeutic effect. The effect of the stem cell treatment is usually evaluated by assessing the rats' behaviour, in tests of sensory or motor performance.[87] Following the evidence gathered in preclinical studies of this kind, stem cell therapies are now being clinically trialled in stroke patients.

Approval for the first trial of a human neural stem-cell-based product in the UK was granted in 2009. A trial in Glasgow is continuing following a positive review of the first patient's progress in December 2010.

In these animal studies, a relatively small proportion of the rat or NHP brain cells are replaced with human-derived cells. Extensions of these methods might involve a greater proportion of cells. We consider the implications of these approaches in Chapter 3.

2.4 Summary

A wide range of genetically altered and chimæric ACHM are in current use in investigational research, as models of disease and in the development, production and testing of therapeutic products. Although there is little public awareness of ACHM (see Box 2.5) their use is long-standing and has made significant contributions across many fields of research. However, the development of animal models of human function and disease is often a gradual process, with models requiring refinement for particular purposes. This can involve iterative research processes spanning several decades. The likelihood of success, and timescales, are difficult to predict. For example the development of humanised monoclonal antibody therapies is one result of over 30 years of intensive research; the development of animals to provide tissue for transplants has not yet yielded clinical benefits after some decades of work.

Box 2.5 Public awareness of research involving ACHM

At the outset of the public dialogue (see Annex III), most participants had little specific knowledge of research involving ACHM, or of the kinds of research that might be possible in the future. However, many participants related such research to other, more familiar approaches (for example the use of animal heart valves transplanted into humans) and were not greatly surprised to learn that such research is taking place.

86 Redmond DE, Jr., *et al.* (2010). *Cellular repair in the parkinsonian nonhuman primate brain*. Rejuvenation Res **13**, 188–94.
87 Pollock K, et al. (2006). *A conditionally immortal clonal stem cell line from human cortical neuroepithelium for the treatment of ischemic stroke*. Exp Neurol 199, 143–55.

3 Future science and implications

3.1 Introduction

The previous chapter described animals containing human genetic or cellular material (ACHM) and illustrated their use in biomedical research. Techniques that enable the transfer of human DNA sequence and the engraftment of human cells into animals or animal cells are well-established. However, continuing advances in the power of the techniques involved are rapidly extending the range and complexity of animal models that can be created. We anticipate that the use of ACHM will continue to expand, as more sophisticated models of human health and disease are developed.

In this section, we consider selected examples to illustrate possible future research directions. We describe two methodological areas in which developments relevant to the creation of animal–human models are apparent.

1. Genetic engineering methods.
2. Stem cell methods.

We also consider three areas in which future research may be particularly sensitive or approach current social, ethical or regulatory boundaries.

1. Research involving the brain.
2. Research involving the reproductive system.
3. Research involving aspects of human appearance or behavioural traits.

These reflect areas highlighted in the public dialogue (Box 3.1).

Box 3.1 Areas of public interest and concern

Overall, a majority of participants in the public dialogue accepted and were ultimately supportive of research using animals containing human material, on the condition that such research is conducted to improve human health or to combat disease. The considerations taken into account by the public when giving their conditional support will be discussed in more detail in Chapter 5 (see Box 5.1).

For the majority of public dialogue participants, *in vitro* experiments such as the creation of animal–human hybrid cells did not cause concern. However, a very small minority of participants objected to this type of *in vitro* research on animal welfare or religious grounds. Some participants raised concerns around the source and disposal of the human tissues, and the risk of unintended release of material, in *in vitro* experiments.

Participants showed greater concern for *in vivo* experiments, and some found such research unacceptable (see Box 5.2). Participants tended to focus on the overall outcome for the research animal involved, in terms of the animal's welfare, capability, and physical appearance, rather than either the proportion of human and animal cells in the resulting animal or its genetic make-up. Internal manipulations, such as the addition of human liver cells to animals, or the development of humanised organs in animals, were generally accepted. However, three areas of particular sensitivity to participants were identified. These were research involving the brain, reproductive tissues or external features (see Boxes 3.9–3.11).

3.2 Genetic alteration of animals

It is now commonplace to genetically alter animals so that their genomes contain up to a few thousand bases of human DNA sequence (see Box 2.2). As genetic technology advances it is becoming possible to manipulate increasingly large sections of DNA, and to modify DNA sequences with greater accuracy. This ability is markedly increasing the range of transgenic models that can be created.[88]

The development of mice generating humanised monoclonal antibodies (see 2.3.2) is underpinned by the ability to transfer extensive sections of DNA which encode the antibody-producing components of the human immune system. In the *Kymouse*™ model around 3 million base pairs of human sequence (approximately 0.1% of the human genome) including coding regions and other DNA sequences essential for B-lymphocyte (antibody producing white blood cell) function will be transferred.[89] The extent of this substitution means that the *Kymouse*™ more closely models the human immune system than previous models, increasing the diversity of human antibodies which the mouse can produce, from which the most specific can be selected for therapeutic development.[90]

A mouse model of Down's syndrome was developed using a chromosome engineering approach and has the largest addition of human DNA of which we are aware.[91] Cells within these mice contain almost all of human chromosome 21 (around 42 million bases of DNA) replicating the 'trisomy' (additional copy) of this chromosome found in human Down's syndrome. The mouse has been developed to study aspects of Down's syndrome which may be treatable (e.g. early-onset Alzheimer's disease). The abnormal development of the mouse's heart (its 'cardiac phenotype') resembles that of humans with Down's syndrome.[92] The mice have been found to have defective blood vessel growth, which is thought to be important in explaining why both the mouse model and people with Down's syndrome have a low risk of some cancers.[93] These phenotypes are probably caused by the imbalance of multiple genes, and may not have been discovered without the transfer of a very large amount of genetic material.

DNA 'regulatory sequences' control the activity of the protein-coding parts of genes, and influence key aspects of gene function, including when and in which tissues a gene is activated, and how much of its product is made (see Box 2.3). Many diseases are caused by mutations in these sequences. There is substantial evidence that changes in regulatory sequences during evolution can underlie species divergence (see 2.3.1). The study of regulatory function will be a major focus over the next decade. Regulatory sequences are often located at a long distance (tens or hundreds of kilobases away) from the protein-coding part of the gene. However, the ability to move extensive stretches of DNA means that the coding sections of human genes can now be transferred together with their corresponding regulatory sequences. This can result in an animal model in which the human gene under investigation is expressed in a human-specific way (only in relevant tissues and at specific times) meaning that the biological function

88 A range of different techniques have been established to transport DNA from one cell into another. For larger amounts of DNA these include the use of vectors, such as bacterial artificial chromosomes (BACs) (which usually carry DNA constructs of 150–350 kilobases (kb)); yeast artificial chromosomes (YACs) (used to clone DNA fragments of 100–3000 kb, and to express proteins that require post-translational modification); mammalian artificial chromosomes (MACSs) (which can carry tens of megabases of DNA). 'Chromosome engineering' includes a range of techniques used to create modifications of DNA at a whole chromosome level including chromosomal duplications, deletions, inversions, or translocations. These rearrangements can span many megabases of DNA and hundreds of genes.

89 Oral evidence from Bradley, A. For information on the *Kymouse*™ see http://www.kymab.com/index.php?option=com_content&view=article&id=52&Itemid=54.

90 Therapeutic products, such as human antibodies or proteins (see 2.3.2) developed in transgenic animals and intended for human application would be subject to pre-clinical safety testing as 'Biotechnology-derived therapeutic products'. See Guidance from the European Medicines Agency http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500002828.pdf

91 O'Doherty A, *et al*. (2005). *An aneuploid mouse strain carrying human chromosome 21 with Down's syndrome phenotypes*. Science **309**, 2033–7.

92 Dunlevy L, *et al*. (2010). *Down's syndrome-like cardiac developmental defects in embryos of the transchromosomic Tc1 mouse*. Cardiovasc Res **88**, 287–95.

93 Reynolds LE, *et al*. (2010). *Tumour angiogenesis is reduced in the Tc1 mouse model of Down's syndrome*. Nature **465**, 813–7.

of the gene is more accurately modelled. The haemoglobin genes and their corresponding regulatory sequences have been intensively studied over recent decades. In one model a 120-kilobase fragment corresponding to the human α-globin region and all its regulators replaced the homologous mouse region so that the mice expressed human α-globin. A regulatory mutation that causes the human blood disorder α-thalassaemia was then recreated in these mice and shown to model the severe human disease accurately.[94,95]

We anticipate that methodological developments will continue to extend the quantity of DNA that can be manipulated.[96] Ultimately, studies may be limited, not by technical challenges, but by the effect of the genetic modifications on the animals involved. For some genes, too much protein product (or its activity) can cause severe defects.[97,98] At a cellular level, when genes from two different species are made within the same cell, as occurs in transgenic animals with human genes, the proteins produced by the different genes need to work together. At very high degrees of transgenesis it seems likely that certain critical human and animal proteins would not interact properly and so compromise the animal's viability.[99] It is known that chromosomes need to 'pair' during meiosis (the special cell divisions that occur in reproductive cell precursors). The presence of a large amount of unpaired DNA, such as a whole extra chromosome, can lead to the failure of meiosis and thus infertility; in the Down's syndrome mouse model the added human chromosome is only transmitted along the female germ line and the male mice are infertile, as are most men with Down's syndrome.[100]

New techniques are enabling models to be created in which the human DNA functions in a more biologically accurate manner. Future developments, for which the α-globin experiment is a forerunner, might include new approaches to:

- Replace (rather than add) genetic material in an animal's genome.
- Control the location in the genome at which copies of transgenes are integrated.
- Precisely control gene expression levels.
- Understand and modify regulatory regions to allow control of temporal and spatial expression of transgenes.
- Translocate sections of human chromosomes onto animal chromosomes.[101]
- Enable germ-line transmission of transgenes (this is currently difficult in some species).[102]

Most transgenic animals carrying human genes are mice; however, gene-targeting methods are now being developed in additional species including the rat and some NHPs, and can in principle be used to introduce human DNA sequence into any animal species (see Box 2.2). There are very few published studies involving transgenic NHPs to date. Early studies reported the creation of a rhesus macaque monkey which expressed a mutant form of the human gene responsible for Huntington's disease, and a marmoset which over-expressed

94 Wallace HA, *et al*. (2007). *Manipulating the mouse genome to engineer precise functional syntenic replacements with human sequence*. Cell **128**, 197–209.
95 Vernimmen D, *et al*. (2009). *Chromosome looping at the human alpha-globin locus is mediated via the major upstream regulatory element (HS-40).* Blood **114**, 4253–60.
96 Written evidence from Wellcome Trust Sanger Institute.
97 Woods KS, *et al*. (2005). *Over- and underdosage of SOX3 is associated with infundibular hypoplasia and hypopituitarism*. Am J Hum Genet **76**, 833–49.
98 Alatzoglou KS, *et al*. (2011). *Increased transactivation associated with SOX3 polyalanine tract deletion in a patient with hypopituitarism*. J Clin Endocrinol Metab **96**, E685–90.
99 If the proteins produced are very similar then too much protein could lead to abnormal phenotypes. Alternatively, even subtle species differences could result in one protein interfering with the function of the other. Gene products, whether proteins or RNA, also function via interactions with other molecules, which can be different proteins, RNA or DNA sequences. A human protein may fail to interact properly with its mouse partner protein or target DNA sequence. It is therefore likely that, if very large amounts of human DNA are incorporated into an animal's genome, one or more of the many human gene products may lead to a deleterious or even lethal phenotype, preventing the establishment of viable transgenic animals.
100 Correspondence from Fisher, E.
101 This approach might enable the development of mouse models containing large sections of human chromosomes with greater viability and stability (e.g. avoiding factors such as the loss of the added chromosome in some tissues over time – creating mosaics). Cell death due to the triggering of an unpaired chromosome in cell division (meiosis) might also be avoided, permitting male germ line transmission of the manipulation.
102 Coors ME, *et al*. (2010). *The ethics of using transgenic non-human primates to study what makes us human*. Nat Rev Genet **11**, 658–62.

(made too much of) the protein 'α-synuclein' to model human Parkinson's disease, although in these models the transgenes did not transmit between NHP generations.[103,104] The introduction of the gene for a protein derived from jellyfish called 'green fluorescent protein' (GFP) into a common marmoset, with germ-line transmission was reported in 2009, and a study in 2010 used viral transfer methods to produce two rhesus monkeys expressing GFP.[105,106,107,108,109] These few reports indicate the imminent possibility of developing transgenic NHP models of human disease, which together with NHP chimæras (see 2.3.4), might be particularly important in studying neurological disorders.

Following recent elucidation of the full genome sequences of many animal species, research is underway to identify sections of the genome that are unique to humans or to our near ancestors. When compared between humans and NHPs, these sections (sometimes called 'human-lineage-specific' sequences) show increases or decreases in the number of copies of a gene, changes in gene sequence (ranging from one or two base pairs, to much larger differences), or altered gene expression patterns. They include genes important in brain development which have been suggested to have a role in the evolutionary enlargement of the human brain.[110,111,112,113]

To fully understand the function of some of these sequences, it is likely to be necessary to insert them into (or delete them from) animals during development, while recognising that this may pose some difficult societal questions. We suggest that manipulation of 'human-lineage-specific' sequences in animals to increase resemblance to the human form, particularly in NHPs, would require particularly careful consideration (see 8.2.2).

3.3 Stem cell research

3.3.1 ACHM and stem cells

The previous section describes modification of animals' genomes to resemble the human, usually by addition of human gene sequence. Creation of chimæras, by mixing human and animal cells, is the second approach that can be used to make ACHM. Many chimæric ACHM are developing using the unique properties of stem cells. These cells can produce specialised (or 'differentiated') cells as well as renewing the stem cell population. These properties enable stem cells to 'colonise' or reconstitute a tissue or organ in a recipient animal.[114] For example, human haematopoietic (blood) stem cells can be grafted into mice, where they replace the mouse immune system with a human–derived (humanised) equivalent (see 2.3.3).[115,116] The rapid recent growth of knowledge about human stem cells is opening many new research

103 Yang SH, *et al*. (2008). *Towards a transgenic model of Huntington's disease in a non-human primate*. Nature **453**, 921–4.
104 Kirik D, *et al*. (2003). *Nigrostriatal α-synucleinopathy induced by viral vector-mediated overexpression of human α-synuclein: a new primate model of Parkinson's disease*. Proc Natl Acad Sci USA **100**, 2884–9.
105 Under certain light, GFP glows and so can be used to 'mark' the cells into which it is integrated, without affecting their function.
106 Chan AW (2004). *Transgenic nonhuman primates for neurodegenerative diseases*. Reprod Biol Endocrinol **2**, 39.
107 Wolfgang MJ, *et al*. (2001). *Rhesus monkey placental transgene expression after lentiviral gene transfer into preimplantation embryos*. Proc Natl Acad Sci USA **98**, 10728–32.
108 Sasaki E, *et al*. (2009). *Generation of transgenic non-human primates with germline transmission*. Nature **459**, 523–7.
109 Niu Y, *et al*. (2010). *Transgenic rhesus monkeys produced by gene transfer into early-cleavage-stage embryos using a simian immunodeficiency virus-based vector*. Proc Natl Acad Sci USA **107**, 17663–7.
110 Evans PD, *et al*. (2004). *Adaptive evolution of ASPM, a major determinant of cerebral cortical size in humans*. Hum Mol Genet **13**, 489–94.
111 Coors ME, *et al*. (2010). *The ethics of using transgenic non-human primates to study what makes us human*. Nat Rev Genet **11**, 658–62.
112 Sikela JM (2006). *The jewels of our genome: the search for the genomic changes underlying the evolutionarily unique capacities of the human brain*. PLoS Genet **2**, e80.
113 Evans PD, *et al*. (2006). *Evidence that the adaptive allele of the brain size gene microcephalin introgressed into Homo sapiens from an archaic Homo lineage*. Proc Natl Acad Sci USA **103**, 18178–83.
114 Unlike many differentiated cell types, such as nerve cells, stem cells retain the ability to divide, producing further stem cells (a process known as self-renewal) as well as cells that go on to specialise.
115 Much of our discussion is focused on stem cells, but we are often using this term loosely also to encompass other progenitor cell types, notably those present in the embryo or fetus, that do not strictly self-renew under normal circumstances. However, their capacity for proliferation and the generation of many differentiated cell types means that they are very similar to stem cells. In addition, their role in promoting growth and development of the embryo can be harnessed in chimæras to substitute tissues in the same way as with stem cells.
116 Haematopoietic (blood) stem cells (HSCs) are found in bone marrow. They are able to self-renew, and to give rise to cells that differentiate into the different forms of blood cells; these include erythroid (red blood) cells and myeloid (white blood) cells such as lymphocytes, which are the key cellular components of the adaptive immune system. Engraftment of human HSCs can therefore be used to reconstitute the immune system of an immune-deficient mouse; these cells colonise the animal giving rise to a 'humanised' immune system.

avenues, based on chimæric animals containing human stem cells.

The same essential properties of stem cells underpin their roles in 'regenerative medicine': the use of cellular therapies to replace damaged or dysfunctional cells in humans (e.g. bone marrow transplants to treat leukaemias or the use of human neural stem cells to repair brain tissue after stroke). The rapidly increasing understanding of stem cell biology is opening up many potential avenues for their use. However, advancement of stem-cell-based treatments is dependent on knowledge of human stem cell biology, and refinement of techniques, which often require prior animal studies.

Stem cell potential

Although much has been learned of the conditions required for the differentiation of several cell types *in vitro*, to fully understand stem cell potential it is still necessary to study them *in vivo* (for further detail on stem cell potential see Box 3.2). Stem cell potential can be assessed to determine either the range of cells a stem cell *normally* gives rise to, or those that it *can* give rise to. The former requires marking a stem cell in its normal location *in vivo* (its 'niche') and following the fate of its progeny over time. The latter can often be explored *in vitro* by varying culture conditions, or *in vivo*, for example by grafting marked stem cells into ectopic sites in an embryo or animal.[117]

It is clearly difficult to conduct such *in vivo* experiments in humans although some information is available, for example after therapeutic grafts of bone marrow cells from a male (XY) donor into a female (XX) host and using Y chromosome DNA as a marker. An alternative is to use animal hosts, although care has to be taken when interpreting results as species differences could affect cell survival or differentiation.

For the *mouse* (and recently for other animals) three techniques have been adopted for testing the potency of stem cell lines such as embryonic stem (ES) cells and induced pluripotent stem (iPS) cells in order to classify them as pluripotent:

1. Growth *in vitro*: by changing the culture conditions ES and iPS cells *can* give rise to a wide range of cell types.
2. Growth *in vivo* in ectopic sites: for example when implanted under the skin, the kidney capsule or into the testis of genetically matched or immuno-compromised mice implants grow into tumours called 'teratomas' or 'teratocarcinomas' which *can* contain a wide range of cell types, and can include some organisation into discrete tissue types (see 3.6.1).
3. Growth and ability to contribute to *normal* embryonic development after reintroduction into an early stage embryo, which is implanted into the uterus of a surrogate mother. This method provides a much stricter test of potential, as it is possible to determine whether the cells contribute to all the tissues of the resulting animal, including the germ line. The ultimate test (which is not used routinely) is tetraploid complementation (see 2.2.2), as this shows whether the stem cells are able to give rise to an entire animal.

Stem cell lines

Embryonic stem cells (ES cells, obtained from an animal or human embryo) can be grown in culture and induced to proliferate indefinitely. It is also possible to derive cell lines from certain tissue specific stem cells (e.g. neural stem cells), although this can be difficult. Unlike ES or iPS cells, differentiated cells and tissue-specific stem cells are usually non-tumorigenic. With prolonged culture, cells (whether stem cells or specialised cells) can pick up mutations, which can make them tumorigenic. For clinical purposes, it is important to avoid tumour formation, so the majority of stem-cell-based treatments use either primary cells (e.g. bone marrow, fetal midbrain cells, limbic cells, skin grafts), or cell lines that have been rigorously tested and shown not to lead to tumours in

117 For stem cells that may be in sites that are difficult to access physically, a range of genetic tools exist, especially in the mouse (which rely on cell-type-specific and conditionally activated reporter transgenes), which can be used in the intact animal.

animals.[118] Development of stem cell therapies is thus dependent on ACHM experiments.

Human stem cells

Human stem cells used in research or for cell-based treatments can be obtained from early-stage human embryos, aborted fetuses, cord blood and some adult tissues.[119] Many cell lines have been derived from human tissue-specific stem cells, and around 400 human ES cell lines have also been derived, mainly from embryos donated by IVF patients. There are now also many human iPS cell lines (see Box 3.3) from both normal individuals and patients carrying a genetic disease. It is illegal to perform the strictest test of pluripotentiality using human embryos (the third test of pluripotency, see above) so the term 'pluripotent', when associated with human ES and human iPS cell lines, should be used with the caveat that it is currently only possible to test this by *in vitro* differentiation (the first test of pluripotency, see above) and/or by the ability to make many tissues in teratomas after grafting the human cells into mice (the second test of pluripotency, see above). However, human ES and human iPS cells are thought to have the potential to give rise to all the tissues of a human embryo, and their creation and use are therefore carefully regulated (see 6.2.7). Realising the potential of human stem cells will thus require ACHM experiments.

Box 3.2 Stem cell potential

Different types of stem cell are found in particular tissues and at different stages of development, and these vary in the range of specialised cells they produce. This property (stem cell 'potential' or 'potency'), is often used to group stem cells, as:

- **Unipotent**: able to give rise to a single specialised cell type.
- **Multipotent**: able to give rise to more than one, or many, specialised cell types.
- **Pluripotent**: able to give rise to all cell types of the developing embryo (e.g. ES and iPS cells, see Box 3.3).[120]

However, assessment of stem cell potential is complicated by several factors, including that:

- Stem cells in adult tissues can be largely 'quiescent' (non-dividing).
- The normal potential of a stem cell type in its 'niche' can differ substantially from its behaviour *in vitro* or in an ectopic location.[121]
- Rather than giving rise immediately to specialised cell types, several stem cell types give rise to 'transit amplifying cells' which divide rapidly and often still have several possible fates. These are not 'true' stem cells because they are set on a path to differentiate and therefore do not strictly self-renew. However, the distinction is often blurred and such transit amplifying cells may revert to quiescence and/or a true stem cell state *in vitro* and in some circumstances *in vivo*. Indeed, current thinking is that it may be a question of probability – the further the cell is from its native tissue environment *in vivo* (its 'niche'), the less likely it is to self-renew and the more committed it becomes.

118 To facilitate the growth of certain stem cells, scientists often 'conditionally-immortalise' them using an oncogene (a growth-promoting gene). Such cells could conceivably be tumorigenic unless the activity of the oncogene were turned off. Thus in these lines the gene activity is tightly controlled by a molecular switch. Some such lines are being approved for clinical trials.

119 With appropriate consent.

120 The additional term 'totipotent' is sometimes used in relation to stem cells. For further discussion see Academy of Medical Sciences (2007). *Inter-species embryos*. http://www.acmedsci.ac.uk/p48prid51.html

121 Or marking it and then transplanting a marked cell back to its niche. The 'niche' for adult stem cells is often complex, requiring several cell types, secreted molecules and perhaps even three-dimensional organisation; consequently we do not yet know how to culture many adult stem cell types in a manner that supports their 'normal' differentiation. Nevertheless, several adult stem cell types are successfully grown *in vitro*, and these are already the basis for several human therapies, such as skin cells for burns victims and limbic stem cells to repair corneal damage.

3.3.2 Stem cells in pre-clinical research, and in the development of therapeutics

In previous sections (see 2.2.3) we have outlined how stem cell methodology has contributed to the development of chimæric humanised animals, which are used for a range of research purposes, for example:

- Engraftment of human haematopoietic cells into immune-deficient mice, used to produce mice with humanised immune systems, susceptible to human-specific diseases including HIV and hepatitis (see 2.3.3).
- To test possible treatments for Parkinson's disease (PD), for example, showing that neurons derived from human iPS cells can reverse symptoms in a rat model of PD.[122]

Stem cell technology is opening up new avenues in regenerative medicine. For several decades, bone marrow (and more recently human cord blood) stem cells have been successfully used to replace the bone marrow after treatment for leukaemia, and skin stem cells grown *in vitro* are used to treat burns victims. Limbic stem cells are being used to treat corneal damage, while the replacement or restoration of damaged tissue using human stem cell lines is now being tested for a much wider range of conditions (e.g. stroke). Both human tissue-specific and human ES cells are current candidates for cell-based clinical therapies. Clinical trials using cells derived from human ES cells are currently underway for spinal cord repair and for macular degeneration.[123] Ultimately, it may prove possible to derive iPS cell treatments from a patient's own somatic cells, so avoiding the problems of immune rejection.

Although the eventual aim of such techniques is to introduce human stem cells into human tissues, animal models will increasingly be required to develop the relevant methodologies (potential, dosage, stem cell handling techniques) and to test human stem cell therapies for their efficacy and safety. For example:

- Human neural stem cells, human mesodermal stem cells, or human haematopoietic stem cells have been investigated for efficacy in rat models of stroke (see 2.3.4).
- Human neural stem cells have been investigated in the NHP brain as a prelude to attempts to correct human developmental disorders such as Batten disease.[124]
- Human enteric nervous system stem cells have been investigated in the fetal gut for Hirschprungs disease.[125,126]
- Studies in NHPs have investigated the potential of human neural stem cells in Parkinson's disease.[127,128]

3.3.3 Current boundaries of research involving human–animal stem cell chimæras

ACHM involving human tissue-specific stem cells

Proper understanding of human stem cell biology, especially stem cell potency, can only be obtained through studying human stem cell types *in vivo*.

Whilst the majority of this research has involved adult animals, there are limited reports in which human tissue-specific stem cells have been introduced into animals at early stages of gestation. For example, human haematopoietic stem cells were introduced into fetal goats, and human mesenchymal stem cells (see Box 3.3) into fetal sheep.[129] The outcomes of such

122 Hargus G, *et al*. (2010). *Differentiated Parkinson patient-derived induced pluripotent stem cells grow in the adult rodent brain and reduce motor asymmetry in Parkinsonian rats*. Proc Natl Acad Sci USA **107**, 15921–6.
123 Carr AJ, *et al*. (2009). *Protective effects of human iPS-derived retinal pigment epithelium cell transplantation in the retinal dystrophic rat.* PLoS One **4**, e8152.
124 See Hayden EC (2008). *California institute to help stem-cell biotechs*. Nature **455**, 436–7.
125 Heanue TA & Pachnis V (2011). *Prospective identification and isolation of enteric nervous system progenitors using Sox2*. Stem Cells **29**, 128–40.
126 Schafer KH, *et al*. (2009). *Neural stem cell transplantation in the enteric nervous system: roadmaps and roadblocks*. Neurogastroenterol Motil **21**, 103–12.
127 Muramatsu S, *et al*. (2009). *Multitracer assessment of dopamine function after transplantation of embryonic stem cell-derived neural stem cells in a primate model of Parkinson's disease*. Synapse **63**, 541–8.
128 Emborg ME, *et al*. (2008). *GDNF-secreting human neural progenitor cells increase tyrosine hydroxylase and VMAT2 expression in MPTP-treated cynomolgus monkeys*. Cell Transplant **17**, 383–95.
129 In 2006, Chinese researchers transferred human haematopoietic (blood) stem cells, extracted from cord blood, into fetal goats during gestation. Analysis at 2 years showed that the stem cells were integrated into the goats' tissues (including blood, bone marrow, spleen, liver, kidney, muscle, lung) and were expressing human genes and proteins. The chimæric goats provide an *in vivo* model to study human blood stem cell differentiation. Similar research has been conducted using another form of human stem cells injected into foetal sheep. See Zeng F, *et al*. (2006). *Multiorgan engraftment and differentiation of human cord blood CD34+ Lin– cells in goats assessed by gene expression profiling*. Proc Natl Acad Sci USA **103**, 7801–6.

Box 3.3 Stem cell types

- **Tissue-specific (or adult) stem cells.** Most adult tissues need a supply of new cells to replace those damaged through normal processes of wear. These new cells are derived from 'tissue-specific' stem cells, which usually contribute only to cells of one tissue type (e.g. blood cells, or skin cells, not both). Some are unipotent (e.g. spermatogonial stem cells usually give rise only to sperm), whereas others are multipotent (e.g. haematopoietic (blood) stem cells in the bone marrow give rise to all the cell types of the blood including red and white cells).
- **Mesenchymal stem cells.** MSCs; sometimes called 'marrow stromal cells' are multipotent stem cells that can differentiate into a variety of cell types, including bone, cartilage, and fat. They can be isolated from several tissues, including fat, and bone marrow. They are the most widely used stem cell types in clinical trials.[130]
- **Fetal stem cells.** The developing embryo contains 'fetal stem cells', which can produce specialised cell types during fetal development. Fetal stem cells tend to have broad potential which becomes reduced ('restricted') as development proceeds, and to change their potential over time. The conditions for culturing some fetal stem cells (e.g. neural stem cells from the developing brain) *in vitro* have been determined. Under these artificial conditions, the fetal stem cells can grow essentially indefinitely (for far longer than they exist *in vivo*) while retaining the ability to differentiate.
- **Embryonic stem (ES) cells.** ES cells correspond to cells in the very early embryo, before any restriction has been made to tissue type within the embryo proper. Research, originally in the mouse, demonstrated that ES cells can give rise to all the cell types of the developing embryo and adult mouse; they are therefore considered 'pluripotent'. They can be maintained essentially indefinitely as a self-renewing cell line *in vitro*; however, any such cell type in the embryo must be very short-lived (if they exist there at all), as this corresponds to a period of very rapid development and ES cells cannot be isolated from an embryo once it begins the process of gastrulation.
- **Extra-embryonic stem cell types.** In the mouse, it is possible to derive stem cells that correspond to the two extra-embryonic stem cell types of the late blastocyst, trophoblast stem cells and extra-embryonic endoderm stem cells. These are able to differentiate into cell types of the placenta and yolk sac respectively, but not to cells of the embryo proper.

Other stem cell types with broad potential.

- **Embryonic germ (EG) cells** can be derived from primordial germ cells (which are normally fated to give eggs or sperm) isolated from embryonic gonadal precursors. EG cells are very similar to ES cells in their potential. Those from the mouse can contribute to normal development after injection into host embryos, and give rise to teratocarcinomas after injection into ectopic sites.[131]
- **Spermatogonial stem cells** (male germline stem cells) are tissue-specific stem cells present from early postnatal stages in the testis. Their self-renewal and differentiation in adulthood enable continuous production of sperm. When grown in specific culture conditions, a minority of spermatogonial stem cells transform into ES-like cells (in a process that may mimic the origin of spontaneous testicular teratocarcinomas).
- **Amniotic stem cells,** obtained by amniocentesis, have a broad potential, variously described as multipotential or pluripotential (although they do not fulfil all the criteria for this as outlined above). They are being investigated as a source of cells for therapies.

130 For examples of clinical trials involving mesenchymal stem cells see: http://www.osiris.com/clinical.php and http://www.nature.com/stemcells/2008/0804/080410/full/stemcells.2008.55.html

131 Because genomic imprinting is erased in the germ line, both of these germ cell-derived stem cell types may not be useful for obtaining certain functional specialised cell types.

- **Cord blood stem cells** are found in umbilical cord blood. Their potency is not yet fully understood. Although they are similar to haematopoietic (blood) stem cells (HSCs), several reports suggest they may be able to give rise to a wider range of cell types, and they probably include a population of MSC-like cells. They have even been reported to give rise to some neurons *in vitro*, although claims that they can do so *in vivo* are controversial.[132] They have been used in treatment to replace bone marrow and blood cells in conditions such as leukaemia since the 1990s.
- **Induced pluripotent stem cells** (iPS cells), do not occur naturally, but are created artificially by 'reprogramming' other cell types, such as adult body somatic cells (e.g. skin cells). For example, iPS cells have been derived by transfection (adding in) of certain genes into adult fibroblasts.[133] iPS cells were first produced in 2006 from mouse cells and from human cells in 2007. Their properties are broadly similar to ES cells; however, individual lines vary in their properties, which may reflect incomplete reprogramming, and some genetic or chromosomal damage. It is not known how relevant these differences will be to their clinical use. For the time being, ES cells are viewed as the 'gold standard' to which iPS cells should be compared. However, iPS cells are very important for research into genetic diseases, in cell culture or after introduction into animals, because they can be derived from specific patients. They are already being used in screens for drugs.

experiments are currently unpredictable. As the human stem cells are merged into the animals at an early stage, there is greater potential for the stem cells to contribute to a wider range of tissues, and there is little control over the types of tissue likely to incorporate the human stem cells. Although the stem cell types involved (haematopoeitic, mesenchymal) were thought to be tissue specific, the actual potential of the stem cells could not be taken for granted before these studies were undertaken.

ACHM involving human embryonic stem cells

It is now technically possible to make animal–human chimæras involving the engraftment of human ES cells into animal embryos. We are aware of only a small amount of such research to date (and this is largely unpublished); however, the development of human ES cell lines, and new approaches to create human pluripotential stem cells, open the way for more work of this kind.

In 2006, researchers claimed that human ES cells could engraft into mouse blastocysts, where they proliferated and differentiated for a few days when these embryos were maintained *in vitro*.[134] However, very few human cells were found within post-gastrulation stage embryos after transfer into surrogate mice, suggesting that the human cells were at a disadvantage compared with the surrounding mouse cells.[135,136] If such chimæras were allowed to be born, it is highly likely that they would have very few or no surviving human cells in most of their tissues. However, because the earlier in development human cells are introduced, the less predictable is the outcome, it remains possible that human cells may not be at a disadvantage in all tissues, so human cells could make a significant contribution to a few cell types in a live-born animal.[137] This might be even more likely if the specific mouse cell types were themselves compromised or eliminated (e.g. similar to the way that mice

132 Bicknese AR, *et al*. (2002). *Human umbilical cord blood cells can be induced to express markers for neurons and glia*. Cell Transplant **11**, 261–4; Lim JY, *et al*. (2011). *Neural differentiation of brain-derived neurotrophic factor-expressing human umbilical cord blood-derived mesenchymal stem cells in culture via TrkB-mediated ERK and β-catenin phosphorylation and following transplantation into the developing brain.* Cell Transplant. *In press*.

133 Notably *Oct4*, *SOX2, K1f4* and *cMyc,* all transcription factors characteristic of pluripotent cells.

134 James D, *et al*. (2006). *Contribution of human embryonic stem cells to mouse blastocysts*. Dev Biol **295**, 90–102.

135 Gastrulation is a phase early in the embryonic development of most animals, during which the single layer of cells called the blastula (or in higher vertebrates the epiblast), is reorganised into a three-layered patterned structure that will go on to form the three primary tissues of the embryo proper (ectoderm, mesoderm, endoderm). In human embryonic development it begins at around 14 days after fertilisation, in the mouse at about 7 days.

136 NHP ES cells have recently been shown also to contribute poorly to early mouse embryos; see Simerly C, *et al*. (2011). *Interspecies chimæra between primate embryonic stem cells and mouse embryos: Monkey ESCs engraft into mouse embryos, but not post-implantation fetuses.* Stem Cell Res **7(1)**, 28-40.

137 See discussion on mice with human immune system or liver 2.3.3.

with human livers or a human immune system are made, or as demonstrated in mice carrying ES-cell-derived rat pancreas (see 3.3.4)).

One concern associated with such studies is that human ES cells may contribute to the germ-line cells in the chimæric mouse, resulting in a mouse with human-derived reproductive cells (see 3.4). In theory, if such an animal were to be bred its offspring could be 'true hybrids'; or if two such animals were to breed, this could result in the fertilisation of a human egg with human sperm. Specific regulation of such experiments is recommended (see 8.2.3).

The evolutionary distance between mouse and humans, and the significant difference in rates of cell division between most human and mouse cell types (human cells are generally significantly slower, which puts them at a competitive disadvantage in a rapidly growing embryo) reduces the chance of human cells surviving in the chimæras. However, if the animal component is one where human cells are less disadvantaged (e.g. as perhaps evidenced by the experiments involving human stem cells introduced into fetal goats), and particularly if NHPs are used, then the concern may increase significantly (see 8.2.2).

3.3.4 Future directions in stem cell research

Several new sources of stem cells are being investigated. iPS cells can be derived and grown essentially indefinitely, from any individual. They provide a novel way to study human genetic disease, where the iPS cells are directed (*in vitro* or in animals) to differentiate into the affected cell type. These can then be used to study the detailed pathology of the disease and to search for treatments. Extending the idea behind iPS cells, several groups are exploring the possibility of direct cell reprogramming, to go from one adult cell type to another.[138,139,140,141]

New imaging techniques are being applied to stem cell biology that will allow increasingly sophisticated observation of cell behaviour *in vivo*. Ultrasound imaging can guide instruments to introduce cells (or DNA) into precise locations within embryos developing *in utero*.[142,143,144,145] This can be done, for example, at early stages of mouse embryos when the developing organs are first seen.[146] Cells to be introduced can be labelled such that that their fate can be followed *in vivo*, using MRI, bioluminescence, fluorescence, positron-emission tomography (PET) scans and X-rays.[147,148,149,150,151,152] Fluorescence imaging allows single cells (or subcellular components, such as nuclei, chromosomes, cell membranes), to be followed after labelling with variously coloured fluorescent proteins.[153,154]

Gene activity can now be manipulated, even within single cells (transplanted or host) within an animal. This can be achieved by, for example, using gene-targeting methods (see Box 2.2) to allow genes to be switched on or off by, for example, a drug, temperature

138 Zhou Q, *et al*. (2008). *In vivo reprogramming of adult pancreatic exocrine cells to β-cells*. Nature **455**, 627–32.
139 Ieda M, *et al*. (2010). *Direct reprogramming of fibroblasts into functional cardiomyocytes by defined factors*. Cell **142**, 375–86.
140 Efe JA, *et al*. (2011). *Conversion of mouse fibroblasts into cardiomyocytes using a direct reprogramming strategy*. Nat Cell Biol **13**, 215–22.
141 Kim J, *et al*. (2011). *Direct reprogramming of mouse fibroblasts to neural progenitors*. Proc Natl Acad Sci USA **108**, 7838–43.
142 Nieman BJ & Turnbull DH (2010). *Ultrasound and magnetic resonance microimaging of mouse development*. Methods Enzymol **476**, 379–400.
143 Pierfelice TJ & Gaiano N (2010). *Ultrasound-guided microinjection into the mouse forebrain in utero at E9.5*. J Vis Exp **13**, 45.
144 Olsson M, *et al*. (1997). *Specification of mouse telencephalic and mid–hindbrain progenitors following heterotopic ultrasound-guided embryonic transplantation.* Neuron **19**, 761–72.
145 Wichterle H, *et al*. (2001). *In utero fate mapping reveals distinct migratory pathways and fates of neurons born in the mammalian basal forebrain*. Development **128**, 3759–71.
146 These are known as organ primordia.
147 Modo M (2008). *Noninvasive imaging of transplanted cells*. Curr Opin Organ Transplant **13**, 654–8.
148 Daadi MM, *et al*. (2009). *Molecular and magnetic resonance imaging of human embryonic stem cell-derived neural stem cell grafts in ischemic rat brain*. Mol Ther **17**, 1282–91.
149 Bible E, *et al*. (2009). *The support of neural stem cells transplanted into stroke-induced brain cavities by PLGA particles*. Biomaterials **30**, 2985–94.
150 Srinivas M, *et al*. (2010). (19)F MRI for quantitative in vivo cell tracking. Trends Biotechnol **28**, 363–70.
151 Daadi MM, *et al*. (2010). *Human neural stem cell grafts modify microglial response and enhance axonal sprouting in neonatal hypoxic–ischemic brain injury*. Stroke **41**, 516–23.
152 Seiler MJ, *et al*. (2010). *Three-dimensional optical coherence tomography imaging of retinal sheet implants in live rats*. J Neurosci Methods **188**, 250–7.
153 Udan RS & Dickinson ME (2010). *Imaging mouse embryonic development*. Methods Enzymol **476**, 329–49.
154 Vermot J, *et al*. (2008). *Fast fluorescence microscopy for imaging the dynamics of embryonic development*. HFSP J **2**, 143–55.

or even light.[155] These methods allow the function of endogenous genes to be assessed, but they can also be used to manipulate cell behaviour, including migration, proliferation and cell death. Many of these techniques are still technically challenging, and most have been applied only in the mouse, but the availability of stem cells will allow much of this to be applied to other species. Indeed this is already occurring with NHP and human stem cells and their differentiated derivatives, and their use in ACHM is likely to increase rapidly.

A recent study showed that rat iPS cells injected into mouse blastocysts lacking the *Pdx1* gene required for pancreas formation, were able to form a fully functional (rat) pancreas in the resulting mice. This is similar in concept to the methods used to derive mice with a human immune system or liver (see 2.3.3), but shows that it can be done with tissues that do not normally regenerate, if the donor cells are introduced at a sufficiently early stage. The availability of human stem cells and sophisticated ways to genetically manipulate host embryos and animals may eventually make it possible to humanise any specific tissue or body system. This could even include parts of the brain, although the challenge of generating functional circuits in rodents from human cells is formidable.

Tissue engineering is also a rapidly expanding discipline, where artificial material or tissue-derived matrices are used to support cells *in vitro* or *in vivo*. Sophisticated chemistry and optical 'etching' techniques can be used to pattern artificial matrices, such as 'Matrigel™', to create three-dimensional substrates that can then be seeded with cells, including stem cells. These can be made to form tissue-like structures, with cells in the correct arrangement, including blood vessels or other structures.[156] These entirely artificial structures could perhaps in future be used to replace lost or damaged tissue or perhaps to decrease dependence on animal models for research.[157]

Decellularised matrix (the extracellular protein and other molecules that comprise the support for cells within a tissue) has been found to have patterning information, such that when re-seeded with a mixture of the appropriate cells (or stem cells) for the tissue from which they were obtained, they can reconstitute a functional tissue or organ. These are already being used clinically to replace small sections of tissue lost through trauma or cancer, e.g. of bladder, ureter and trachea.[158,159,160,161] It may become possible to use such techniques to rebuild more complex organs and tissues, such as the heart, or parts of the brain.[162] To show that these engineered human structures are functional and safe will require testing in animals.

3.3.5 Current boundaries and controversies in stem cell research and application

We discuss below several areas currently under intense investigation in the development of potential therapies based on stem cells. The resolution of almost all of these issues will most likely involve testing of human cells in animals.

Sources of stem cells

There is considerable debate about how 'good' each stem cell type (e.g. ES, fetal, cord blood, adult, iPS) is with respect to research and therapeutic potential (including safety). It

155 Recently developed 'optogenetic' techniques use light to trigger genetic or molecular changes, and are increasingly being applied to study neural function and connectivity because they can be used not only to mark cells, but also to induce activity or inactivity of ion channels, nerve conductance and synaptic function. See Kravitz AV & Kreitzer AC (2011). *Optogenetic manipulation of neural circuitry in vivo*. Curr Opin Neurobiol; Tonnesen J, *et al*. (2011). *Functional integration of grafted neural stem cell-derived dopaminergic neurons monitored by optogenetics in an in vitro Parkinson model*. PLoS One **6**, e17560; Carter ME & de Lecea L (2011). *Optogenetic investigation of neural circuits in vivo*. Trends Mol Med **17**, 197–206.

156 Moon JJ, *et al*. (2010). *Biomimetic hydrogels with pro-angiogenic properties*. Biomaterials **31**, 3840–7.

157 Bible E, *et al*. (2009). *The support of neural stem cells transplanted into stroke-induced brain cavities by PLGA particles*. Biomaterials **30**, 2985–94.

158 Macchiarini P, *et al*. (2008). *Clinical transplantation of a tissue-engineered airway*. Lancet **372**, 2023–30.

159 Orlando G, *et al*. (2010). *Regenerative medicine applied to solid organ transplantation: where do we stand?* Transplant Proc **42**, 1011–3.

160 Tian H, *et al*. (2010). *Differentiation of human bone marrow mesenchymal stem cells into bladder cells: potential for urological tissue engineering*. Tissue Eng Part A **16**, 1769–79.

161 Badylak SF, *et al*. (2010). *Whole-Organ Tissue Engineering: Decellularization and Recellularization of Three-Dimensional Matrix Scaffolds*. Annu Rev Biomed Eng. *In press*.

162 Iyer RK, *et al*. (2011). *Engineered cardiac tissues*. Curr Opin Biotechnol. *In press*.

seems likely that each source of cells will find specific applications depending on the tissue and the problem to be solved.

In developing cell based therapies it is important to consider whether it is more effective to transplant stem cells, or a form of cell derived from them (e.g. transit amplifying cells, committed progenitors or fully differentiated cells). The most effective approach is likely to be specific to the tissue requiring repair. For example, tissue-specific stem cells are likely to be better where production of many new cells is required over an extended period (e.g. to make new skin), while in other cases, such as the retina, there is evidence that post-mitotic (i.e. no longer dividing) cells are necessary.[163,164]

Selection and derivation of cells

It can be difficult to obtain specific cell types from some stem cells. Certain differentiation protocols (e.g. specific growth factors or inhibitors added to the cultures) can be used that favour the production or survival of one cell type over another. Antibodies that recognise molecules on the cell surface can be used to select for or against specific cell types.[165]

With the mouse (and increasingly for other animals) it is often possible to genetically engineer ES cells (or the animal from which the stem cells are to be derived) to introduce a marker gene (e.g. encoding a protein that is fluorescent or confers drug-resistance), to allow purification of the relevant cell type *in vitro*. Clearly it is not an option to genetically engineer humans for this purpose, and introducing marker genes directly into stem cell types including human ES and iPS cells, is often difficult. In addition, regulatory authorities are concerned about the use of modified cells as each alteration carries a risk of damaging an endogenous gene, perhaps promoting cancer. Demonstrating the safety of a cell line is costly and time-consuming, and though this might be justifiable where a single cell line could treat many patients, in other cases, especially for 'personalised' treatment, it may prove a barrier.

Compared with other stem cell types, pluripotent stem cells grow well in culture and have greater potential, allowing many different cell types to be derived from a single source. While an advantage in many respects, and essential if the cell type in question is specified relatively early in the embryo (e.g. motor neurons), this can cause difficulty in separating out the required cell type. It has been difficult to use *in vitro* differentiation of pluripotent cells to obtain fully mature functional cells, even if these are grafted into an appropriate *in vivo* site, but because we know that mouse pluripotent stem cells can form functional tissue in chimæras or even give rise to entire adult mice, any inability to obtain mature cell types possibly reflects our current lack of knowledge, rather than an intrinsic problem of the pluripotent stem cells.[166,167,168] In contrast, adult stem cells are thought to be better able to give mature cell types, but such stem cells are often difficult to isolate and grow *in vitro*. This may again reflect limitations in our understanding, but in some cases it could be due to an intrinsic property of the adult stem cells, which are often largely quiescent in their niche *in vivo.*

Risks of therapeutic uses of stem cells

The risk of having abnormal cell types (especially cancer-causing cells) present within a stem cell line, varies according to stem cell type. Even a single ES cell is able to give rise to a teratocarcinoma, so any protocol to derive cells for transplant has to be very efficient at removing these.[169,170] Various protocols have been established for trials based on ES cell-derived cell types (notably oligodendrocyte precursors

163 Lapouge G & Blanpain C (2008). *Medical applications of epidermal stem cells.*
164 West EL, *et al*. (2009). *Cell transplantation strategies for retinal repair*. Prog Brain Res **175**, 3–21.
165 This can be done with techniques such as fluorescence-activated cell sorting, magnetic bead separation or complement-mediated cell killing.
166 Mignone JL, *et al*. (2010). *Cardiogenesis from human embryonic stem cells*. Circ J **74**, 2517–26.
167 Vidarsson H, *et al*. (2010). *Differentiation of human embryonic stem cells to cardiomyocytes for in vitro and in vivo applications*. Stem Cell Rev **6**, 108–20.
168 We know that certain cell types have fetal and adult forms, where the latter only arise postnatally from an undifferentiated precursor (e.g. blood stem cells and Leydig cells), and protocols developed to date may favour isolation of the fetal rather than the adult cell type.
169 Blum B & Benvenisty N (2009). *The tumorigenicity of diploid and aneuploid human pluripotent stem cells*. Cell Cycle **8**, 3822–30.
170 Lindgren AG, *et al*. (2011). *Loss of Pten causes tumor initiation following differentiation of murine pluripotent stem cells due to failed repression of Nanog*. PLoS One **6**, e16478.

for acute spinal cord repair and pigmented retina cells for macular degeneration). With the notable exception of spermatogonial stem cells, there is little or no risk of teratocarcinomas from tissue-specific stem cells, especially when these are obtained from adults; however, these may show signs of ageing (such as short telomeres and somatic mutations), and therefore have an increased risk of carrying tumour-promoting genetic abnormalities compared with an embryonic cell type.

Potential risks associated with iPS cells are even greater. If derived from an adult cell, they could carry mutations. The iPS cells are as efficient as ES cells at making teratocarcinomas. Moreover, there are some concerns about incorrect reprogramming and genetic damage in iPS cells.[171,172] Incomplete reprogramming appears to be common, and can result in the iPS cells retaining a 'memory' of the starting cell type, which might compromise their ability to differentiate into the desired cells.[173] The factors added to reprogramme the cells are often oncogenic (tumour-promoting); moreover, they turn cells that may be relatively quiescent into ones that divide rapidly, which can lead to 'replicative stress' and to chromosome abnormalities and other mutations. The original methods to obtain iPS cells relied on the integration of retroviral vectors carrying the four reprogramming genes[174], and this could also lead to mutation of endogenous genes. New methods to induce reprogramming without integration of vectors will overcome this problem, but not necessarily others associated with the process.[175,176,177]

Regardless of the source of stem cells, there are issues related to the quantities of cells required for therapies. Problems of 'scale-up' include risks of contamination and the appearance of mutations giving a replicative advantage, where the latter may be associated with a loss of function and increased cancer risk.[178]

Another important issue with respect to source of stem cells to be used for transplants is how to avoid immune rejection. Some organs such as the central nervous system are thought to be sufficiently hidden from the immune system ('privileged' sites) that tissue (human leukocyte antigen (HLA)) matching is not essential. However, this may not be entirely true and immune damage may confuse the interpretation of results.[179] For other organs and tissue types, immune rejection is a clear problem. To overcome this a variety of options is being explored including immune suppressants, inducing tolerance or use of closely HLA-matched or even autologous cell sources (derived from the patient to be treated).[180,181] The last option can include tissue-specific stem cells (assuming that there are sufficient remaining in the patient to be useful), direct reprogramming or iPS cells, even though personalised treatments are costly and a regulatory challenge. There are efforts to derive a minimal set of ES and iPS cells that would allow at least majority of patients to be treated with closely matched cells; however, many hundreds of lines would still not cover more than 90% of people.

How should potential stem cell therapies be tested?

Rigorous assays of quality, safety and efficacy are a regulatory requirement for all treatments, including those based on cell lines. Until there are validated *in vitro* surrogates, it is likely that human stem cells and their differentiated derivatives will need to be tested in appropriate

171 Hussein SM, *et al*. (2011). *Copy number variation and selection during reprogramming to pluripotency*. Nature **471**, 58–62.
172 Howden SE, *et al*. (2011). *Genetic correction and analysis of induced pluripotent stem cells from a patient with gyrate atrophy*. Proc Natl Acad Sci USA **108**, 6537–42.
173 Barrero MJ & Izpisua Belmonte JC (2011). *iPS cells forgive but do not forget*. Nat Cell Biol **13**, 523–5.
174 See Footnote 125
175 Chen G, *et al*. (2011). *Chemically defined conditions for human iPSC derivation and culture*. Nat Methods **8**, 424–9.
176 Okita K, *et al*. (2011). *A more efficient method to generate integration-free human iPS cells*. Nat Methods **8**, 409–12.
177 Anokye-Danso F, *et al*. (2011). *Highly efficient miRNA-mediated reprogramming of mouse and human somatic cells to pluripotency*. Cell Stem Cell **8**, 376–88.
178 Olariu V, *et al*. (2010). *Modeling the evolution of culture-adapted human embryonic stem cells*. Stem Cell Res **4**, 50–6.
179 Chen Z, *et al*. (2011). *MHC mismatch inhibits neurogenesis and neuron maturation in stem cell allografts*. PLoS One **6**, e14787.
180 Lui KO, *et al*. (2009). *Embryonic stem cells: overcoming the immunological barriers to cell replacement therapy*. Curr Stem Cell Res Ther **4**, 70–80.
181 Autologous transfer refers to the movement of cells or tissue from one part of the body to another in the same individual.

animal models. As outlined in 3.3.4, there are now many ways to follow transplanted cells in live animals. Consideration of the type of animal model is appropriate: rodents, large animals, such as pigs or sheep, or NHPs? This may depend on the body system to be treated, where the animal model is chosen according to the similarity in physiology and/or size and complexity of the relevant organ to that of humans. However, this might seem to imply that NHPs should always be the model of choice for assessing treatments for brain disease or trauma. Most studies so far have made use of rodent models, and these seem appropriate to give at least general answers – such as can the cells engraft, do they promote any functional repair? However, they may not predict what will happen in a more complex brain.

How should clinical trials be conducted?
There are still relatively few clinical applications for stem cell transplants. Protocols have been established for conducting trials of applications relying on bone marrow or cord blood stem cells, which can be introduced into the circulatory system, as well as some that involve grafts to surface epithelia (skin and cornea). For many other tissues it is less clear how to introduce cells, and how to design clinical trials. The problem is perhaps most acute for the central nervous system. Grafts of fetal brain cells to people with Parkinson's disease provide an interesting case history, where extensive preclinical data in animal models led to some promising first-in-man experiments, but then larger trials gave results that were conflicting and hard to interpret, in part because of significant variation in the protocols used (see also 2.3.4).[182,183,184]

Some of the questions that need to be considered are:

- Should the trials be double-blind? If so, what treatment should control patients receive?
- How should patients be chosen: likelihood of benefit, age or whether terminally ill?
- How will transplanted cells be followed in the patients: through short term labels, through genetic engineering to introduce markers for *in vivo* imaging, or post-mortem?
- Should the stem cells be engineered to enable them to be destroyed in some way, in case something goes wrong?

Ethical and societal problems
There are several ethical and social issues that affect work in this area, such as the question of the ownership of stem cells and patent rights to procedures involving them; questions about the proper use of these cells, not merely to cure disease or trauma but also to extend life span and as a route to genetic enhancement; and, fundamentally, questions about the acceptability of research that uses human embryonic stem cells. Because these issues are not specific to work involving ACHM and have been much debated elsewhere, we have chosen not to pursue them here. For further discussion of these issues, see reports from the Hinxton Group (see 7.4.2).[185]

3.4 Research involving the brain

Many animal models of human diseases involving the brain have been developed. These include transgenic mice used to study prion diseases and dementias.[186] A few transgenic NHP models have also been developed (see 3.2). Attitudes to research involving the brain expressed in the public dialogue are summarised in Box 3.9.

Chimæric models that involve the implantation of human neural stem cells into an animal's brain are already used in research. For example, rats engrafted with human neural stem cells are used to study the potential of these cells for repairing damage caused by stroke (2.3.4).[187] In research to develop treatments for

182 Brundin P, *et al*. (2010). *Neural grafting in Parkinson's disease. Problems and possibilities*. Prog Brain Res **184**, 265–94.
183 Dunnett SB, (2010). *Neural transplantation. Handbook of Clinical Neurology* **95**, 885–912.
184 Loewenbruck K & Storch A (2011). *Stem cell-based therapies in Parkinson's disease: future hope or current treatment option?* J Neurol **258**, S346–53.
185 See www.hinxton group.org and Caulfield T, *et al*. (2010). *Stem cell research policy and iPS cells*. Nat Methods **7**, 28–33.
186 Prion diseases include conditions such as variant Creutzfeldt–Jakob disease (vCJD), a human neurodegenerative condition.
187 Pollock K, *et al*. (2006). *A conditionally immortal clonal stem cell line from human cortical neuroepithelium for the treatment of ischemic stroke*. Exp Neurol **199**, 143–55.

Parkinson's disease, NHPs with Parkinsonian-like brain lesions have had human neural stem cells implanted in their brains. These studies have provided understanding of the ways in which stem cells migrate towards the sites of damage in the primate brain.[188] We are not aware of evidence that the addition of human-derived cells into an animal's brain in studies of this kind has resulted in any obvious changes in the cognitive abilities of the animals involved.

We have described methods whereby cells within an animal's organ, such as the liver, can be replaced by human cells (see 2.3.3). Equivalent studies involving the brain have been the subject of considerable ethical discussion. The predominant question is whether populating an animal's brain with human-derived cells could result in the production of an animal with human 'cognitive capacity' (i.e. some aspect of 'consciousness', 'awareness' or 'sentience') or 'human-like' behavioural capabilities.[189]

In 2000, Dr Irving Weissman (at Stanford University, USA) proposed an experiment to create what has become known as the 'human neuron mouse', which would involve a far greater degree of substitution of the mouse brain with human-derived cells. The proposal was to use mice with a condition causing death several days before birth owing to the loss of most or all of the developing neurons in the fetal mouse brain. Weissman suggested transplanting human brain stem cells into the fetal mice, just as their own neurons were dying, with the intention of producing a mouse with a functional brain made up of mouse glial (supporting) cells and human neurons, to enable the study of human neurons *in vivo*. The proposed experiment was voluntarily subjected to ethical analysis by an independent study group, (led by Professor Greely, Stanford University, USA) which recommended that the experiment could be performed ethically.[190] However, the experiment has not as yet been performed.[191]

The balance of opinion on the working group is that, even if an experiment of this type produced a functional brain, it would be very unlikely to result in a mouse with human cognitive characteristics, as a mouse brain is much smaller and could not develop the complex interconnections that occur in human brains. It would lack much of the sensory input (e.g. through the visual system) received by the human brain and the distinctive motor outputs that characterise human motor behaviour. As one submission to our study indicated, *'If these cells do make effective connections then the signals that pass through them will be the signals of the host. Thus human nerve cells within a mouse would receive signals from the mouse's sensory organs (e.g. auditory signals about high frequency sound, vision adapted to dim illumination but not colour, touch from whiskers and olfactory input from the mouse's sensory world). Conversely, these cells would link to cells controlling the movement of four legs, and not to human hands or facial movement (speech).'* [192] The extent to which mouse glial cells could support normal human neural function is also undetermined. The development of human capacities of sentience and cognition are also crucially dependent on developmental pathways, from conception to adulthood, which would obviously be fundamentally different in a rodent model. However, the precise effects of this modification on the animal's phenotype cannot be fully

188 Bjugstad KB, *et al*. (2008). *Human neural stem cells migrate along the nigrostriatal pathway in a primate model of Parkinson's disease*. Exp Neurol **211**, 362–9.

189 In its broadest sense, human 'cognition' can be defined as the 'faculty of knowing', to include aspects such as knowledge, reason, intelligence, understanding, sensation, perception and conception (as distinguished from feeling and volition). In 3.4.1 we describe how experimental measures could act as a proxy for assessment of human 'cognition' in animals.

190 The original ethical analysis is unpublished; however, its findings were summarised in Greely HT, *et al*. (2007). *Thinking about the human neuron mouse*. Am J Bioeth **7**, 27–40. The group examined the potential costs or risks of the experiment, considered factors to mitigate these, and weighed risks against the possible benefits. Risks included the source of the human brain stem cells from aborted human fetuses; potential for pain and suffering to the mice; propriety of this use of human tissues; risks of conferring some degree of humanity on another species; risks to public support of science. Benefits focused on the potential uses of animal containing human neurons for basic science and for clinical applications. The group concluded that the experiments could proceed ethically, subject to careful staging and monitoring. Recommendations included that human brain stem cells only be used with appropriate consent; the experiments should be performed in stages and should be carefully monitored; the experiments should be done in an open manner with appropriate information conveyed to the press; the mice should be disposed of appropriately and should not be allowed to breed.

191 Greely HT, *et al*. (2007). *Response to open peer commentaries on "Thinking about the human neuron mouse"*. Am J Bioeth **7**, W4–6.

192 Written evidence from Parker, A.

predicted without more experimental evidence.

The potential consequences of a similar experiment conducted in a larger animal, for example a sheep or pig are more debatable; even more so in an NHP, which has sensory and motor capabilities more similar to the human. If an NHP modified in some such way came to approximate the cognitive capacity of a Great Ape (common chimpanzee, bonobo, orang-utan and gorilla), would it no longer be deemed appropriate for use in experimentation, given that research on Great Apes is not currently permitted in the UK (see 5.6 and Box 6.1)?

In 2005, a multi-disciplinary working group considered ethical issues arising from the transplantation of human neural stem cells into the brains of NHPs.[193] This group concluded that such research should minimise the risk that an animal would develop human-like cognitive capacities, and it set out a series of factors that should be considered in reviewing proposals for such research (Box 3.4). Analogous questions could be asked about transplantation of NHP neural stem cells into other animals.

3.4.1 Approaches to assessing alteration in cognition

It is difficult to predict confidently the outcomes of experiments such as those described above, until further evidence is available. However, we can begin to consider which aspects of brain function might be considered particularly 'human', and how these could be monitored. Measures of this kind could perhaps act as a proxy for human 'sentience' and provide a practical basis for assessing change within such chimæric brains.

Neuroscience has made important advances in defining aspects of brain function and in developing methods to assess these functions in humans and other species. Although we intuitively think of human brain function and 'thought' as unique to humans, studies indicate that human and animal brain function have much in common. Some relatively sophisticated aspects of brain function are evident in a range of mammalian species (see Box 3.5).

Box 3.4 'Moral issues of human-non-human primate neural grafting' [194]

In relation to the introduction/integration of human neural stem cells into NHP brain, Greene *et al*. concluded *'we support the National Academy's recommendation that human-NHP neural grafting experiments be subject to special review'* and recommended that *'experiments involving human-NHP neural grafting be required, wherever possible, to look for and report changes in cognitive function. Explicit data collection on cognition and behavior will help to ensure that ethical guidelines can be developed appropriately as the field advances.'*

The group proposed *'six factors that research oversight committees and other review groups should use as a starting framework'.* These were:

1. The proportion of engrafted human cells.
2. The stage of neural development.[195]
3. NHP species.
4. Brain size.
5. Site of integration.
6. Brain pathology.[196]

193 Greene M, *et al*. (2005). *Ethics: moral issues of human–non-human primate neural grafting*. Science **309**, 385–6.
194 *Ibid.*
195 Higher proportions of engrafted cells were considered likely to be achieved by implantation early in neural development; such cells were also considered likely to have greater functional influence.
196 The condition of the recipient brain might affect the influence of the graft – for example damage to neural structures in adult animals, intended to model neurological disease, might give greater scope for engrafted human cells to colonise and in turn effect cognitive capacities. However, such models would also be impaired, and so perhaps less likely to acquire human-like function.

In limited areas, cognitive abilities of some animals approach, or arguably even exceed, those of humans. Macaque monkeys are the most commonly used experimental NHPs. Visual memory (identification of objects that they have, or have not, seen previously) is highly developed in macaques, and they can out-perform people with Alzheimer's disease and even many healthy adults in some tests.[197] This kind of memory in macaques can also be enhanced, for example by cognitive-enhancing drugs such as AMPA-kines. Enhancement of other functions can be achieved through behavioural approaches (e.g. Japanese monkeys have been shown to acquire the ability to use sensory tools such as endoscopes, through training).[198,199] We distinguish between this type of *quantitative shift* in existing animal cognitive capacities and *qualitative change* towards 'uniquely human' capacities. Merely demonstrating quantitative enhancement of one aspect of an animal's cognitive function does not imply its cognitive capacity is approaching that of the human. Conferring an increase in cognitive capacity on an animal through the addition of human cells or DNA would not necessarily hold any greater significance than equivalent effects obtained through drug or behavioural manipulation.

Certain aspects of brain function are, however, only evident in humans and others are mainly present in humans and marginally in the Great Apes. In these areas, we can begin to identify the types of brain function that may distinguish humans from other species (see Box 3.6).

Box 3.5 Set shifting in humans, primates and rodents

The Wisconsin card-sorting test is used in human neuropsychology. It measures the subject's ability to sort cards according to given rules (e.g. by the colour, shape or number of objects on the card) on the basis of feedback – and importantly to *adapt* as the rules are changed. The test has been described as an assessment of 'set-shifting' ability, which may be considered a form of 'executive function' (higher brain processes associated with planning and abstract thinking). Normal human subjects adapt quickly, but people with brain disorders are slower to identify and adapt to new rules.

The CANTAB ID-ED test has been developed as an equivalent test for monkeys, based on a screen touch system. When presented with a series of paired shapes and lines, marmoset monkeys show the ability to learn to respond to particular shapes, as well as the ability to shift from responding to shapes, to lines (i.e. they have the ability to learn the concept of 'classes' of shape and the capacity to set-shift). Rhesus monkeys are superior to marmosets in performance on this task.[200]

Studies using olfactory or textual cues have demonstrated that mice and rats can also set-shift. The brain regions that underpin this ability in rodents may be equivalent to those used in humans.

197 Basile BM & Hampton RR (2011). *Monkeys recall and reproduce simple shapes from memory*. Curr Biol **21**, 774–8.
198 Yamazaki Y, *et al*. (2009). *Acquisition of an externalized eye by Japanese monkeys*. Exp Brain Res **194**, 131–42.
199 Sensory tools are those used to acquire sensory information or to augment sensory function, including tools such as endoscopes.
200 Weed MR, *et al*. (1999). *Performance norms for a rhesus monkey neuropsychological testing battery: acquisition and long-term performance.* Brain Res Cogn Brain Res **8**, 185–201.

Box 3.6 Aspects of brain function that may distinguish humans and the Great Apes from other species

1. **Episodic memory**. This is sometimes called 'autobiographical memory' or memory of events. Operational aspects of episodic memory (recall of what, where and when) have been demonstrated in species such as corvids (crows) and apes.[201] However, it is suggested that the 'subjective component' of episodic memory (an awareness of personal involvement in previous events) is a uniquely human function.
2. **Planning**. Humans have the capacity for 'planning', the ability to recognise and address future needs (sometimes even when these conflict with immediate need). Apes and chimpanzees are believed to be capable of selecting tools for future use.
3. **Numerosity**. The ability to work with numbers greater than 5 and to represent large numbers is extremely limited even in apes. Studies suggest that other monkeys (including macaques) can only work with small numbers.
4. **Language**. The capacity for language in NHPs is a classical controversy. Case studies in chimpanzees, including 'Washoe (1965–2007)' and 'Nim Chimpsky (1973–2000)', are inconclusive.
5. **Theory of mind**. Evidence for this function (the ability to identify and attribute mental states, e.g. beliefs, intents, desires, pretending, knowledge of yourself and others, and the capacity to recognise that the mental states of others can differ from your own) in NHPs is controversial.[202]
6. **Social cognition**. A task known as the 'ultimatum game' has been used to explore aspects of social cognition such as the willingness to accept injustice and social inequality in humans. In a variation of this task, chimpanzees have been found to lack a sense of 'fairness'.[203]

Further insight into the cognitive qualities that differ between humans and other primates has come from studies comparing the abilities of 2-year-old (pre-speech) human children, chimpanzees and orang-utans.[204] Although the human children only slightly out-perform chimpanzees and orang-utans on 'physical domain' tests (e.g. spatial memory and tool use), they significantly out-perform apes in 'social domain tests' (e.g. social learning and comprehension).

Comparative psychologists and ethologists have developed 'test batteries' to assess primate cognition, grouped into physical and social 'domains' (see Box 3.7). Test batteries of this kind could, in principle, be used to assess experimental animals for aspects of cognition that are indicators of relevant alterations in cognitive capacity.

Neuroanatomical correlates

Study of neuroanatomical and imaging correlates of brain function is now beginning to identify brain regions involved in aspects of social learning in NHPs. For example, research using neural recording techniques in monkeys has indicated a role for a region called the medial pre-frontal cortex in capturing a representation of the actions of another animal.[205,206] Studies in humans, sheep and macaques indicate a role for the medial frontal lobes and temporal lobes in tasks such as face perception.[207] Further developments may eventually provide useful diagnostic markers of altered cognitive capacity in experimental animals.

201 Martin-Ordas G, *et al*. (2010). *Keeping track of time: evidence for episodic-like memory in Great Apes*. Anim Cogn **13**, 331–40.
202 Penn DC & Povinelli DJ (2007). *On the lack of evidence that non-human animals possess anything remotely resembling a 'theory of mind'*. Philos Trans R Soc B **362**, 731–44.
203 For further detail see Jensen K, *et al*. (2007). *Chimpanzees are rational maximizers in an ultimatum game*. Science **318**, 107–9.
204 Herrmann E, *et al*. (2007). *Humans have evolved specialised skills of social cognition: the cultural intelligence hypothesis*. Science **317**, 1360–6.
205 See Quallo MM, *et al*. (2009). *Gray and white matter changes associated with tool-use learning in macaque monkeys*. Proc Natl Acad Sci USA **106**, 18379–84; this study shows use of magnetic resonance imaging and additional techniques to reveal brain region changes during learning of rake tool use in macaques.
206 See Yoshida K, *et al*. (2011). *Representation of others' action by neurons in monkey medial frontal cortex*. Curr Biol **21**, 249-53; this study uses neural recording in monkeys to identify where in the brain the action of others is represented.
207 Peirce JW, *et al*. (2001). *Human face recognition in sheep: lack of configurational coding and right hemisphere advantage*. Behav Processes **55**, 13–26.

Box 3.7 Test batteries for assessing aspects of primate cognition

Humans out-perform NHPs on social domain tests, whereas differences in abilities between humans and apes are less distinct in physical domains.[208]

Physical domains and tests	Social domains and tests
• Space – spatial memory	• Social learning
• Space – object permanence	• Communication – comprehension
• Space – rotation	• Communication – pointing
• Space – transposition	• Communication – attentional state
• Quantities – numerosity	• Theory of mind – gaze following
• Quantities – addition	• Theory of mind – intention

3.4.2 Adopting an incremental approach

Because of difficulty in predicting the outcome of human–animal chimæric brain experiments, particularly in larger animals, some might suggest that such experiments should not be pursued. However, there are important reasons for seeking to determine how neurons derived from human neural stem cells, or other cell types, can potentially integrate into, and function in, a damaged brain. Before transplanting such cells into the brains of humans suffering from brain disorders, it is essential to investigate possible safety issues, and to have good evidence of likely efficacy; both these are likely to involve some testing on animals. Authorisation of research of this type should (at least for some time) be based on careful, case-by-case evaluation to ensure that, as in all research, the use of animals can be justified by the potential benefit and the lack of satisfactory alternative research strategies. Many experiments will involve such a low level of engraftment of human cells into the animal brain that they will cause little concern, and can confidently be regulated under ASPA with no additional oversight (see 8.2.1).

We suggest that experiments where there is doubt as to the potential functional effect of modification of the brain, particularly in larger animals and NHP's, should be subject to additional oversight by an expert national body (see 8.2.2), and may need to be carried out on an incremental basis (see Box 3.8).

208 A domain is a specialised sphere of activity or knowledge. See Herrmann E, *et al*. (2007). *Humans have evolved specialised skills of social cognition: the cultural intelligence hypothesis*. Science **317**, 1360–6.

Box 3.8 Expert assessment/incremental approach

- For some forms of experiment (as set out in 8.2.2) an incremental research approach should be agreed at the outset between researchers, inspectors and the national expert body.
- Initial experiments should usually be undertaken using 'lowest' feasible species not previously studied, in small numbers. Where possible there should be a graduated approach to the amount/proportion of human material added.[209]
- Each animal should be tested according to a pre-agreed protocol with clear end-points. Tests appropriate to the different research situations and species should be used to detect any modification/loss of the animal's usual cognitive capacities and behaviours. Close monitoring of the animals should take place, with due regard to minimising observer bias.[210]
- Once experience is gained, studies involving larger numbers of animals, a greater proportion of cellular replacement, and more advanced species could be undertaken.
- For example, research intended to study the effect of incorporating human neurons into an NHP brain, could start with evidence based on modest neuronal incorporation into rodents, and proceed by degrees to experiments involving larger scale replacement in NHPs. In this case, the monitored effects might relate to the development of human-like cognitive capacities. A range of tests, from which a protocol could be developed are set out in Box 3.7.
- Unusual and 'first of a kind' experiments will need to be judged on an individual basis; but as experience is gained, guidance could be developed so that some classes of experiment may be undertaken with lower levels of regulatory scrutiny (see Chapter 8).

Box 3.9 Two ways of viewing the brain

Some participants in the public dialogue appeared to adopt a dual conceptualisation of the brain, in which it was seen as both a purely physical organ, as simply 'tissue', and secondly as the source of consciousness and thought 'greater than the sum of its parts'. When considering scientific research, participants often tended to think about the brain in the first way, and few people appeared to believe that small changes to an animal's brain at the cellular level would have a discernable impact on its cognitive function: *' … a mouse brain is so much smaller, I don't think a little brain will be able to sit there and "think therefore I am" …'*

However, in considering the possible implications of manipulating the brain as a whole, the second view tended to be adopted. From this viewpoint, some participants expressed a clear sense of unease around research involving the brain, and its potential outcomes. Some participants suggested that research that might make an animal's brain more similar to a human brain would be unacceptable: *'I don't have a problem with it until it gets to the brain … but bits to do with memories, that would be too far – it's a human thing to have a memory.'*

209 The basis of the incremental approach should be carefully considered in each situation, and should not mandate additional studies where clear scientific justification is not evident. For example, work on lower species should not be required where previous evidence is already adequate (e.g. from cell-based studies). Good evidence from previous work should always be taken into account in planning and licensing experiments.

210 For example, the use of double blinding, and or automated observation techniques.

3.5 Research involving the reproductive system

There are important differences in reproductive biology between mammalian species. The relatively high frequency of infertility, details of placental development, and menopause are largely specific to humans.[211] Compared with most animal species, the reproductive system of humans is prone to problems such as premature ovarian failure, endometriosis, and cancers of the ovary, testis, cervix and breast. The exploration of inter-species differences has often been very illuminating; however, because of these differences, unmodified animals are frequently unsuitable models for the human. Despite this, animal research has contributed significantly to knowledge in this area, and to the development of treatments for reproductive disorders. For example, many human-assisted reproductive techniques, including *in vitro* fertilisation (IVF), were initially developed in other mammalian species, particularly the rabbit and mouse.[212]

Models involving ACHM have been, and are likely to continue to be, particularly important for research in this field. The fertilisation of animal eggs by human sperm was an important test of male fertility and these are still used to explore mechanisms associated with fertilisation (see 2.2.3). Animals containing human DNA are used to explore the role of specific human genes (and their regulatory sequences) on many human-specific aspects of reproductive function, at any stage from gamete development to parturition (the process of giving birth). Chimæric animals carrying human germ cells (sperm or eggs) or other reproductive system tissues (e.g. endometrium (womb lining)) can provide important investigative models in reproductive research, though they appear to be contentious (see Box 3.10 and 5.7.2).

3.5.1 Germ cell development and function

Male germ cells

Abnormalities of fetal testis development and function can predispose men to disorders that become evident in adulthood, such as testicular germ cell cancers and low sperm counts, disorders that are increasing in incidence for unknown reasons. The fetal origins of these conditions cannot be investigated in adult patients, and it would be unethical and impractical to conduct studies on live human fetuses. To study these conditions, small pieces of testicular tissue, taken with permission from legally aborted fetuses are implanted under the skin of immune-deficient mice. The implants grow and develop normally, and provide a way of dynamically studying the developing human testis allowing investigation of the effects of chemical exposures or other interventions.[213,214] This model is used in investigating the fetal origins of testicular germ cell cancers (the commonest cancer of young men) and in assessing the effects of exposure to environmental chemicals (such as phthalates, used in plastics).[215] Investigations of this type may also yield new insights into the mechanisms regulating human male germ cell proliferation and differentiation, which could be used both for fertility treatments and the development of male contraceptives.[216]

Research is underway to develop procedures to preserve testicular germ cells from boys who are being treated for cancer with therapies that may cause sterility.[217] One approach under consideration is to graft tissue or cells from human testis biopsies, collected before therapy, into mice and to allow the human cells to survive and/or proliferate, with the aim of either

211 Other factors largely unique to human reproduction include poor rates of early embryo development and implantation, and a short gestation period relative to neonatal size.
212 Fauser BC & Edwards RG (2005). *The early days of IVF*. Hum Reprod Update **11**, 437–8.
213 Mitchell RT, *et al*. (2008). *Germ cell differentiation in the marmoset (Callithrix jacchus) during fetal and neonatal life closely parallels that in the human*. Hum Reprod **23**, 2755–65.
214 Scott HM, *et al*. (2009). *Steroidogenesis in the fetal testis and its susceptibility to disruption by exogenous compounds*. Endocr Rev **30**, 883–925.
215 Mitchell RT, *et al.* (2010). *Xenografting of human fetal testis tissue: a new approach to study fetal testis development and germ cell differentiation*. Hum Reprod **25**, 2405–14.
216 Mitchell RT, *et al*. (2009). *Male fertility and strategies for fertility preservation following childhood cancer treatment*. Endocr Dev **15**, 101–34.
217 Although it has recently been shown possible to obtain functional mouse sperm by culturing pieces of neonatal testis *in vitro*, the technique has not yet been developed for human testis; moreover, it is not clear that it will work with the relatively early stages of fetal testes that can be obtained from aborted embryos. If it does work, it could replace some of the ACHM experiments of the type described here. See Sato T, *et al*. (2011). *In vitro production of functional sperm in cultured neonatal mouse testes*. Nature **471**, 504–7.

transplanting the cells back into the donor after the patient's recovery, or to use mature germ cells (if these can be grown in the xenografts) for *in vitro* fertilisation.[218] Various studies, including one using childhood tissue, have shown the potential utility of this approach but further development is needed to develop a clinical treatment.[219] Clinical application would also require the development of methods to prevent animal–human disease transmission (see 4.2). Further policy and ethical consideration would also be appropriate. Under the HFE Act (2008), human sperm (or eggs) derived in animals or *in vitro* may be classified as 'non-permitted gametes'; if so, it would be possible to use them for *in vitro* tests of fertilisation and early embryo development (under licence) but not for clinical purposes (see Box 6.4).

Female germ cells

Factors affecting the development and maturation of human egg cells have, similarly, been studied in immune-deficient mice engrafted with human ovarian tissues. Initial studies demonstrating that frozen human ovarian follicles (egg precursor cells) were able to continue development were first made by re-implanting these tissues into immune-deficient mice.[220,221,222] These studies found that the human grafts were able to resume apparently normal follicle growth and maturation, in the mice.[223,224] With such a model it was recently found that when an inhibitor of PTEN (part of a molecular pathway known to block oocyte development) was given to the mice, human primordial follicles (the earliest egg stage) present in the graft can develop all the way to mature pre-ovulatory stages, and contained oocytes able to undergo maturation (and perhaps fertilisation). This process took 6 months (something that would be difficult to achieve *in vitro*), but it is a potentially important way to generate large numbers of human oocytes for research or even potentially for fertility treatments (subject to the possible legal restrictions described above).

Studies using engrafted human ovary played an important role in improving cryo-preservation (freezing) techniques used to store ovarian tissue from people at risk of losing their fertility (e.g. due to cancer therapy).[225,226,227,228] Ovarian tissue banking has since been offered in many oncology centres, and although few transplants of thawed tissue have been reported, there have been successful live-births following this procedure.[229] Xenograft models have also been used to investigate the effects of anti-cancer drugs on follicles within the ovarian tissue, e.g. to determine the treatment least likely to lead to infertility as a side-effect.[230]

Reproductive disorder with genetic origins

Chromosome abnormalities arising during germ cell development leading to extra (or missing) chromosomes are thought to be one of the causes of the high rates of early embryo loss seen in humans, for example as early miscarriages, and are responsible for several syndromes affecting liveborn individuals, including Down's and Turner syndromes. The incidence of some of these abnormalities

218 Mitchell RT, *et al*. (2009). *Male fertility and strategies for fertility preservation following childhood cancer treatment*. Endocr Dev **15**, 101–34.
219 Mitchell RT, *et al*. (2010). *Xenografting of human fetal testis tissue: a new approach to study fetal testis development and germ cell differentiation*. Hum Reprod **25**, 2405–14.
220 Gook DA, *et al*. (2001). *Development of antral follicles in human cryopreserved ovarian tissue following xenografting*. Hum Reprod **16**, 417–22.
221 Oktay K, *et al*. (2000). *Transplantation of cryopreserved human ovarian tissue results in follicle growth initiation in SCID mice.* Fertil Steril **73**, 599–603.
222 Gosden RG, *et al*. (1994). *Follicular development from ovarian xenografts in SCID mice*. J Reprod Fertil **101**, 619-23.
223 Oktay K, *et al*. (2000). *Transplantation of cryopreserved human ovarian tissue results in follicle growth initiation in SCID mice*. Fertil Steril **73**, 599–603.
224 Gook DA, *et al*. (2003). *Oocyte maturation, follicle rupture and luteinization in human cryopreserved ovarian tissue following xenografting*. Hum Reprod **18**, 1772–81.
225 Newton H, *et al*. (1996). *Low temperature storage and grafting of human ovarian tissue*. Hum Reprod **11**, 1487–91.
226 Soleimani R, *et al*. (2010). *Xenotransplantation of cryopreserved human ovarian tissue into murine back muscle*. Hum Reprod **25**, 1458–70.
227 Rahimi G, *et al*. (2010). *Re-vascularisation in human ovarian tissue after conventional freezing or vitrification and xenotransplantation*. Eur J Obstet Gynecol Reprod Biol **149**, 63–7.
228 Van Eyck AS, *et al*. (2010). *Both host and graft vessels contribute to revascularization of xenografted human ovarian tissue in a murine model*. Fertil Steril **93**, 1676–85.
229 Donnez J, *et al*. (2004). *Livebirth after orthotopic transplantation of cryopreserved ovarian tissue*. Lancet **364**, 1405–10.
230 Oktem O & Oktay K (2007). *A novel ovarian xenografting model to characterize the impact of chemotherapy agents on human primordial follicle reserve*. Cancer Res **67**, 10159–62.

increases significantly with age.[231,232,233,234] It can be difficult to study factors that may predispose to these abnormal cells, especially during the first meiotic division in oocytes as this begins during fetal ovary development. Progress is being made on *in vitro* growth and maturation of primary follicles, which could be used to study later stages of egg cell division, but these methods are not yet sufficiently robust.[235] Animals carrying grafts of human ovarian (or testicular) tissue may allow detailed, longitudinal studies on factors (hormonal, toxic or age-related) affecting chromosome segregation.

3.5.2 Endometrial development and pathology

Experimental approaches involving ACHM are currently being established to study the normal physiology of human endometrial tissue, as well as its malfunction in conditions such as endometriosis, a condition that may affect more than 10% of women.[236, 237] These studies involve the engraftment (usually into the peritoneal cavity) of small sections of endometrial tissue, taken from a healthy human donor, or a patient with endometriosis, into ovariectomised, immune-deficient mice.[238] The mice are treated with a course of endocrine hormones which imitate the human female menstrual cycle. The engrafted human tissue allows study of factors such as tissue morphology and gene expression. Bioluminescent or other markers can also be introduced into the endometrial cells to allow their growth to be monitored *in vivo*.[239] The effects of repeated hormone cycles could be studied. These models are being used to screen for molecules that might reduce endometrial cell proliferation or might prevent attachment and spread of endometriosis.[240,241] Recently, using such mouse models, at least three types of drug have been claimed to reduce growth of human endometrial tissue.[242,243,244,245,246] Apart from giving promising leads towards therapy, these mouse studies replace the use of other animals, notably baboons, that are also used in research on endometriosis.[247]

3.5.3 Implantation and placenta development

Two important areas where ACHM may allow future research are embryo implantation into the lining of the uterus and placental development.

There is a significant loss of human embryos (perhaps as high as 70%) during pre-implantation development and around implantation. This very high rate appears specific to humans, and although chromosomal abnormalities account for a significant proportion (see 3.5.1), most causes are unknown. A failure of interaction between the embryo and the endometrium into which it implants is probably also a common cause. The underlying defect could be intrinsic to the embryo, the endometrium or the hormone system that makes the endometrium receptive.[248] It is, however, very difficult to carry out relevant experiments on human material. Some research

231 Hunt P & Hassold T (2010). *Female meiosis: coming unglued with age*. Curr Biol **20**, R699–702.
232 Thomas NS, *et al*. (2010). *De novo apparently balanced translocations in man are predominantly paternal in origin and associated with a significant increase in paternal age*. J Med Genet **47**, 112–5.
233 Thomas NS, *et al*. (2001). *Maternal sex chromosome non-disjunction: evidence for X chromosome-specific risk factors*. Hum Mol Genet **10**, 243–50.
234 Muhlhauser A, *et al*. (2009). *Bisphenol A effects on the growing mouse oocyte are influenced by diet*. Biol Reprod **80**, 1066–71235.
235 Jin SY, *et al.* (2010). *A novel two-step strategy for in vitro culture of early-stage ovarian follicles in the mouse.* Fertil Steril **93**, 2633–9.
236 Olive DL & Schwartz LB (1993). *Endometriosis*. N Engl J Med **328**, 1759–69.
237 Giudice LC & Kao LC (2004). *Endometriosis*. Lancet **364**, 1789–99.
238 Ovariectomy (removal of the ovaries) is used to remove the mouse's own secretion of endocrine hormones. See Masuda H, *et al*. (2007). *Noninvasive and real-time assessment of reconstructed functional human endometrium in NOD/SCID/gamma c(null) immunodeficient mice*. Proc Natl Acad Sci USA **104**, 1925–30.
239 Defrere S, *et al*. (2009). *Review: luminescence as a tool to assess pelvic endometriosis development in murine models*. Reprod Sci **16**, 1117–24.
240 Hull ML, *et al*. (2008). *Endometrial–peritoneal interactions during endometriotic lesion establishment*. Am J Pathol **173**, 700–15.
241 Collins NH, *et al*. (2009). *Characterization of antiestrogenic activity of the Chinese herb, prunella vulgaris, using in vitro and in vivo (Mouse Xenograft) models*. Biol Reprod **80**, 375–83.
242 The drugs are: simvastatin, a cannabinoid agonist which inhibits the Akt signalling pathway; Raloxifene, a selective estrogen receptor modulator; and an antibody based protein, 'icon', that inactivates a growth factor.
243 Bruner-Tran KL, *et a*l. (2009). *Simvastatin protects against the development of endometriosis in a nude mouse model*. J Clin Endocrinol Metab **94**, 2489–94.
244 Leconte M, *et al*. (2010). *Antiproliferative effects of cannabinoid agonists on deep infiltrating endometriosis*. Am J Pathol **177**, 2963–70.
245 Chen YJ, *et al*. (2010). *Oestrogen-induced epithelial–mesenchymal transition of endometrial epithelial cells contributes to the development of adenomyosis*. J Pathol **222**, 261–70.
246 Krikun G, *et al*. (2010). *The immunoconjugate "icon" targets aberrantly expressed endothelial tissue factor causing regression of endometriosis*. Am J Pathol **176**, 1050–6.
247 Tirado-Gonzalez I, *et al*. (2010). *Endometriosis research: animal models for the study of a complex disease*. J Reprod Immunol **86**, 141–7.
248 Singh M, *et al.* (2011). *Bridging endometrial receptivity and implantation: Network of hormones, cytokines, and growth factors*. J Endocrinol. *In press.*

on implantation is currently conducted in animals such as the baboon, where mechanisms of implantation are thought to be fairly similar to those in humans. There are attempts to derive *in vitro* systems to look at implantation using cultures of endometrium, but these do not replicate the complex three-dimensional architecture or physiology of the womb.

ACHM experiments involving grafts of human uterus or endometrium into animals, or, if the identities of the human genes required for receptivity are known, transgenic animals expressing such genes within their uterus, might allow human embryo implantation to be studied. This would not be permitted under the HFE Act (2008) (see 6.5); however, it might be possible to use disabled embryos, such as trophoblast vesicles or tetraploid embryos.

Different mammals often have very different types of placenta. Some studies can be done in rodents on placental cell types that appear similar to those in the human placenta, and some molecular and genetic pathways are conserved, but to understand many details of human placental development and physiology fully requires studies in humans or closely related species.[249] Although it would be difficult to study entire human placental development in animals, it is possible to study the role of specific human genes in transgenic animals, or to introduce specific human placental cell types into the placenta of animals *in utero* and to determine their effects on placenta function and physiology.

3.5.4 Other studies involving reproductive tissues and general concerns

ACHM may be appropriate to study a wide range of questions about human reproduction, from eclampsia and birth timing to menopause. In such studies, human reproductive tissues are usually implanted into the recipient animal 'ectopically' (e.g. under the skin of a mouse rather than into its own reproductive system), and there is very little possibility of the eggs or sperm contacting another germ cell and being fertilised. However, some experiments of this type do result in the presence of functional human sperm and/or egg cells in animals, which raises the possibility that fertilisation between human and animal germ cells (or even between human eggs and sperm) may inadvertently occur within an animal.

At least one study has reported grafting pieces of human ovary under the membrane of mice ovaries, creating the possibility that human oocytes might enter the reproductive tract of female mice.[250] The females were not allowed to mate, but if they had (with male mice), there would be very little chance of hybrid embryo development or implantation. Human sperm do not normally penetrate the mouse zona pellucida (a type of protective shell around the egg), as species-specific molecules are required. Transgenic mice expressing human zona pellucida proteins are being used to search for the relevant molecules.[251,252] To achieve cross-species fertilisation, for example in tests of human male fertility using hamster or mouse eggs, it is necessary to remove the zona pellucida or to use ICSI.[253] Such tests are usually terminated at the two-cell embryo stage, although 'true-hybrid' embryos may be allowed to develop for 14 days (see Box 6.6).[254] We have briefly discussed the problems of studying human embryo implantation and human placental development (3.5.3). Would it be possible to transplant a human uterus into an animal and then use this to implant human embryos? Given that uterus transplants are being considered

249 For example, at least two retroviral elements, HERV-W and HERV-FRD, both of which lead to the expression of viral envelope proteins (termed Syncytin and Syncytin2, respectively) in the human placenta, are specific to the primate lineage. The Syncytin proteins lead to cell fusion and to the formation of a specialised tissue, termed syncytiotrophoblast, which plays an important role in the maternal–fetal interchange of nutrients. Although some rodents also have syncytiotrophoblast, this appears to be due to the activity of rodent-specific retroviral genes. Other retroviral integrations are thought to have affected the expression of endogenous genes involved in human placental development, such as the Insulin-like 4 gene *(INSL4)*.

250 Dath C, *et al*. (2010). *Xenotransplantation of human ovarian tissue to nude mice: comparison between four grafting sites*. Hum Reprod **25**, 1734–43.

251 Xu YN, *et al*. (2010). *DNA synthesis and epigenetic modification during mouse oocyte fertilization by human or hamster sperm injection*. J Assist Reprod Genet. *In press.*

252 Yauger B, *et al*. (2011). *Human ZP4 is not sufficient for taxon-specific sperm recognition of the zona pellucida in transgenic mice*. Reproduction **141**, 313–9.

253 Intra-cytoplasmic sperm injection (ICSI) involves injecting a single sperm directly into an egg in order to fertilise it.

254 However, subsequent development is unlikely to occur owing to epigenetic defects, aneuploidy, and species-specific factors controlling implantation.

as an alternative to surrogacy in humans, this is not necessarily a remote possibility.[255] Careful thought would need to be given about such experiments from scientific and ethical perspectives. Licensing of any animal experiment where there is a chance of human embryo or true hybrid development should address the precautions taken to avoid this (see 8.2.2).

3.6 Research involving human appearance or behavioural traits

Current ACHM do not show overt human-like appearance or behaviours; the alterations are seen at a biochemical or pathological level. Transgenic mice have the appearance of ordinary mice; chimæric goats engrafted with human stem cells look like 'ordinary' goats. Even the most extensive of current genetic modifications, such as the addition of a human chromosome to mice in the Down's syndrome model (see 3.2.), do not markedly alter the appearance of the animals to a casual human observer.[256]

Participants in the public dialogue expressed particular concern that the incorporation of human material into experimental animals might result in the creation of animals with 'human-like' appearance or characteristics (see Box 3.11). There are some cardinal phenotypic features that are intuitively recognised as essentially human, such as facial appearance and skin texture, and behaviours including speech. Experiments that confer these properties on animals may be expected to attract public interest (see for example the reaction to the Vacanti Earmouse, Box 3.12). The societal and ethical bases of such concern are discussed in Chapter 5.

Box 3.10 Public views on research involving human reproductive tissues

The creation of animal models including human reproductive tissue was a very sensitive area for public participants. Compared with other human tissues, the use of animal models involving human reproductive cells was regarded as acceptable by the fewest number of participants in the quantitative survey (42%).

'*… that is so far out there, just awful. Perhaps if there was no sperm left on earth, but otherwise no way.'*

Dialogue discussions identified several possible explanations for these responses, including:

- The cultural significance of reproductive cells (through associations with sex, the production of children, birth experiences and development, and familial characteristics).
- A suggestion that even small changes to a single reproductive cell might produce profound effects (reproductive cells were seen as easy to 'abuse', and contrasted with the brain, where 'changing a few cells might not matter').
- A view that the consequences of research involving human reproductive cells might be experienced not only by the animal involved, but potentially by resulting human offspring.

Box 3.11 Public views on research involving human-like appearance

Research involving external body parts, such as the use of human hair, skin, or the possible development of human-like limbs on animals, was often met with distaste by dialogue participants. This type of response was attributed to participants' ability to imagine and visualise the resulting animal as 'unnatural'. The physical appearance of animals was found to be an important way in which participants identified animals as different 'kinds', and changes to external features might be seen to blur these well-recognised visible distinctions between species.

255 Grynberg M, *et al*. (2011). *Uterine transplantation: a promising surrogate to surrogacy?* Ann N Y Acad Sci **1221**, 47–53.
256 Correspondence from Fisher, E.

3.6.1 Human external appearance

Studies involving the transplantation of human skin onto animals are undertaken for several research purposes. Exposure of human skin to radiation (such as ultraviolet B in sunlight) can lead to DNA damage and skin cancer, and mouse models have been developed for use in research to understand the mechanistic basis of cancers of this kind. Human skin with different pigmentation types, and skin from cancer-prone patients with the disease *Xeroderma pigmentosum*, have been transplanted onto immune-deficient mice, allowing the effects of radiation exposure to be investigated, and potential therapeutics tested.[257]

Mice are used in the study of psoriasis, a human skin condition that results in the development of scaly, red patches on the skin. Some forms of psoriasis result from disorders of the immune system, and mice transplanted with skin grafts from psoriatic patients have been used to understand the malfunctioning relationship between the epidermal (skin) cells and the immune system.[258] Mice with human skin grafts have also been used to improve grafting techniques (e.g. for use with burns patients) and to investigate approaches to reduce the immune rejection of skin grafts.[259] In such studies only a small area of human skin is grafted onto the recipient mice.

Transgenic animal models are contributing to understanding of the genetic basis of limb development. For example, the *Prx1* gene codes for a DNA-regulating protein important for the growth of limb bones. The regulatory sequences affecting *Prx1* expression are now known in several species, including the mouse and bat. In studies to investigate their function, the mouse regulatory sequences were exchanged for the equivalent regulatory regions from the bat. The resulting transgenic mice had elongated forelimbs.[260] Mutation of a human gene regulatory sequence results in the development of extra digits ('pre-axial polydactyly'). Mice carrying the same genetic mutation are also born with extra digits. The mice have contributed significantly to the identification of the developmental basis of this condition, which is now known to result from extra expression of a protein in a small patch on one side of the developing limb.[261] Animal and human limbs are composed of similar cell types making similar proteins; the different shapes of human and animal limbs presumably reflect differences in gene regulation. Testing this could in theory lead to transgenic animals with human-like hands or feet. This could give basic understanding that has clinical importance, but it may cause some disquiet and it would, of course, have consequences for the animals, including mismatch between hard-wired behavioural patterns and what the new limbs can do.

In future, genes underlying the development of other body parts, perhaps including facial features, may also be studied in animals. Such experiments may require consideration from both socio-ethical and animal welfare perspectives. An animal may be distressed by an unusual body part, may suffer rejection by its own species, or elicit unusual response from those charged with its care (see 4.1.2). Attempts should be made to anticipate such effects, in the design and licensing of the work.

Recognisable fragments of teeth, hair and other tissues can sometimes arise in naturally occurring tumours, known as 'teratomas', which are occasionally found in humans and other species. They arise from remnants of very early stem cells, capable of differentiating into different body tissues. Similar teratomas are often created in stem cell research (see 3.3.1), when human or other embryonic stem cells are implanted into mice, for example to test the cells' developmental potential, resulting in the presence of, for example, fragments of human tooth or hair (within the tumour) in the mouse.[262]

257 Sun XZ, *et al*. (2008). *Animal models of xeroderma pigmentosum*. Adv Exp Med Biol **637**, 152–60.
258 Guerrero-Aspizua S, *et al*. (2010). *Development of a bioengineered skin-humanized mouse model for psoriasis: dissecting epidermal-lymphocyte interacting pathways*. Am J Pathol **177**, 3112–24.
259 Issa F, *et al*. (2010). *Ex vivo-expanded human regulatory T cells prevent the rejection of skin allografts in a humanized mouse model*. Transplantation **90**, 1321–7.
260 Cretekos CJ, *et al*. (2008). *Regulatory divergence modifies limb length between mammals*. Genes Dev **22**, 141–51.
261 Lettice LA, *et al*. (2008). *Point mutations in a distant sonic hedgehog cis-regulator generate a variable regulatory output responsible for preaxial polydactyly*. Hum Mol Genet **17**, 978–85.

Box 3.12 The Vacanti ear-mouse

The 'Vacanti ear-mouse', which *appeared* to have a human ear grown on its back, was created in 1997. The mouse was created to demonstrate a method of fabricating cartilage structures for transplantation into human patients. The 'ear' was actually a cartilage structure, grown by seeding cow cartilage cells into a biodegradable, ear-shaped, polyester fabric mould, which was then implanted under the mouse's skin. Although the Vacanti mouse did not contain any human tissue, and was not functional, its human-like appearance evoked a strong public interest and is still widely remembered today (erroneously) as an example of an animal containing a human organ. [262,263]

3.6.2 Human behavioural traits

It is hard to argue that many behavioural traits are individually unique to humans, although large brains and manual dexterity allow us to generate sophisticated tools, which then influence aspects of our behaviour, such as writing and reading, playing and appreciating music, and playing sports.

Recent research to investigate language-related disorders identified a mutation in a gene (known as *FOXP2*), which was found to be associated with an inherited form of speech and language disorder in humans. The *FOXP2* sequence was found to be different between humans and Great Apes (and other mammals), leading to the suggestion that these changes may be partly responsible for the acquisition of speech during human evolution.[263] Furthermore, when the human equivalent sequences were introduced into mice, they developed vocalisations different from those of non-modified mice.[264,265] These studies provide some evidence to suggest roles for genes such as *FOXP2* in the processes underpinning speech and language development.

However, it is important to distinguish vocalisation (making sound) from speech and language (the complex human system of communication). Parrots are capable of complex vocalisation and 'mimickry'. The capacity to make sounds is not the same as the possession of language, which involves many cognitive processes (e.g. memory symbolisation, a shared communicative structure of signs and a process of learning in interaction with adults at crucial developmental stages). Evidence for true language acquisition, even in higher NHP species such as the chimpanzee, is controversial and inconclusive (see Box 3.6). It is likely that more genes underpinning speech development will be identified in future. However, even if all the genes underlying these processes could be introduced into an NHP, it remains a matter of speculation whether the brain of the modified animal would then be capable of language acquisition. Although in some studies carefully trained NHPs have developed some aspects of communication (see Box 3.6), is it not clear that even a modified NHP brain would have the capacity for complex human communication in its true sense.[266]

Creating characteristics such as speech and behaviour in animals would be very complex, probably requiring manipulation of environmental as well as biological factors. Authorisation of such work would need to be justified by considerable potential benefit and the lack of satisfactory alternative research strategies. Measures to determine and respond to public sensitivity should be considered before licensing such research.

262 Cao Y et al. (1997). *Transplantation of chondrocytes utilizing a polymer-cell construct to produce tissue-engineered cartilage in the shape of a human ear.* Plast Reconstr Surg **100(2)**, 297-302.
263 Hardin J (1998). *Producing tissue-engineered cartilage in the shape of a human ear.* Plast Reconstr Surg **101(6)**, 1745.
264 Reimers-Kipping S, *et al.* (2011). *Humanized Foxp2 specifically affects cortico-basal ganglia circuits*. Neuroscience **175**, 75–84.
265 Enard W (2011). *FOXP2 and the role of cortico-basal ganglia circuits in speech and language evolution*. Curr Opin Neurobiol. *In press.*
266 Human communication conveys meaning and intent, which requires a concept of the mental state of others to whom you are communicating. There are reported instances of human children who have not developed language. Such findings suggest that, although the capacity for human language might have a biological basis, its realisation depends on immersion in complex human communities from birth. For example,

see 'Genie' – http://en.wikipedia.org/wiki/Genie_(feral_child) and 'Oxana' http://www.mymultiplesclerosis.co.uk/misc/feral-children.html.

4 Welfare and safety aspects of ACHM

4.1 Welfare

The protection of animals is central to the operation of the UK's Animals (Scientific Procedures) Act 1986 (ASPA), which is intended to ensure that animals used in research are not subject to unnecessary pain, suffering, distress or lasting harm (see 6.2.1, and for a wider international perspective see 7.3.1).[267] Under ASPA, all experiments involving 'protected' animals must be licensed, and they can be licensed only if there are no scientifically suitable alternatives that *replace* animal use, *reduce* the number of animals needed or *refine* the procedures used to cause less suffering (principles known as the '3Rs' see 6.2.1).[268] Decisions to license research must take into account the likely benefits (to humans, other animals or the environment), weighed against the likely welfare costs to the animals involved.[269] Additional requirements apply to particular research, such as that involving genetically altered animals or species including NHPs (see Box 6.1).[270] This long-standing framework underpins the close governance of animal research in the UK, which is more carefully scrutinised than other uses of animals such as in agriculture, or as companion animals (pets). (See Box 4.1 for public views on animal welfare.)

Application of animal welfare principles is an obligation on individuals and institutions under the Home Office licensing system, and is monitored both locally, for example by 'named animal care and welfare officers', and by the Home Office inspectorate. Further improvements are encouraged and taken forward in the UK through the work of the National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs), and other bodies.[271] An important aspect of such work is the development of guidelines for best practice, in areas such as welfare assessment of genetically modified rodents, and in defining the welfare needs of particular animal species.[272] **We emphasise that research involving ACHM should be subject to scrutiny, and advancement from the perspective of animal welfare, in a manner no different from other animal research.**

Here we introduce two aspects of animal welfare relating *specifically* to ACHM:

- The possibility that the creation or use of ACHM raises specific welfare concerns.
- The potential of ACHM research to contribute to advancement of the 3Rs (replacement, reduction and refinement, see above).

Further consideration is included in Chapter 5 (5.5).

4.1.1 ACHM and animal welfare

In principle, the use of ACHM that closely model human biology increases the likely benefit of the research and so contributes to the refinement of experimental techniques. ACHM use can support animal welfare principles by enabling researchers to use species likely to experience less pain, suffering or harm, or to reduce the numbers of animals used in some experimental situations.[273]

267 New legislation, intended in part to bring harmonisation in animal welfare practices across Europe, has recently been adopted (see 7.3.1).
268 See Guidance on the Operation of the Animals (Scientific Procedures) Act 1986, Section 2.3. http://www.archive.official-documents.co.uk/document/hoc/321/321.htm
269 Animal Procedures Committee (2003). *Review of the cost-benefit assessment in the use of animals in research.* http://www.homeoffice.gov.uk/publications/agencies-public-bodies/apc/key-reports/
270 Research involving NHPs is only permissible where there is strong scientific justification, and where no other species are suitable for the purposes of the programme of work, or where it is not practicable to obtain animals of any other species that are suitable for those purposes. See Guidance on the Operation of the Animals (Scientific Procedures) Act 1986, Section 5.22.
271 The National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs) is an independent scientific organisation, tasked by Government with supporting the UK science base through the application of the 3Rs. See www.nc3rs.org.uk
272 For example see, 'Mouse Welfare Terms' http://www.mousewelfareterms.org/doku.php?id=home developed by the Medical Research Council Harwell and Wellcome Trust Sanger Institute Cambridge; Wells *et al. (2006) Full report of GA mouse welfare assessment working group.* Lab Animals **40,**111–114; Ellegaard L, *et al.* (2010). *Welfare of the minipig with special reference to use in regulatory toxicology studies.* J Pharmacol Toxicol Methods **62,** 167–83; RETHINK http://www.rethink-eu.dk/index.php?page=one&id=8
273 Use of a lower species (phylogenetic reduction) is often considered to be refinement, but such a judgement can only be made if assessment of the available scientific evidence suggests that the lower species is less sentient/likely to suffer less. See http://www.nc3rs.org.uk/category.asp?catID=78

Research involving ACHM, particularly mice with humanised organs such as the liver, or the immune system, can be used as an alternative to NHPs in investigating infectious diseases. For example, they have facilitated studies of HIV and hepatitis, to which unmodified mice are not susceptible, and for which NHPs have previously been used (see 2.3.3). ACHM approaches also have potential application in drug development and testing; for example the use of transgenic mice susceptible to polio virus through incorporation of the human *CD155* gene has been approved as an alternative to NHP use in polio vaccine testing.[274] A protocol developed using a chimæric mouse model which recapitulates forms of human cancer facilitates a significant reduction in the number of mice used compared with previous approaches. This is due to the more faithful development of cancer in the chimæric model, and elimination of the need for breeding programmes.[275] The recent increase in the development of antibody therapies (see 2.3.2) has resulted in an increase of the use of NHPs in toxicity testing of these therapeutics. This is an important avenue for future study in which mice with humanised immune systems may reduce (though not fully replace) NHP use. For other types of human condition, for example those affecting cognitive abilities, it may be that NHP models incorporating human material are so much better than similar rodent models that they will allow an overall reduction and refinement, and lead more rapidly to treatments. An example where this might be the case (although no treatments are yet available) is Huntington's disease.[276]

We anticipate that the use of animals containing human material is likely to present further avenues for advancement of the 3Rs. We support their development and use, while emphasising the view put forward in evidence that '*… the development of an 'improved' model needs to be followed by a rigorous and critical appraisal of the value of existing models by research funders, scientists and regulators.*'[277] However, we do not anticipate that research involving ACHM would decrease the *overall use* of animals in medical research in the short term, in part because the development of ACHM will open up new research avenues.

4.1.2 Specific welfare concerns

We have considered whether the incorporation of human genetic or cellular material into an animal might in itself have the potential to cause a distinct dimension of 'pain, suffering or lasting harm' to the animal involved. Our general conclusion is that, although individual experiments may give rise to particular types of animal suffering, the techniques in themselves do not raise distinct types of animal welfare concern.

Social aspects of animal welfare are increasingly taken into account, and research animals are housed in appropriate and species-specific environments, which often involve 'group-housing' (e.g. of NHPs). We considered whether, by conferring a human characteristic onto an animal (such as appearance, e.g. through engraftment of human skin (see 3.6), or a behavioural trait) an animal might suffer distress or harm, resulting from the actions of others of its own species, or those responsible for its care. Although the potential for suffering brought about in this way is plausible, it does not represent a 'unique' dimension of suffering that is specific to the creation of ACHM, because similar situations can arise (and need to be taken into account in assessing welfare issues) in other types of research. Evidence submitted to the study indicated that there is: '*no rationale,* specifically on animal welfare grounds, *for moving to regulate this type of research differently from other animal research*' and that '*research involving ACHM is not significantly different to other areas of animal research from an animal*

274 Humans and primates express a protein (CD155) on their neurons which confers susceptibility to infection by the polio virus. Batches of live polio vaccine for human use cannot be tested to determine their activity (virulence) on species that lack the CD155 protein (including mice) and are therefore tested on NHPs. See Shultz LD, *et al.* (2007). *Humanized mice in translational biomedical research.* Nat Rev Immunol **7**, 118–30; Mendelsohn CL, *et al.* (1989). *Cellular receptor for poliovirus: molecular cloning, nucleotide sequence, and expression of a new member of the immunoglobulin superfamily.* Cell **56**, 855–65; Dragunsky EM, *et al.* (2006). *Further development of a new transgenic mouse test for the evaluation of the immunogenicity and protective properties of inactivated poliovirus vaccine.* J Infect Dis **194**, 804–7.

275 Zhou Y, *et al.* (2010). *Chimeric mouse tumor models reveal differences in pathway activation between ERBB family- and KRAS-dependent lung adenocarcinomas.* Nat Biotechnol **28**, 71–8.

276 Yang SH & Chan AW (2011). *Transgenic Animal Models of Huntington's Disease.* Curr Top Behav Neurosci **7**, 61–85.

277 Written evidence from the Royal Society for the Protection of Cruelty to Animals (RSPCA).

welfare perspective.'[278,279] Although we do not currently see any reason for this aspect of animal welfare to be treated differently in ACHM experiments compared with other animal experimentation, this matter should be kept under review as techniques evolve.

We considered whether, through incorporation of human neurons into its brain, an animal might in some way be made more 'self-aware' and therefore capable of experiencing a greater degree of suffering (see 3.4.1 and 5.6.2). The same issues would potentially apply to any situation where neural cells from a more self-aware species are introduced into one that is less self-aware, such as chimpanzee into macaque, or macaque into marmoset. However, as humans are probably the most self-aware species (at least we like to think so), then ACHM pose the greatest risk of this happening. We are not aware of any evidence that self-awareness has been altered in such experiments, but researchers and regulators should be aware of the possibilities.

The effect of animal experimentation on those directly responsible for the day-to-day care of research animals is often underestimated.[280,281] Although ACHM in general are unlikely to pose additional concerns in this respect, it is conceivable that some individual carers might react differently to animals containing large amounts of human material, or with altered appearance or behaviour, whether or not the animals were actually more 'human-like'. There could be positive or negative effects on either the animals or their carers. This is a topic that could be explored, especially as there is an increasing tendency for animal technicians to become more directly involved in the design and interpretation of experiments.

Box 4.1 Public concern for animal welfare

The views of participants in the public dialogue on animal welfare emerged in several ways. Although the dialogue was not intended to explore attitudes to the general use of animals in research, animal welfare concerns were consistently expressed, and participants often transferred broad concerns for the welfare of research animals directly onto research using ACHM.

Overall, as described in Box 3.1, participants expressed conditional support for ACHM. Animal welfare was one of the considerations which they took into account when thinking about whether such research would be justified. (See Box 5.1 for more discussion of these considerations.)

In the quantitative survey, animal welfare was the reason most often given by those who found introducing human material into animals unacceptable. When participants were asked about the welfare aspects relating specifically to ACHM, there were a few suggestions that a new kind of suffering might result from the creation of ACHM. These included concerns that modifying an animal's external organs to cause them to appear human in some way might cause the animal distress, or that research involving the brain might alter an animal's perception of its own circumstances and so increase its suffering. However, for the most part, participants did not feel that the creation of ACHM would produce greater suffering than other types of research involving animals.

'It's a great deal of suffering. The fact that it has human material makes no difference really.'

This concerned but fundamentally supportive view of animal experimentation, if carried out for medical advancement, is in agreement with recent trends in public polling on the topic – see *The 2010 Ipsos MORI Report on Public Attitudes towards Animal Experimentation.*[282]

278 Oral evidence from the Royal Society for the Protection of Cruelty to Animals (RSPCA).
279 Oral evidence from Robinson V., National Centre for the Replacement, Refinement and Reduction of Animals in Research
280 Herzog H (2002). *Ethical aspects of relationships between humans and research animals.* ILAR J **43**, 27–32.
281 Coleman K (2011). *Caring for nonhuman primates in biomedical research facilities: scientific, moral and emotional considerations.* Am J Primatol 73, 220–5.
282 Ipsos MORI (2011). *Views on Animal Experimentation (BIS research) Alternatives to Animal Experimentation (NC3R research).* http://www.ipsos-mori.com/researchspecialisms/socialresearch/specareas/nhspublichealth/attitudestowardsanimalexperimentation.aspx

4.2 Safety

4.2.1 Introduction

Research involving ACHM is subject to safety controls that apply to all biomedical research. These include practices set out in legislation and guidance to protect against hazards to human health and to the environment (e.g. principles of occupational safety and hygiene, and good laboratory practice).[283] Further precautions apply to studies involving genetically altered animals, relating to their containment or deliberate release into the environment (see 6.2.4).

We are grateful for the advice of experts outside the working group, which has aided us in this consideration.[284]

4.2.2 Safety issues considered

Some ACHM experiments will raise safety issues related to the individual experiments being proposed, for example those where animals are made susceptible to infectious agents normally confined to humans, including viruses, bacteria, parasites and prions (see 2.3.3).[285] Such hazards must be considered and managed in order to protect those handling the animals, the animals themselves (from inadvertent infection) and the public, notably from infection or the escape of animals which might act as a reservoir of infection. Neither the issues nor the methods of managing them are different in ACHM experiments from those regularly encountered when dealing with other types of experiment involving infectious agents or other biohazards in animals or cell cultures. For example, ferrets are susceptible to human influenza viruses, but they are routinely used to study the viruses in facilities with a high level of containment. Similarly, how work with human cells or tissue *in vitro* is conducted will depend on the nature of any hazards that might be generated by the experiments proposed, but the minimum conditions used are those that protect both the cells and the researchers from adventitious infection. All such experiments should be assessed in advance for potential risk by researchers and regulators, and managed accordingly; exactly the same considerations apply to ACHM work.

We considered whether there are additional, generic safety issues applicable to research involving ACHM. The major potential issues identified arise from the fact that complex genomes (both human and animal) carry within them integrated viral genomes (endogenous retroviruses or proviruses). These may be quiescent and only able to replicate under certain conditions; indeed many are inactivated because during evolution either the viruses have lost an essential component that enables them to replicate satisfactorily, or the host has lost cell-surface receptors or intracellular factors essential for viral entry and infection of new cells. Specific intracellular host factors (known as restriction factors) can also produce resistance to infectious agents such as viruses; these are species specific and can confer species resistance to particular infectious agents such as HIV.[286] This is a further barrier to cross-species transmission of infectious agents. It is, however, known that infectious agents can, occasionally, change their host specificity (e.g. owing to mutation and/or recombination with other related viruses) such that they become able to infect species previously not liable to infection. Such events are likely to have been involved in influenza strains moving from birds to humans, or from pigs to humans (swine 'flu). HIV might have moved into humans from NHP hosts by similar mechanisms in the wild. We therefore considered whether ACHM experiments could lead to an increased likelihood of reactivation of quiescent viruses or to changes of host specificity of infectious agents.

283 See Health and Safety Executive. *The Scientific Advisory Committee on Genetic Modification (SACGM) Compendium of guidance,* Part 3 (Containment and control of activities involving genetically modified microorganisms). http://www.hse.gov.uk/biosafety/gmo/acgm/acgmcomp/index.htm; Organisation for Economic Co-operation and Development (OECD) Principles on Good Laboratory Practice http://www.oecd.org/officialdocuments/displaydocumentpdf/?cote=env/mc/chem(98)17&doclanguage=en

284 Correspondence from Lever A., Stoye J., Bradley A., Weiss R., and Weissmann C.

285 A prion is an infectious agent composed of protein in a misfolded form.

286 Lever AM & Jeang KT (2011). *Insights into cellular factors that regulate HIV-1 replication in human cells.* Biochemistry **50**, 920–31.

4.2.3 Inter-species chimæras

Making chimæras involves mixing cells or tissues containing whole genomes (including their integrated viruses) of different species. We considered whether such an intimate admixture of human and animal cells and tissues might lead to reactivation of infectious particles, such as retroviruses or other pathogens; or to alter their host specificity so that they become infectious to humans. Very similar issues have been extensively discussed in the debate around the transplanting of animal tissues into humans, referred to as xenotransplantation.

Humans and animals have lived in close proximity for a long time, and although examples of viral transfer between human and other species are well known, they are relatively infrequent.[287] Animal tissues have been introduced into people (e.g. pig heart valve transplants, baboon hearts), although in small numbers. What limited evidence is available on humans who have received living pig cells indicates that no infection by porcine viruses has taken place.[288,289,290] A number of studies have, however, shown transient appearances of foreign virus in humans or animals who had received cellular material from other species, suggesting that this issue must be kept under careful review.[291,292,293,294] Any move from experimental into clinical systems will, as with any new therapy, need very careful assessment of safety including infectious risk.

There have been years of experience, and large numbers of experiments, grafting human tissues such as tumours into other species. Human tumour tissue transplanted into immunodeficient mice is known to become infected by endogenous mouse retrovirus. We know of no proven incidents of transmitted infectious events hazardous to man.[295] Mice with human immune systems or mostly human livers have been produced for studying specific infections, and have therefore been closely monitored. To our knowledge there have been no reports that these animals have developed any problems due to activation of proviruses or to novel infections.

Inter-specific cell hybrids involve an even closer association of cells than is generally the case in chimæras because they involve the fusion of whole cells, which can be from a range of species including animal–human combinations. These have been cultured in laboratories for decades, without any generic safety issues of this sort arising.

Chimæras that comprise a mixture of many different cell types, both human and animal, may possibly pose a slightly greater risk than the examples above. This is partly because specific molecules on the cell surface (referred to as receptors) to which viruses and other pathogens attach are often cell-type specific (e.g. influenza viruses tend to infect cells lining the upper respiratory tract, other pathogens target cells in the gut). The greater the range of cell types present from the two species, the greater the chance of any virus finding its appropriate receptor. Moreover, cell fusion does occur naturally in some tissues, such as placenta and muscle, so that inter-species chimæras may also contain inter-specific cell hybrids, increasing the chance of viral recombination events.

Factors relating to the animal host may affect the probability of an adverse event occurring. For example, the longer cell types from two species co-exist the more opportunity there may be for rare events to occur, so that chimæras with a long life span may deserve

287 For example, see Shukla P, *et al.* (2011). *Cross-species infections of cultured cells by hepatitis E virus and discovery of an infectious virus–host recombinant.* Proc Natl Acad Sci USA **108**, 2438–43.
288 Ekser B, *et al.* (2009). *Xenotransplantation of solid organs in the pig-to-primate model.* Transpl Immunol **21**, 87–92.
289 Paradis K, *et al.* (1999). *Search for cross-species transmission of porcine endogenous retrovirus in patients treated with living pig tissue. The XEN 111 Study Group.* Science **285**, 1236–41.
290 Weiss RA (1998). *Transgenic pigs and virus adaptation.* Nature **391**, 327–8.
291 Teotia SS, *et al.* (2005). *Prevention, detection, and management of early bacterial and fungal infections in a preclinical cardiac xenotransplantation model that achieves prolonged survival.* Xenotransplantation **12**, 127–33.
292 Michaels MG, *et al.* (2004). *Baboon bone-marrow xenotransplant in a patient with advanced HIV disease: case report and 8-year follow-up.* Transplantation **78**, 1582–9.
293 Michaels MG, *et al.* (2001). *Detection of infectious baboon cytomegalovirus after baboon-to-human liver xenotransplantation.* J Virol **75**, 2825–8.
294 Stoye JP & Coffin JM (1995). *The dangers of xenotransplantation.* Nat Med **1**, 1100.
295 We are aware of claims that some cases of prostate cancer and myalgic encephalopathy have been associated with murine derived retrovirus. These claims remain scientifically contentious and it is not clear that, even if true, they are related to ACHM. See Urisman A, *et al.* (2006). *Identification of a novel Gammaretrovirus in prostate tumors of patients homozygous for R462Q RNASEL variant.* PLoS Pathog **2**, e25; Hue S, *et al.* (2010). *Disease-associated XMRV sequences are consistent with laboratory contamination.* Retrovirology **7**, 111.

closer attention, especially as aged animals can show reduced immune function. Such events might be more likely to occur in animals that are immune deficient.

It is conceivable that human cells isolated from animal–human chimæras and grown in the laboratory might have acquired replication-competent retroviruses from the animal host. Such animal viruses do not usually cause problems to humans when they are made by animal cells, because they have animal-type coat modifications (alpha-gal epitopes) that would lead to them being detected and destroyed or severely damaged by anti-alpha-gal antibodies that are present in humans. However, if the viruses had moved from the animal to the human cells within a chimæric animal and these human cells were then isolated and grown in culture, the viruses could be competent to infect human cells.[296,297,298]

Any future attempts to use material derived from chimæric animals for therapeutic purposes would need to be very carefully assessed for safety (as is the case with any proposed new therapeutic) and particularly for risk of viral transmission.

Researchers studying pathogens are more likely to consider the infection risks than those who do not. It follows that there needs to be some general awareness of potential infection risks when chimæric animals have been modified in a way that may make them susceptible to human pathogens, but where the study of the latter is not the primary purpose. For example, human respiratory tract cells introduced into animals to study disease such as cystic fibrosis may be susceptible to strains of influenza that could be passed to them by humans, and subsequently passed back. Transmission directly between humans during an epidemic is more likely, but the animals would also need protection.

On balance, we consider the overall risk of an event of this type to be small, though not zero. The types of risk are, however, not unique to ACHM and there are well established methods for risk management. It is important that researchers and regulators bear these risks in mind, particularly when contemplating novel classes of experiment, and act appropriately to manage any possible hazards.

4.2.4 Transgenic and genetically altered animals

Transgenic experiments in which unusually large amounts of genomic material (such as a whole chromosome) are transferred between species (see 3.2) raise similar issues as chimaeras, as it will be difficult to know *a priori* whether the sequences contain proviruses that could be activated or genes that are critical for pathogen infection. However, the great majority of transgenic experiments do not raise these issues because the transfer of one or a few specific known gene sequences should not lead to transfer of viral sequences into an unusual environment, unless it is part of the experimental design.

Modification of cell surfaces can produce or modify viral or other pathogen receptors, leading animal (or human) cells to alter their 'tropism' (ability to be infected by the pathogen).[299] This approach has been used deliberately to develop animals expressing specific human receptors, to study human-specific viruses and infectious agents (see 2.3.3). For example, transgenic mice have been made that express the human cell-surface receptor for polio virus, so that the modified mice become susceptible.[300] Mice susceptible to hepatitis virus have also been developed (see 3.3), and a similar approach for the study of HIV is under investigation.[301]

296 Hara K, *et al.* (2008). *Neural progenitor NT2N cell lines from teratocarcinoma for transplantation therapy in stroke.* Prog Neurobiol **85**, 318–34.
297 Newman MB, *et al.* (2005). *Tumorigenicity issues of embryonic carcinoma-derived stem cells: relevance to surgical trials using NT2 and hNT neural cells.* Stem Cells Dev **14**, 29–43.
298 Nelson PT, *et al.* (2002). *Clonal human (hNT) neuron grafts for stroke therapy: neuropathology in a patient 27 months after implantation.* Am J Pathol **160**, 1201–6.
299 Tissue tropism is a term used in virology to define the cells and tissues of a host which support growth of a particular virus. Bacteria and other parasites may also be referred to as having a tissue tropism.
300 Ren RB, *et al.* (1990). *Transgenic mice expressing a human poliovirus receptor: a new model for poliomyelitis.* Cell **63**, 353–62; Koike S, *et al.* (1994). *Characterization of three different transgenic mouse lines that carry human poliovirus receptor gene-–—influence of the transgene expression on pathogenesis.* Arch Virol **139**, 351–63; Dragunsky E, *et al.* (2003). *Transgenic mice as an alternative to monkeys for neurovirulence testing of live oral poliovirus vaccine: validation by a WHO collaborative study.* Bull World Health Organ **81**, 251–60.
301 Shultz LD, *et al.* (2007). *Humanized mice in translational biomedical research.* Nat Rev Immunol **7**, 118–30.

We have described the generation of strains of mice with humanised immune systems (2.3.3) and have considered whether these systems may allow rodent viruses to become selected for the ability to escape human immune systems, and so encourage their ability to cross species barriers. Expert consensus is that this is an extremely unlikely scenario. All such mouse strains should in any event be kept in appropriate containment.

Virus inactivation can occur by the same mechanism as the hyperacute rejection of xenografts.[302] Lysis of animal retroviruses is triggered by the binding of human anti-alpha-gal antibodies to alpha-gal epitopes expressed on the viral envelope (outer shell of the virus). Virus grown *in vitro* in non-primate cells is inactivated by human blood serum, but the same virus cultured in human cells is not. This is because the virus makes its envelope by budding out from the cells it grows in – only when alpha-gal is present on the host cells is the viral envelope sensitive to antibody-dependent, complement-mediated lysis by components of human serum. It follows that modifications to the alpha-gal system to make pig xenografts resistant to hyperacute rejection may make enveloped pig viruses resistant to destruction by humans.[303,304] Two of the three complement regulatory proteins are also receptors for human viral pathogens: CD46 is a cell-surface receptor for measles virus, and CD55 can serve as a binding receptor for Echo and Coxsackie B picornaviruses.[305] Transgenic animals expressing human CD46 and CD55 would therefore be vulnerable to infection from humans with these viruses (this is a welfare concern for the animals), but a greater concern is that such transgenic animals may increase the opportunities for animal viruses to adapt to a human host range. For example, in transgenic pigs that express both pig and human forms of the CD55, picornaviruses that use the porcine CD55 equivalent might readily adapt to recognise human CD55. These viruses would be pre-adapted for transmission to a xenograft recipient, and for human–human transmission.

Where the genes under manipulation carry any risk of modifying viral receptors or aspects of the intracellular environment in a way that risks affecting endogenous pathogens, the same precautions are required as in other experiments involving potentially infectious agents: prior risk assessment and appropriate risk management, including containment strategies. In our view, provided proper vigilance is exercised in the design and licensing of relevant ACHM experiments, current knowledge makes it unlikely that important safety issues of this sort would arise accidentally. These considerations only apply to a small minority of ACHM experiments, but it is very important that proper vigilance is maintained in the design and regulation of these experiments.

In summary, although the use of humanised animals could theoretically lead to adaptation or recombination of viruses, we concur with broader guidance that such risk is low:

'*… if an animal line was produced which was modified to contain a receptor for a human virus, these animals may act as a novel reservoir for human disease. Although the possibility of such additional hazards to humans must always be considered, it is recognised that, in most cases, the activities will not pose any extra hazards to humans.*'[306]

We also consider that any risk to other animals (especially those outside any research facility) is very low.

Any manipulation that is known to, or could, alter viral or other pathogen recognition sites, or in any other way affect susceptibility to pathogens or

302 Magre S, *et al.* (2004). *Reduced sensitivity to human serum inactivation of enveloped viruses produced by pig cells transgenic for human CD55 or deficient for the galactosyl-alpha(1-3) galactosyl epitope.* J Virol **78**, 5812–9. In this study amphotropic murine leukaemia virus, porcine endogenous retrovirus, and vesicular stomatitis virus were tested.
303 Destruction in this context refers specifically to antibody-dependent, complement-mediated lysis of enveloped virus particles.
304 Weiss RA (1998). *Transgenic pigs and virus adaptation.* Nature **391**, 327–8.
305 *Ibid.*
306 See Health and Safety Executive. *The Scientific Advisory Committee on Genetic Modification (SACGM) Compendium of guidance,* Part 5 (Genetic modification of animals), Clause 38. http://www.hse.gov.uk/biosafety/gmo/acgm/acgmcomp/index.htm

that deliberately involves the activation of human and animal proviruses within the same ACHM (such that they could recombine) should be carefully risk-assessed by researchers and regulators and appropriate control mechanisms should be put in place (see 8.5).[307]

4.2.5 Accidental or deliberate release

We have considered potential issues relating to the accidental or deliberate release of ACHM into the environment. Accidental release would be mainly relevant to animals that are less easily contained, such as rodents, those with small free-living eggs or larval forms, or those with flight.[308] The release of large or non-endemic animals would be more apparent and recapture more likely.

Chimæric animals containing human cells are very unlikely to pose any specific hazard, unless they are also infected with an animal or human pathogen as part of a research programme or are very likely to pick up such a pathogen in the wild. We do not consider such ACHM to pose risks different from conventionally infected animals used in research.

Animals containing human DNA sequence may transmit these modifications to offspring. However, there are well-established protocols for containing genetically modified animals, which would equally apply to ACHM (see 6.2.4). Competition to breed outside a contained environment is usually high and evidence suggests that laboratory strains are less able to compete and breed in the wild.[309] If there was concern around a specific human DNA alteration, and a risk of interbreeding in the wild, then inclusion of a genetic alteration to prevent survival or fertility should also be considered in designing and reviewing the experimental protocol.[310]

Good practice requires that ACHM should be kept under appropriate containment, and any deliberate release should only be contemplated after full risk assessment, and with appropriate regulatory permission (see 6.2.4).

4.2.6 Other considerations

ACHM and the food chain

We have considered whether it is feasible that ACHM may be consumed by other organisms (by intention, or accident) and whether there may be safety concerns associated with ACHM entering the animal or human food chain. For example, the possibility of genetically engineering cows to express human milk proteins has been considered and some progress reported.[311,312]

There are general arguments related to the use of genetically altered animals in agriculture, beyond the scope of the current study (see 1.1), which we do not replicate here. As a specific subset of such animals, it is not evident that the consumption of animals (e.g. sheep or goats) carrying human DNA would merit concern from a safety perspective above that of genetically modified animals in general, unless the particular genetic modification itself created a hazard. We therefore see no additional considerations that should be applied to such animals, except in limited cases that relate to the specific modifications involved.[313]

Although we have considered only safety issues in this section, we stress that deliberate introduction of any such materials into the human food chain could only be contemplated after full public discussion of all the issues involved; and with appropriate evaluation and authorisation under the relevant European frameworks for genetically modified and novel foods. These are administered in the UK by the Food Standards Agency and enforced by local authorities.[314]

307 It is critical that the provenance of human material to be used clinically is known and considered during the risk assessment.
308 Such as aquatic species including *Ciona* (sea squirt), fish and frogs, insects (e.g. *Drosophila*) and birds.
309 See Meagher S, *et al.* (2000). *Male-male competition magnifies inbreeding depression in wild house mice.* Proc Natl Acad Sci USA **97**, 3324–9; Jimenez JA, *et al.* (1994). *An experimental study of inbreeding depression in a natural habitat.* Science **266**, 271–3.
310 For example, the animal could be modified to become dependent on administration of a drug.
311 Wang J, *et al.* (2008). *Expression and characterization of bioactive recombinant human alpha-lactalbumin in the milk of transgenic cloned cows.* J Dairy Sci **91**, 4466–76.
312 Yang B, *et al.* (2011). *Characterization of bioactive recombinant human lysozyme expressed in milk of cloned transgenic cattle.* PLoS One **6**, e17593.
313 For example, animals that have been modified to render them susceptible to carry human pathogens, or human prions, would require very stringent control.
314 There are two pieces of relevant European legislation in this area: Regulation (EC) No 1829/2003 on genetically modified food and feed, which would apply to genetically altered animals, and Regulation (EC) No 258/97 concerning novel foods and novel food ingredients, which would apply to chimæras.

Biological weapons
ACHM could, in theory, be applied to the development of biological weapons or to development of antidotes or countermeasures, but it is not obvious that it creates important novel hazards, nor do we see that it raises concepts that have not already been covered elsewhere in this report.

Concerns about the safety of ACHM raised by participants in the public dialogue are set out in Box 4.2.

Box 4.2 Public concerns about the safety of the use of ACHM

Participants' safety concerns around ACHM fell into two categories: immediate and future risks.

Immediate risks related to unintended release of modified animals and the consequences for humans, animals or the environment. Concerns included:

- Triggering disease epidemics (some participants related this to the origin of HIV through human-primate contact).
- 'Contamination' of the food chain.
- Permanent alteration or loss of existing species due to breeding with released animals.
- Unpredicted impacts of modified animals on existing flora, fauna and the ecosystem.

Future risks concerned events such as the creation of species for terrorism or warfare, which participants felt might ultimately result from the decision to permit certain types of research now (sometimes described as the 'slippery slope' argument).

5 Ethical and social concerns

5.1 Ethical principles and biomedical research

Biomedical research seeks to determine the normal processes of life, to advance the understanding of health, and to identify and develop new methods of promoting health and preventing illness. This research deals with conditions which affect humans and therefore at some stage entails investigations of human subjects; ideally, the research ultimately leads to new interventions that need to be tested on human subjects before they can enter clinical practice. So the involvement of human subjects in medical research is inescapable. But it is also constrained by the rights and interests of the human subjects, and where medical research poses serious risks to humans it is important to minimise these risks by undertaking other kinds of research before research involving human subjects is undertaken.

5.1.1 The contested domain of animal research and our working assumption

The way of pursuing this objective which is under examination here, involves the use of animals which have been modified to contain human genetic or cellular material. It may be objected at once that the acceptability of such research can be challenged on the grounds that all research involving the use of animals is unethical, except where the research involves procedures which benefit the animals involved. We do not attempt to enter directly into these arguments here; for a recent survey of the issues and arguments in this highly contested area, we commend the 2005 report by the Nuffield Council on Bioethics on '*The ethics of research involving animals*'[315] and two previous reports led by the Academy of Medical Sciences '*The use of non-human primates in research'* (2006)[316] and '*Inter-species embryos*' (2007).[317,318] But in Chapter 6 we describe current legislation and practice in the UK under which some types of animal research (such as the use of Great Apes) are not undertaken, and in which use of animals is licensed only where principles such as the 3Rs (see 6.2.1) are followed and it is judged that the potential benefits of the research outweigh the harm done to the animals involved; and we assume here that these practices are broadly acceptable. We recognise that not everyone will agree with this assumption (see Box 5.2); but our aim is to focus specifically on the issues raised by the use of animals which include human genetic or cellular material (ACHM) and these issues are best addressed in the context of present practices.

5.1.2 Three ethical perspectives: utilitarianism, deontology and virtue ethics

Although we start here from the assumption that the use of animals in the course of medical research is morally acceptable where its benefits outweigh the harm done, and thus from a position that in this respect addresses moral questions from a broadly utilitarian perspective, we accept that moral thought often includes 'deontological' duties to others whose basis lies in their status and our relationships with them rather than in the relative value of the consequences of action. So the approach taken here is to be understood to allow for consideration of similar duties to animals which would place limits on the ways in which animals may be used in medical research.[319] We also recognise the importance of the ethical perspective characteristic of virtue ethics, which invites us to reflect on the kind of person we aim to be, in addition to considering the justifications for and defences of the actions we undertake. This perspective is manifest in the ways in which we think about other people; for we do not just evaluate the acceptability of their actions – we also care about their character, their motivations, dispositions and aspirations. This, then, is an ethical perspective which approaches

315 Nuffield Council on Bioethics (2005). *The ethics of research involving animals.* Nuffield Council on Bioethics, London.
316 Weatherall D (2006). *The use of non-human primates in research.* http://www.acmedsci.ac.uk/images/project/nhpdownl.pdf
317 Academy of Medical Sciences (2007). *Inter species embryos.* http://www.acmedsci.ac.uk/p48prid51.html
318 See also Box 5.2 for the views obtained through our public dialogue.
319 For further discussion of 'deontological' considerations of this kind, see Fiester A (2009). *Ethical issues in transgenesis.* In Taupitz J. & Neschka M., Chimbrids. Springer, Berlin.

the moral questions raised by the use of animals in research, not only by reference to the rights and wrongs of the research, but also by reference to what it shows about the character and relationships of those involved in it and of the societies which practice it.

5.2 The significance of the distinction between animals and humans

We begin by reflecting briefly on traditional attitudes to the distinction between humans and animals. Over the last two million years of human history people have been profoundly affected by their encounters and relationships with animals, especially those on which they have come to depend for their way of life and those which threaten it. The distinctions that have been drawn between humans and animals, and among groups of animals, have been central to the values and culture of almost all human societies of which we have records. Some of these distinctions may be arbitrary, such as that between animals which we eat and those we refuse to eat; many just reflect human interests, such as the categorisation of some animals as pets and others as vermin. But the understanding of the relationship between humans and animals always has a special status: in many cultures it defines what it is to be human, informing social rituals and taboos, shaping what humans may do, and determining those to whom special responsibilities are owed by defining the limits of those who are considered human. The fact that the ways this distinction is made may sometimes seem to us to be irrational, unstable or hard to define does not rob it of importance, though it indicates that its significance is often a matter of social practice, and hence of cultural and historical specificity.[320]

5.2.1 The special 'dignity' of man

We use palaeontology and molecular genetics to distinguish between the species to which we belong, *Homo sapiens sapiens,* and other hominid apes; with continuing debate about the status of Neanderthal man and other earlier creatures we see as significantly near-relations of ours. The ethical and symbolic significance of this distinction, and that between humans and animals generally, is normally explained by reference to capacities which are central to our sense of what gives special value to human life, such as the capacity for rationality and self-consciousness, for free will and moral sensibility, or for language and culture. And one term, 'dignity', has come to symbolise the thought that human life has a special value. Kant famously maintained that only humans have the kind of self-conscious rationality which gives them dignity as 'ends-in-themselves' and entitles them to respect from others;[321] and following Kant, 'human dignity' is regularly invoked in declarations and charters of human rights (see 7.4.1).[322]

5.2.2 Challenging the moral boundary between animals and humans

But these explanations, and the boundaries that come with them, can be challenged. In the 19th century Jeremy Bentham argued that it is the capacity for suffering that is of fundamental ethical significance, and that once this is recognised the moral boundary between humans and animals should be erased:

'The day may come, when the rest of the animal creation may acquire those rights which never could have been withholden from them but by the hand of tyranny. … It may come one day to be recognized, that the number of the legs, the villosity of the skin, or the termination of the os sacrum, are reasons equally insufficient for abandoning a sensitive being to the same fate. What else is it that should trace the insuperable line? Is it the faculty of reason, or, perhaps, the faculty of discourse? But a full-grown horse or dog is beyond comparison a more rational, as well as a more conversable animal, than an infant of a day, or a week, or even a month, old. But suppose the case were otherwise, what would it avail? the question is not, Can they reason? nor, Can they talk? but, Can they suffer?'[323]

320 The classic account of this topic is Douglas M (1966). *Purity and danger: an analysis of concepts of pollution and taboo.* Routledge Classics, London.
321 Kant I (1785). *Groundwork of the metaphysics of morals.*
322 See, for example, articles 1 and 2 of the 1997 UNESCO Declaration on the Human Genome and Human Rights. http://www.unesco.org/new/en/social-and-human-sciences/themes/bioethics/human-genome-and-human-rights/
323 See Chapter 17, section IV, note 122 of Bentham J (1823). *Introduction to the principles of morals and legislation.*

Bentham did not convert his contemporaries to his radical point of view. But in our own time Bentham's challenge has been renewed by philosophers such as Peter Singer and Tom Regan, and there is no doubt that through their writings they have managed to broaden support for a Benthamite animal-rights movement.[324] Many important issues are raised here concerning the ways in which animals are viewed and treated in contemporary life, in agriculture, in domestic contexts, in protected natural habitats as well as in the course of medical research; and we recognise the importance of the continuing debates on these issues. As we have indicated earlier in this report we do not seek to enter into these broad debates, our focus is on the question of whether the use of ACHM makes a significant difference to the acceptability of research involving them. But there is one project championed by Singer which merits some attention here, – his 'Great Ape project' which aims to secure a legal status for Great Apes comparable to that of humans.[325] For it is an explicit aim of Singer's project to establish a ban on the use of Great Apes in medical research.

Research involving Great Apes has not in fact been undertaken in the UK in the past 50 years (unlike research on human subjects); nonetheless the issue of a complete ban remains controversial. Opponents of a complete ban such as Colin Blakemore argue that the use of Great Apes for research needs to be retained as an option for cases where there is a pressing medical need involving a serious disease whose control requires research that cannot be carried out in any other way.[326] In this report we accept that there are powerful moral reasons for being very reluctant to use Great Apes for medical research; but we argue that it is reasonable to hope that the issue of a complete ban can be set to one side by the use of other transgenic animals containing human materials (see 4.1). Nonetheless the fundamental issue between animal-rights advocates and their opponents is whether there is a moral boundary between humans and (other) Great Apes. Where Singer's Great Ape Project is explicitly founded on the claim that there is no such boundary, Blakemore took the opposite position: '*I worry about the principle of where the moral boundaries lie. There is only one very secure definition that can be made and that is between our species and others.*'[327] In our discussion below of the use of primates in medical research, we too find ourselves drawn into this debate.

5.3 Humanised animals in fiction

The phrase 'humanised animal' is often used in scientific literature to describe transgenic animals or chimæras in which human genetic material or cells have been incorporated. For those who know the origin of the phrase 'humanised animal' the use of this description will be disconcerting. It was coined by H G Wells to describe the results of the cruel activities of the fictional vivisectionist Dr. Moreau whose project of creating 'humanised animals' is described in *The Island of Dr. Moreau.*[328] But because the 'humanisation' inherent in the work of today's researchers is not at all like that attempted by H G Wells' Dr. Moreau, who sought to turn animals of other species into quasi-humans, any direct association between the two would be misguided and unfair.

5.3.1 Frankenstein and his 'monster'

Wells's book is not well-known these days. But popular discussions often allude to Mary Shelley's *Frankenstein.* Unlike Wells's Dr Moreau, Shelley's Victor Frankenstein is not represented as engaged in a deliberately vicious project – instead he is carried along by a thoughtless, obsessive wish to bring life back to a human corpse, or rather to a creature assembled from several human corpses.

324 Singer's most famous work in this area is Singer P (1976). *Animal liberation.* Cape, London. Tom Regan's writings include Regan T (1983). *The case for animal rights.* University of California Press, Berkeley.
325 Singer P & Cavalieri P eds (1994). *The Great Ape project: equality beyond humanity.* Fourth Estate, London.
326 For a recent statement to this effect, see the transcript of Blakemore's ARZone discussion (19 February 2011): http://arzone.ning.com/profiles/blogs/transcript-of-prof-colin
327 Owen J & Lean G (2006). *Leave our apes alone.* The Independent. http://www.independent.co.uk/environment/leave-our-apes-alone-481035.html
328 Wells H G (1962). *The island of Dr. Moreau.* Penguin Books, Harmondsworth.

The horrendous consequences of his success are then the substance of Mary Shelley's extraordinary story. Although Frankenstein's 'monster' is not a humanised animal, Shelley's depiction of the monster's thoughts and feelings, and of the attitudes of the humans whom the monster encounters to his advances, brilliantly captures a natural fear concerning humanised animals, especially humanised primates: the fear that although through their humanisation they become so close subjectively to humans to merit treatment as humans, their appearance and behaviour gives rise to revulsion and horror as a result of which they turn against their human creators.

5.3.2 Children's fiction

These stories by Wells and Shelley are of course just the tip of the iceberg when it comes to fictional explorations of variations of the boundary between humans and animals. From Aesop's Fables to Maurice Sendak's '*Where the Wild Things Are*', stories for children have been populated with animals, familiar or imaginary, which take on human capacities for thought and feeling and also human virtues and vices.[329] Quite why stories about animals are so well-suited as ways of introducing children to human characters and situations is a deep question for child psychology which we do not attempt to investigate here.[330] But there is no doubt that our attitudes to animals and sympathies for them are affected by these stories, even when we recognise that they are fanciful and that we are prone to the 'pathetic fallacy'[331] of projecting human sentiments into animals that are not capable of them.

The temptation when faced with fictional and mythical explorations of humanised animals is to regard them as intriguing exercises of the imagination, often charming though sometimes frightening, but not especially revealing when it comes to a serious understanding of animals, which requires instead more austere scientific research. But that may be too quick. It is often through our relationships with the animals with which we share our homes, our 'pets', that we learn to appreciate something of their subjectivity even when we recognise the truth of Montaigne's famous remark '*When I play with my cat, who knows if I am not a pastime to her more than she is to me*'.[332] Our capacity to understand and engage with each other draws upon an intuitive 'theory of mind' which is more a matter of empathetic simulation than of overt reasoning,[333] and there is every reason to suppose that a similar capacity is engaged in our direct relationships with animals.[334] So although fiction no doubt exaggerates the empathetic projection of human sentiments into animals, it draws on a capacity which is fundamental to our understanding of each other.

5.3.3 Kafka's animals

Two stories by Kafka exemplify these types of fiction.[335] In his well-known story, 'Metamorphosis', the hero, Gregor, is mysteriously transformed into a cockroach; and the story then imaginatively explores Gregor's terrifying predicament and the attitudes of his family to the giant cockroach who shares their small apartment. Wonderful though this story is, it tells us nothing about cockroaches. But Kafka wrote another short story, whose title, 'A Report to the Academy' is nicely appropriate for this report. In this story Kafka writes from the point of view of a humanised chimpanzee about the life of a circus ape who has learnt to speak. It is not a comforting story and Kafka clearly writes to make one wonder what 'it might be like' for a chimpanzee to be in this situation.[336] So there is a 'Kafkaesque concern' which we need to take seriously, alongside what one might call the 'Frankenstein fear' that the medical research which creates 'humanised' animals is going to generate 'monsters'.

329 Sendak M (1963). *Where the wild things are.* Harper & Row, New York.
330 Bruno Bettelheim developed an influential Freudian approach to this issue in Bettelheim B (1976). *The uses of enchantment.* Knopf, New York. For a critical discussion of Bettelheim's position see Zipes J (1979). *Breaking the magic spell: radical theories of folk and fairy tales.* University of Texas Press, Austin.
331 The phrase is Ruskin's; see volume 3, part 4, of Ruskin J (1856). *Modern painters.* Smith, Elder, London.
332 Montaigne M (1580). *An apology for Raymond Sebond* (Essays Book 2, Chapter 12).
333 For discussion of the issues here see Carruthers P & Smith PK (eds) (1996). *Theories of theories of mind.* Cambridge University Press, Cambridge.
334 The issues here are explored in Haraway D (2008). *When species meet.* University of Minnesota Press, Minnesota.
335 Both stories are included in Kafka F (1996). *The metamorphosis and other stories.* Dover, New York.
336 *ibid*.

5.4 'Playing God'

'*God made all the animals and then he made man to be in charge of animals and take charge of the world. We have the ability to do that.*' Public dialogue participant, London.

One of the themes of Mary Shelley's novel is that the terrible consequences of Frankenstein's success in acquiring a God-like power to overcome death show the need for humility in the exercise of power gained through scientific research. In a similar way, it might be said that by creating animals with significant human genetic and cellular components contemporary scientists are 'playing God'. This is not a specifically religious objection, although some may make it on religious grounds; the phrase carries a more general sense that scientists are possessed of a certain hubris, a false belief in their own powers and their own rights to exercise them in pursuit of their own projects, hence abusing their capacities without proper consideration of the consequences, in this case the transgression of the boundaries between humans and other animals.

5.4.1 Humanity's stewardship responsibility

There are two ways in which this complaint can be made more specific. From one direction, it might be said that by creating humanised animals scientists threaten the distinctive dignity of man; from the other direction, it might be argued that the process of humanising an animal undermines the integrity of the animal's inherent life-form. We discuss the first point in this section and come back to the second in the next section. But in both cases we start from the thought that humans have a general ethical responsibility to act as 'stewards' of the natural world, valuing and caring for the environment, including plants, fish and animals, instead of just treating them as a resource to be exploited for the benefit of one species, mankind. We take it that the exercise of this stewardship responsibility can be thought of as a virtue which should inform our relationships with the natural world, bringing with it duties that are appropriate to these relationships.

This report is not the place for a detailed exploration of these duties whose exercise enters into a great number of activities, but we take it that they do not preclude research which leads to the creation of animals which cross the boundaries between species, as long as the research is conducted in a way which attends to the interests of the animals involved and to the health of the broader environment. However, when one of the species is man, an extra deontological moral claim comes into play, the 'dignity' of humans (see 5.2.1); and the first claim above was that by humanising animals and thus blurring the distinction between animals and humans, scientists threaten the special dignity of man.

5.4.2 Humanised animals and human dignity

We have already observed that the presumption that humans have this distinctive status can be questioned by comparing humans with other animals, especially Great Apes; and we return to this point below. But setting it aside for the moment, it has long been accepted that the dignity of man does not rule out many ways in which animal and human materials are combined. After all, most humans eat meat or drink milk. Of course, some people are vegans on moral grounds, but these grounds are not that the very idea of combining human and animal materials is wrong, but that it is wrong to kill animals for human consumption, that dairy farming is exploitative and so on. Again, humans are not demeaned by the incorporation of parts of non-human animals (such as heart valves from pigs) through xenotransplantation, though it is possible to object to this practice on other grounds.[337] Similarly, therefore, the creation by another form of xenotransplantation of animals which include significant human elements cannot be held to threaten human dignity just because it humanises the animals involved. In particular, the creation of reliable animal models for human disease poses no threat to human dignity. Perhaps this practice imposes unacceptable harm on the animals involved; but that is a different argument which will be considered in the next section.

337 For a further recent work about human–animal xenotransplantation, see Blackman M (1997). *Pig heart boy.* Doubleday, London.

5.4.3 Extending human dignity

But what, one might suggest more speculatively (and this is the Kafkaesque concern of the previous section), about the creation of animals, especially primates, with the types of capacity that are more central to human dignity, such as a capacity for practical reasoning, a sense of their own identity and the ability to understand and engage with others? On reflection, however, what this possibility would undermine is not the dignity of human life, but its supposed *distinctive* dignity, in a way that extends the central claim of Singer's Great Ape project that there is no moral boundary between humans and Great Apes (see 5.2.2 above). For the more such enhanced primates come to have the capacities that have been regarded as characteristically human, the more unacceptable it would be to maintain a firm moral boundary between them and ourselves.[338]

In the present context, this conclusion cuts two ways. It refutes the complaint that it is an insult to human dignity to create animals which include significant human materials. But it also suggests that it would be right to hold that such enhanced primates should be accorded much the same moral status that we take ourselves to have, and thus that there are deontological grounds for opposing their use for research, at least in any way in which we would not use humans for research. In section 5.6 we return to these difficult issues.

5.5 Animal welfare

We now turn to the second point raised earlier, that the process of humanising an animal interferes with it in a way which is destructive of its integrity. In Chapters 2 and 3 we reviewed the ways in which current medical research involves the use of animals which include significant amounts of human material. Much of this research is directed to the development of animal models for human disorders to make it possible to undertake fundamental research into the causes of these conditions and possible treatments for them which cannot be properly carried out on human subjects. In the course of this research, therefore, animals such as mice are modified in such a way that they become susceptible to disorders such as variant Creutzfeldt–Jakob disease, Huntington's disease, Parkinson's, diabetes, Down's syndrome, β-thalassaemia, human cancers and so on. While this list shows the potential of this approach to medical research, from the point of view of animal welfare it is depressing: for the research precisely involves finding ways of transmitting the worst of human disorders to animals that are not normally afflicted by them. While no doubt the animals are treated 'humanely' (a strange word in this context), the whole process is intended to transform these animals into living laboratories for research into these human disorders.

In thinking further about this, there are two questions which one can raise. The first question arises from a utilitarian ethical perspective and looks both to the interests of the animals involved and to the interest of the humans who might benefit; it asks whether medical research, which involves ACHM, makes things distinctively worse for the animals involved as compared with other forms of medical research which use animals and compares this with the benefits that might accrue to humans. The second question arises from the 'stewardship' virtue ethics perspective described earlier (see 5.4.1) and looks to the relationship between humans and animals implicit in this kind of medical research; it asks whether humanising animals, so that they can be used as models for human disorders, introduces a new level of exploitation into the relationship between humans and animals which is unjustified by the correlated benefits to humans.

5.5.1 Comparing welfare

'*A mouse feels the same pain. I'm not saying protect the millions of them. But I feel pain is pain to be honest*' Public Dialogue, London.

338 See Harris J (2011). *Taking the "human" out of human rights.* The Cambridge Quarterly of Healthcare Ethics **20**(1), 9–20.

'*It's a great deal of suffering. The fact that it has human material makes no difference*'. Public Dialogue, Newcastle.

The familiar way of answering the first question is to apply the approach which is characteristic of the existing rules which govern the use of animals in medical research and concentrate primarily on the levels of suffering to which the animals are exposed. Thinking about this requires comparisons which cannot be precise, but the salient points appear to be the following:

1. The specific techniques involved in creating transgenic and chimæric animals involving human material do not themselves bring any great suffering to the animals involved, nor is their quality of life seriously compromised by these transformations, at least as compared with that which is normal for experimental animals (see 4.1.2).
2. But, the use of these animals for research which could not otherwise be conducted into human disorders, including in principle the worst that we experience, does often impose significant suffering on the animals.
3. Equally some current animal research necessarily involves the infliction of suffering on animals, and a minority of research very great suffering, including that mandated by our human safety regulations.
4. In fact (see 4.1.1), research indicates that it should be possible to undertake some types of research and testing (including some toxicity testing) on transgenic mice rather than on species such as primates whose suffering is of more concern to us because of their greater cognitive capacity, but which are currently the best indicators of human reactions.

The last two points here are significant, for from the point of view of animal welfare, it is extreme suffering that is most objectionable, and if this new research makes it possible to limit the need for tests which involve it, or to mitigate the suffering involved in them, then that is an important animal welfare benefit (we return to this point in the next section). The second point above should then be set against this benefit, but it is hard to see that it implies that this work significantly increases the level of suffering experienced by the animals involved as compared with that experienced by animals in other kinds of medical research.

In considering the impact of this research on the animals involved, however, it is not sufficient to take account of the familiar question about the level of suffering involved, since further questions about animal welfare are raised by the process of humanisation itself. But as long as the condition mentioned in the first point above is met, namely that the quality of life of these humanised animals, for example that of breeding colonies of transgenic mice, is not seriously compromised by their humanisation, at least as compared with that which is normal for experimental animals, this kind of research does not appear to bring with it any new animal welfare consideration. What it does open up instead is the challenge inherent in the second question above, namely that humanising animals so that they can be used as models for human disorders introduces a new level of exploitation into the relationship between humans and animals which runs contrary to the values inherent in our stewardship responsibility to animals.

5.5.2 Stewardship, humanisation and exploitation

The process of humanising an animal is not necessarily harmful to it: it could be part of a process of enhancement which endows the animal with greater physical abilities or resistance to disease. Yet although there are no doubt possibilities of this kind, especially where primates are concerned (to which we return in the next section), it would be disingenuous to pretend that a significant part of the work described in Chapters 2 and 3 is of this kind. The type of humanisation of animals we are considering here is undertaken primarily to facilitate medical research for the benefit of the human species.

The issue which this challenge throws into relief is that of the ethical significance of the 'human' dimension of the process of humanisation when it is considered in the context of the assumption that the use of animals for medical research is in principle acceptable under certain conditions (see 5.1.1). Is it the introduction of significant human materials into animals which is thought to make the process especially exploitative? Or is it just the fact that the process is undertaken primarily for the benefit of humans? If the former claim is made, then it needs to be explained why the presence of the human materials (cells or genes) is by itself of decisive significance. Suppose that it is discovered that there are ways of genetically modifying mice which do not involve the insertion of human genes but which provide equally valuable models for human disorders, and that all the animal welfare issues are much as they are for humanised mice: would that kind of practice be ethically preferable to that which we are considering here?[339] We find it hard to see what reason one could have for such a preference beyond the symbolic absence of human materials from the animals in the hypothetical case; yet given that the animal welfare issues are supposed to be the same, it is hard to see why this justifies a moral distinction between the two cases (and if one thinks that it does, suppose that the hypothetical procedure leads to a greater cost in animal welfare; which procedure is then preferable?). If, alternatively, it is just the fact that the primary goal of this research is the promotion of human welfare that is supposed to make it exploitative, then there is no reason to hold that this kind of research is ethically more problematic than other types of medical research which use animals for the benefit of research into human disorders.

There is another way in which the ethical significance of the 'human' dimension of the process of humanisation might be elucidated, namely by supposing that where it involves neuronal cells, it transfers significant human psychological capacities and abilities to the animals involved. But we set that aside for now since we shall discuss the issue to which this hypothesis gives rise in the next section.

5.5.3 A preliminary conclusion

The conclusion that we have arrived at so far is that the practice of humanising animals for the purpose of medical research does not bring significant new ethical problems as compared with other kinds of medical research which use animals. As we have explained, as far as animal welfare is concerned, there are in fact grounds to hope that the new practice will make it possible to decrease the amount of suffering required for some tests (and we say more about this in the next section). The further charge was that humanising animals specifically to benefit humans introduces a new level of exploitation into the relationship between humans and animals. On examination, however, this charge does not stand up: once the symbolic value of the introduction of human materials into animals is set aside, the basis of the charge is that the whole practice is undertaken for the benefit of humans. That should indeed be admitted, but in this respect the new kind of research is not different from others which use animals for medical research without humanising them.

5.5.4 Our conflicting responsibilities

One might respond that the conclusion to be drawn from this argument is that the whole practice of using animals for medical research whose primary goal is the treatment of human disorders is exploitative and runs counter to the stewardship responsibility which ideally guides man's dealings with animals. But that response opens up the general issue of justifying this practice, an issue which we have here set to one side. Our basic presumption is that alongside our stewardship responsibility to animals there is a general social responsibility to facilitate medical research. Thus we face in this area a conflict of responsibilities where the use of animals for medical research provides the best, and perhaps the only, acceptable way of attempting to understand, diagnose and treat some terrible human disorders. It is,

339 In fact, as is noted in 2.2.1, in some cases ACHM-type animals contain no human cells or DNA even though their genome has been modified to mimic relevant parts of the human genome.

we think, reasonable to believe that success in this endeavour would bring a very great benefit, just as withholding or postponing that benefit would risk bringing significant suffering and premature death to very large numbers of people; and our working assumption is that this benefit is sufficient to justify the harm done to the animals involved. We recognise that not everyone shares this assumption, and we ourselves accept that it would be wonderful to be able to make progress in medical research without harming either animals or human subjects. But in our judgement that is not the world we inhabit.

5.6 Non-human primates

'*I don't have a problem with it until it gets to the brain – liver, heart, etc. are all fine. It's the brain which makes people humans*' Public Dialogue, Newcastle.

We are ourselves primates. For this reason the use in medical research of NHPs as substitutes for humans gives rise to a dilemma. Their biological proximity to us implies that they generally provide more reliable models for human disorders and reactions than other animals, which makes them especially suitable for use in medical research; yet it also implies that their capacities and abilities are more similar to ours than those of other animals, and as a result some of the deontological considerations we have for not conducting medical experiments on unconsenting humans apply also to them. It is not our task to explore and debate this dilemma, though we commend the discussion of it in the Academy's 2006 report on '*The use of non-human primates in research*', undertaken by a working group chaired by Sir David Weatherall.[340] For us the question is just what difference is made by the development and use of animals containing significant amounts of human material, which is not a question directly addressed in that report.

5.6.1 Substitutes for NHPs

One striking fact highlighted in the Weatherall report is that the great majority (about 75%) of the NHPs currently used in medical research in the UK are used for the purpose of testing the toxicity of drugs.[341] The explanation for this is that testing drugs on primates has been a much more reliable guide to the effects of drugs on humans than testing the drugs on other animals, such as mice.[342] But, as we mentioned above, the situation is now changing, and it is reasonable to hope that suitable humanised mice, or similar animals, could be developed as effective substitutes for NHPs for the purpose of many toxicity tests. Such a change could therefore eventually lead to a reduction in the number of NHPs used for this type of medical research, which we take to be an important potential change for the better because the primate's greater cognitive abilities imply that it is likely to experience greater suffering and distress in toxicity tests than a mouse.

Similar reasons apply to the potential substitution of transgenic humanised mice for NHPs in research concerning diseases such as HIV, tuberculosis and hepatitis. And here the benefit of substitution is especially important, since in some cases (e.g. hepatitis) the dilemma of primate research applies especially sharply: on the one hand, it is only the primates biologically closest to humans, chimpanzees, which provide a naturally effective model for the human disease; but just because they are so close to humans, with highly developed cognitive abilities and affective sensibilities, their use for medical research is morally very problematic and has not been undertaken in the UK for the last 50 years.[343] Hence the possibility of carrying out research with mice and other similar animals containing human material should make it possible to take forward research concerning these devastating diseases without incurring the moral injury of inflicting them on NHPs.

340 Weatherall D (2006). *The use of non-human primates in research* http://www.acmedsci.ac.uk/images/project/nhpdownl.pdf.
341 *Ibid* Chapter 8.
342 It is important to recall that the value of pre-clinical testing is limited by differences between species. In March 2006, a study of the antibody TGN1412, which had been pre-clinically tested in species including NHPs, caused severe adverse reactions in six trial participants. An expert inquiry into the trial concluded '… the pre-clinical development studies that were performed … did not predict a safe dose for use in humans, even though current regulatory requirements were met.' http://www.dh.gov.uk/prod_consum_dh/groups/dh_digitalassets/@dh/@en/documents/digitalasset/dh_073165.pdf
343 See 3.4 for more detailed discussion and comparison of the abilities of humans, Great Apes and other primates.

5.6.2 The challenge of using NHPs for research into neurodegenerative disorders

But there are areas of medical research where substitution of this kind is not likely to be helpful, especially that concerning neurodegenerative disorders. Because the brains of mice are very much simpler than those of primates, it is judged very unlikely that they will provide satisfactory models for these human disorders. In this area, therefore, medical research is beginning to use monkeys such as marmosets and macaques (evolutionarily further from humans than the Great Apes) both for fundamental research and as models for human disorders such as Parkinson's and Alzheimer's, and in some cases this research has involved the introduction of human neural stem cells into NHPs.[344] A related development has been work which showed the possibility of germline inheritance of genetic modifications introduced into marmosets, thus holding out the possibility of creating a breeding colony of transgenic humanised monkeys.[345] The issue which this kind of research now raises is whether this kind of 'neural humanisation' of an NHP endows it with added cognitive abilities or affective sensibilities which make it improper to use it for potentially distressing medical research, such as that into Parkinson's or Alzheimer's disease.[346]

As ever, the dilemma of primate research opens up: by humanising these monkeys to make them useful as models of human neurodegenerative disorders, one may endow them with capacities and abilities which make it even more problematic to carry out the research. It is not possible to resolve this dilemma at present. To be confident about a judgement, one needs answers to the following questions concerning this proposed neural humanisation of NHPs:

1. Will it be possible to create useful models for human disorders such as Parkinson's and thereby facilitate research which cannot now be undertaken?
2. Could an NHP, once modified as a model for a disorder such as Parkinson's, lead a life whose quality is acceptable when assessed by the normal standards for experimental animals?
3. But (assuming that the answer to (2) is positive) will the neural humanisation of an NHP enhance its cognitive and affective abilities in such a way that these become comparable to those of Great Apes?

Despite some early work in which transgenic rhesus macaques were developed to model Huntington's disease, it is too early to answer the first question.[347] But it ought to be possible to answer the second question once this work has progressed. If it turned out that the monkeys were seriously impaired by their neural adaptation, or that the quality of life of breeding colonies of transgenic humanised monkeys were significantly impaired by their humanisation (perhaps by their becoming more aware of their confinement), then these would be powerful reasons for halting the research. But assuming that the answer to the second question is positive, we are led to the third, speculative question; and if the answer to this turned out to be positive, then, from the other direction, there would also be reason for halting the research, since it would imply that the reasons we have for not licensing medical research which uses chimpanzees and other Great Apes apply also to research which uses these genetically enhanced monkeys.

It is not straightforward to envisage how this third question is to be settled. One can be confident that the introduction of some human neural stem cells would not endow a monkey[348] with a human-type self-consciousness, since that requires a capacity for higher-order thoughts associated with language, and it is fanciful to suppose that this capacity might be produced in a monkey simply by the introduction of some human neural stem cells into its brain. But once one recognises that

344 Redmond DE *et al.* (2010). *Cellular repair in the parkinsonian nonhuman primate brain.* Rejuvenation Res **13**, 188–94.
345 Sasaki E et al. (2009). *Generation of transgenic non-human primates with germline transmission.* Nature **459**, 523–27. As critics have observed, Sasaki's research was not without costs to the animals involved: to get a single case of germline transmission he used eighty modified marmoset embryos (the modification was the inclusion of the enhanced green fluorescent protein transgene).
346 For a preliminary discussion of these issues, see Olsson I & Sandøe P (2010). *What's wrong with my monkey? Ethical perspectives on germline transgenesis in marmosets.* Transgen Res **19**(2), 181–6.
347 Yang S *et al.* (2008). *Towards a transgenic model of Huntingdon's disease in a non-human primate.* Nature **453**, 921–4.
348 In this section, the term 'monkey' is used to refer to primates other than both man and the Great Apes.

the important comparison here is with Great Apes, then the uncertainties that affect our understanding of their cognitive abilities also affect procedures for comparing their abilities to those of enhanced monkeys.[349] Hence if work of this kind with monkeys proceeds it would be important to study some neurally humanised monkeys before potentially damaging medical research on them is undertaken so that an informed assessment of their abilities can be undertaken.[350]

5.7 Public concerns

The public dialogue we carried out brought out several areas of concern (see Boxes 5.1–5.3 in this chapter and others throughout the report).[351] One was that this research should be carried out in a way which advances the public good and not primarily the interests of business enterprises which have invested in it. This point is indeed implicit in our discussion: given that the practice of using animals for medical research is justified (insofar as it is) by its benefits for human health, the practice clearly needs to be organised in a way which ensures that these benefits are available to the public without excessive cost. Another area of concern was research involving the brain, especially those of monkeys; some participants expressed the kind of unease concerning the transfer of human capabilities to monkeys which we have just discussed here. But there were two further areas of concern which we have not addressed.

5.7.1 Humanising the appearance of an animal

One concern arose from the possibility of humanising the external appearance of an animal in such a way that it strongly resembled some aspect of a human being, an example would be endowing a primate with human-type skin in order to learn something about human skin disorders that could not be investigated in any other way.[352]

Many participants expressed strong distaste concerning possibilities of this kind, even when they were content with experiments which humanised the internal organs of animals of the same kind (see 3.6 and Box 3.11). Hence the issue here is whether this reaction itself provides a strong reason for not permitting the research in question in a situation in which the research is potentially important and it has been established that the condition (e.g. the humanised skin), including its appearance, is not distressing to the primate itself or to others with which it is living.

In thinking about this, the issue is what significance one should attach to the distaste at the visible appearance of a humanised animal. One suggestion might be that this distaste, or repugnance, reveals an ethical truth, the profound error of blurring the boundary between humans and animals.[353] The objection to this suggestion, however, is that once it is acknowledged that the same distaste is not manifested towards substantial internal humanisations of an animal, the reaction appears to be irrational. Instead one can compare this distaste at the humanised appearance of an animal with the common reaction of unease at the sight of human disfigurement. This is a primitive reaction which has no inherent 'wisdom'. Nonetheless, given the likely hostility to research which endows animals such as primates with a humanised appearance, there are pragmatic reasons of public policy for requiring that special consideration be given to proposals for research of this kind.[354]

349 The discussion of this issue in 3.4 makes these uncertainties very clear.

350 These considerations connect with those discussed by Greely and others in their paper: Greely HT *et al.* (2007). *Thinking about the human neuron mouse.* Am J Bioeth **7**, 27–40 in connection with the speculative 'Mouse Neuron Project' first proposed in 2000 by Dr Irving Weissman (see Box 3.4). Whereas working with mice was never likely to yield a useful model for human neurodegenerative disorders, it is quite possible that monkeys will provide useful models; so it is important to begin 'thinking about the human neuron monkey'. Some preliminary considerations were discussed in Greene M *et al.* (2005). *Moral issues of human-non-human primate neural grafting.* Science **309**, 385–6.

351 Ipsos MORI (2010). *Exploring the boundaries: public dialogue on animals containing human material* http://www.acmedsci.ac.uk/index.php?pid=209.

352 *Ibid* p29.

353 This suggestion takes its lead from Leon Kass's thesis of 'the wisdom of repugnance'; Kass L (2002). *Life, liberty, and the defense of dignity.* Encounter Books, San Francisco.

354 For further discussion of animal welfare issues of this kind, see Coors ME *et al.* (2010). *The ethics of using transgenic non-human primates to study what makes us human.* Nature Rev Genet **11**, 658–62.

5.7.2 Research involving reproductive cells

The other area of public concern arose from research which involves introducing human reproductive tissues and cells into animals (see 3.5 and Box 3.10).[355] Although it was not clear quite what kinds of research gave rise to this concern, it is easy to understand anxieties about the possibility of creating human–animal hybrid embryos. In fact the main area of research here involves the grafting of human reproductive tissues such as ovarian tissue into mice or other animals in order to understand reproductive biology, the causes of infertility, and to develop methods for preserving the reproductive potential of young people, for example those whose therapeutic treatment poses a threat to the viability of their reproductive system (see 3.5).[356] By itself this technique is not ethically problematic: on the contrary the research aims to provide a way of enabling those who are undergoing an invasive treatment to recover their reproductive ability once the treatment is over and the tissues in question are replaced in their own bodies. So the issue here is whether there is a significant chance that while these human reproductive tissues are lodged within a mouse or similar animal, some human germ cells might migrate within the host animal to that animal's own reproductive system and then lead to the creation of a hybrid human–animal embryo. In principle it appears that an event of this kind could occur, albeit unlikely. So far as we know, no such event has occurred in the context of current research; but we share the public's concern that this should not happen. There will be many ways of rationalising opposition to the creation of such an embryo, but for us it is sufficient to observe that it could never lead to the birth of a biologically coherent animal. So research that involves placing human reproductive tissues in non-human animals needs to be conducted in a way which avoids the risk of fertilisation inside the animal.

5.8 Conclusion

'*Going into the discussion I think I was very against any kind of animal research, but having heard about what it is and what it is for, I have completely reversed my position*'. Public dialogue – interview with Newcastle respondent.

We accept that the use of animals for medical research remains controversial, and we have not attempted here to justify the practice. Our attention has been directed at the distinctive ethical issues raised by the use of animals which include human genetic or cellular material. In discussing these we have addressed a variety of concerns – including utilitarian concerns about animal welfare, deontological concerns arising from the capacities which underlie human dignity, and considerations arising from our stewardship responsibility towards animals. We have not prioritised any one of these ethical perspectives in our attempt to capture the complexity of the cross-cutting ethical considerations that are in play in this issue. Our conclusion is that this work does not give rise to principled new concerns which provide reasons for curtailing it, and indeed that it offers the prospect of reducing the use of primates and similar animals in damaging experiments such as toxicity tests. Nonetheless, this work does have some troubling features which can be justified only by the prospect of facilitating the development of effective treatments for serious human disorders. In the few areas we have highlighted, such as neural experimentation with monkeys in order to advance the understanding and treatment of neurodegenerative disorders, such work needs to be accompanied by a careful assessment of the abilities of any humanised NHPs and of the ways in which their involvement in research affects their quality of life.

355 See page 31 of Ipsos MORI (2010) '*Exploring the boundaries*'. *Public dialogue on animals containing human material.* http://www.acmedsci.ac.uk/index.php?pid=209

356 For a survey of some recent work, see Dath C *et al.* (2010). *Xenotransplantation of human ovarian tissue to nude mice: comparison between four grafting sites.* Hum Reprod **25**(7), 1734–43.

Box 5.1 Conditional support for research involving ACHM

The majority of participants in the public dialogue accepted and were supportive of research using animals containing human materials (see Box 3.1). However, this support came with conditions attached – the majority of participants gave their support on the understanding that it is conducted to improve human health or combat disease.

In considering examples of research, participants were found to 'trade-off' the anticipated benefits or purpose of the research against concerns about the process.

The purpose of the research was judged on its perceived value against two main factors:

- Tangibility: research with more immediate or certain benefits received most support.
- Severity of the health issue: research addressing common terminal, debilitating or painful diseases found greatest acceptance, followed by research into conditions causing disfigurement or impacting on quality of life.

Key concerns that participants set against the value of the research included:

- Novelty: animal modifications that were seen as extensions of existing techniques were generally more accepted than new approaches, or the creation of new entities.
- The type of entity created: *in vitro* research caused fewer concerns than research involving whole animals (see Box 3.1).
- Tissue type: human-like modifications of an animal's brain, reproductive system, or external features were less accepted than modification of internal organs (see Box 3.1).
- Experimental species: particular concerns were expressed in relation to the use of pigs and monkeys (and especially chimpanzees).
- Animal welfare concerns were important for many participants (see Box 4.1).
- Safety: perceived current and future risks were both a concern (see Box 4.2).
- Animal–human boundaries: some examples raised ethical concerns, such as how partly human experimental remains should be treated, and whether animals with elements of human capacity (particularly cognition) should gain human rights.
- Who would benefit: it was important to some participants that research benefits would be distributed equitably.

Box 5.2 Opposition to research involving ACHM

The dialogue identified a group of participants who did not find research involving animals containing human material acceptable, even to address human health problems. Survey data indicated that this view is held by around 15% of the British population. Around two-thirds of this group in the survey also opposed any form of animal research, and a similar proportion did not trust UK regulation of research involving animals containing human material. Workshop participants who opposed research involving animals containing human material expressed doubt whether such research would deliver benefits, or would achieve its aims.

In the qualitative survey the most frequent reasons for finding such research unacceptable among this group were concerns for animal welfare, that it was against their personal views or that it was unnatural.

Box 5.3 Focus group findings and demographics

Three groups whose views were anticipated to be more distinct than those of the wider public were included in the dialogue:

- Patients and carers of those with serious illness (potential beneficiaries of medical research). Although concerned for animal welfare, this group welcomed all research with clear medical objectives and strongly supported the continuation of research using ACHM.
- Those who indicated religious faith played an important role in their daily life. An underlying view that human life has a pre-eminent value strongly influenced this group. Participants were highly supportive of research seen to enhance human life, and did not voice specific theological objections to research involving ACHM.
- Those with strong concern for animal welfare. This group broadly opposed research involving ACHM. Besides welfare concerns and a belief that animal experiments are unethical, the group expressed wider concerns including that research benefits would not be fairly distributed. Alternative priorities, including addressing poverty, global warming and causes of disease, were suggested.

The dialogue did not find sufficient evidence to indicate that views varied between participants of different ethnicities, or from different regions of the UK. However, there were some differences in views on animals containing human material research across demographics:

- Gender: survey data indicated men were more likely to find research acceptable than women.
- Age: older people were slightly more supportive of the research than younger people.
- Educational level: participants with higher education were more likely to express strongly polarised views, either in favour of or opposing the research. Survey data indicated that those with higher qualifications were more likely to find such research acceptable.

6 Legal and regulatory considerations

6.1 Introduction

No single piece of legislation specifically governs the creation or use of ACHM in medical research within the UK. However, several pieces of UK law are relevant to particular aspects of this research.

The most significant is the Animals (Scientific Procedures) Act 1986 (ASPA) which regulates the use of animals in research. Also relevant are the Human Fertilisation and Embryology Act 1990 (as amended in 2008) (HFE Act), which governs research involving human gametes, human embryos and human admixed embryos, and the Human Tissue Act 2004 (HT Act), which governs the use of human tissue containing cells and human DNA.

Research involving ACHM will generally fall under one or more of these pieces of legislation, and therefore be within the remit of one or more UK regulatory body, depending on the specific nature of the experiments involved. It may also be subject to other UK laws in some instances, including regulations relating to the use of genetically modified organisms, property and intellectual property (patent) law, and the Data Protection Act (DPA). In addition to rules, standards and procedures defined in law, research involving ACHM is also governed by professional guidelines or codes of conduct.

The complexity of the regulatory background is mirrored in the number of Government Departments with some function related to research using ACHM. The Department of Health supports health research and its translation into better healthcare. Its role as sponsor for the independent bodies that regulate the use of human embryos and human tissue sits alongside this broader function.

Responsibility for ensuring a sustainable science base rests with the Department for Business, Innovation and Skills. In contrast to Department of Health and Department for Business, Innovation and Skills, facilitating biomedical research is not a core objective of the Home Office but it has a specific role in the regulation of the research use of animals so its activities impact on the work of many researchers in the biomedical field. Other Government departments also have a role in relation to safety issues (see 4.2.5, 4.2.6).

Some consideration of the UK regulation of research involving ACHM was undertaken in the context of a wider review of the regulation of transgenic and cloned animals, by a working group of the Animal Procedures Committee (APC) in 2001.[357]

This chapter reviews the current UK regulatory environment for the creation and use of ACHM, and considers the interfaces between the relevant legislative instruments.[358] The factors that the public involved in the dialogue felt were important for the regulation of ACHM are outlined in Box 6.8.

6.2 Overview of the current UK legal and regulatory environment

6.2.1 Animals (Scientific Procedures) Act 1986

Scope and purpose

Scientific experimentation conducted in the UK using 'protected animals' is regulated by the Animals (Scientific Procedures) Act 1986 (ASPA), the principal purpose of which is to ensure that animals used in research are not subject to unnecessary pain, suffering, distress or lasting harm.[359,360] ASPA operates a

357 Animal Procedures Committee (2001). *Report on Biotechnology*. http://www.homeoffice.gov.uk/publications/agencies-public-bodies/apc/key-reports/biotechnology?view=Binary

358 The pathway of regulation and governance of research involving human participants, their tissue or data is addressed in a report from the Academy of Medical Sciences (2011). *A new pathway for the regulation and governance of health research*. http://www.acmedsci.ac.uk/p47prid88.html

359 The Animals (Scientific Procedures) Act 1986 is available at http://www.legislation.gov.uk/ukpga/1986/14/contents. For the associated Guidance on the Operation of the Animals (Scientific Procedures) Act 1986 see http://www.archive.official-documents.co.uk/document/hoc/321/321.htm

360 'Pain, suffering, distress and lasting harm' encompass any material disturbance to normal health (defined as the physical, mental and social well-being of the animal). They include disease, injury and physiological or psychological discomfort, whether immediately (such as at the time of an injection) or in the longer term (such as the consequences of the application of a carcinogen). Guidance on the operation of ASPA, Section 2.14.

licensing and inspection system, which governs experimental or other scientific procedures applied to 'protected animals'.[361]

'Protected animals' are defined as 'any living vertebrate, other than man', and '*Octopus vulgaris*' (the common octopus).[362] The Act applies to these types of animal if they are used, or survive into, any of the following stages of their development:

- Mammals, birds and reptiles: from half-way through the gestation or incubation period.
- Fish, amphibia and *Octopus vulgaris*: from the time at which they become capable of independent feeding.[363]

Vertebrates and *Octopus vulgaris* that do not survive beyond these developmental stages, and all other invertebrates, are not 'protected animals' under ASPA. Use of these life forms in research is not specifically regulated beyond the Genetically Modified (GM) (contained use) regulations, the GM (deliberate release) regulations, and other general health and safety requirements (see 6.2.4).

Application of ASPA to ACHM research

Although research involving ACHM is not explicitly described within ASPA or its associated guidance, in practice, almost all such research is governed by ASPA because it involves 'regulated procedures' applied to 'protected animals'. Moreover, the regulatory safeguards established under ASPA apply to animals genetically altered for the purposes of research and their progeny, howsoever produced, throughout their lives.[364,365]

ASPA licensing system

ASPA operates through a three-part licensing system.[366] The Act sets out an exhaustive list of the purposes for which project licences may be granted (Box 6.1).

The decision to license research is based on an analysis in which the potential benefits (to human welfare or knowledge, to the welfare of other animals or to the environment) are weighed against the likely welfare costs to the animals involved.[367] Research can only be authorised if there are no scientifically suitable alternatives that replace animal use, reduce the number of animals needed or refine the procedures used to cause less suffering – principles known as the 3Rs. Additional conditions apply for research involving particular species or purposes (Box 6.1).

The focus of ASPA and its implementation is on animal welfare and the 3Rs. The legislation was designed and is principally intended to ensure the protection of animals rather than to examine ethics, societal issues, or emerging research. Although these wider issues are considered in the weighing process described above, ASPA was not designed with the complex ethics and societal issues described in Chapter 5 in mind. As respondents to our call for evidence indicated, all animals used in research under ASPA are treated in a manner appropriate to their welfare needs, whether or not they contain human material: '*animal technicians … and researchers will assess the health of animals in their care equally, regardless of whether human materials have been incorporated into the animals' bodies or not'*.[368]

361 These are a defined as 'regulated procedures'. Guidance on the operation of ASPA, Sections 2.13–2.23.
362 The term 'man' is not defined in this context, but could be considered to include certain predominantly human human–animal entities. For discussion see 6.2.2.
363 For example, licences are required for research involving embryonated bird eggs if the embryo is allowed to survive into the second half of the incubation period. Guidance on the operation of ASPA, Section 2.8.
364 Animal Scientific Procedures Committee (2007): *Consideration for the discharge of GA animals from Animal (Scientific Procedures) Act 1986*. A genetically altered animal is defined as an animal in which the heritable DNA has been intentionally altered, or which carries a genetic mutation recognised as harmful, or the progeny of such an animal. This includes animals produced by genetic modification (as defined in the Genetically Modified Organisms (Contained Use) Regulations 2000); animals produced by induced mutagenesis; animals created by nuclear transfer procedures; animals created by the use of certain selective breeding strategies; harmful mutant lines arising from spontaneous mutations. It excludes animals with changes that are not heritable, such as somatic gene therapy or DNA immunisation.
365 It is in theory possible for such animals to be released from the requirements of ASPA once the research has been completed if the Home Office is satisfied this is appropriate on animal welfare grounds and has satisfied itself on any environmental or health and safety issues. In practice this has never happened, though it has been discussed by the APC (see 6.2.4 and Guidance on the operation of ASPA, Section 8.14). The approval of Defra would also be required to release such animals from the controls of the GM regulations.
366 Those carrying out any regulated procedure must hold a *personal licence*, all procedures must be part of a programme of research specified in a *project licence*, and research must be carried out at a designated scientific procedure *establishment*. See Guidance on the operation of ASPA, Section 2.36.
367 See Guidance on the operation of ASPA, Sections 5.10–5.12.
368 Written evidence from Wellcome Trust Sanger Institute.

Enforcement of ASPA and the role of the APC

Enforcement of ASPA, including the issue of licences, is the direct responsibility of the Secretary of State for the Home Office. The Animal and Scientific Procedures Division of the Home Office operates the licensing system on the Secretary of State's behalf, as well as providing the primary source of policy advice. The Animals (Scientific Procedures) Inspectorate provides advice to the Secretary of State as to whether, and on what terms, licences should be granted, and provides the primary assessment of licence applications.[369] The Animal Procedures Committee (APC) is an advisory non-departmental public body, set up to provide strategic advice to the Secretary of State on policy, practice, ethics, science and animal welfare related to ASPA.[370] Neither the Inspectorate nor the APC have executive powers. Their advice to the Secretary of State is not legally binding, though failure of the Secretary of State to have regard to it may be subject to judicial review.

The APC and the ASPA system more broadly operate on a case-by-case basis rather than through the development and application of policy. Typically, the Committee considers fewer than ten applications per year. The Committee reviews any applications referred to it by the Inspectorate and can review further applications on request. It automatically reviews all applications that fall within four categories agreed with the Home Secretary (see Box 6.2). These four categories are principally based around animal welfare issues of particular sensitivity or concern, although the fourth ('applications of any kind raising novel or contentious issues, or giving rise to serious societal concerns'), which is not defined, may be interpreted more broadly.

In conducting a review, the APC must have 'regard both to the legitimate requirements of science and industry and to the protection of animals against avoidable suffering and unnecessary use in scientific procedures'.[371] The Committee does not have any of the broader functions conferred on some of the statutory regulators (for example the functions of issuing guidance, monitoring new developments and engaging with external stakeholders of the Human Fertilisation and Embryology Authority and the Human Tissue Authority).

Some consideration was given to ACHM by the APC's Biotechnology working group in 2001, in the context of a wider review.[372] Their report highlighted concerns that emerged through consultation about experiments involving the humanisation of animals. It recommended that research involving some chimæric and hybrid forms should not be licensed. '*The true worry is about the creation of creatures with overtly human properties, or conversely the production of human-born entities with 'animal' properties. … Concern may be partly for the fate of such hybrids. But there may be a deeper repugnance at the thought of chimæras and hybrids: the wrong may not be in how we would treat them if they did exist but in their existing at all …*'[373] However, these recommendations have not been developed into specific rules and advice by the APC and decisions by the Home Secretary about such research continues to be issued on an *ad hoc* basis.

Local ethical review processes

In addition to the licensing system, ASPA requires every designated user and breeding/supplying establishment involved in animal research to have a local ethical review process. The purposes of the ethical review process are to ensure that all use of animals in an establishment is 'carefully considered and justified, that proper account is taken of the 3Rs, and that high standards of accommodation

369 The Inspectorate is also responsible for conducting inspections of premises where regulated procedures are performed, and where animals are bred or kept, to monitor standards and compliance with the Act. See Guidance on the Operation on ASPA, Sections 2.90–2.92.
370 See http://www.homeoffice.gov.uk/agencies-public-bodies/apc/
371 See Guidance on the Operation on ASPA, Section 2.93.
372 Animal Procedures Committee (2001). *Report on Biotechnology*. http://www.homeoffice.gov.uk/publications/agencies-public-bodies/apc/key-reports/biotechnology?view=Binary. The working group was established to consider 'the adequacy and appropriateness of the present regulatory regime under ASPA in regard to transgenic and cloned animals' in the light of current and likely scientific developments at that time.
373 *Ibid*. The report recommended that 'no licences should be issued for the production of embryo aggregation chimæras especially not cross-species chimæras between humans and other animals, nor of hybrids which involve a significant degree of hybridisation between animals of very dissimilar kinds.'

and care are achieved'.[374] Whilst the ethical review process is intended to be specific and appropriate to each individual establishment, common aims and functions are defined in Home Office guidance (see Box 6.3).

Responsibility for operation of the ethical review process rests with a named 'certificate holder' at each establishment. Membership of any ethical review group should, where practicable, include a veterinary surgeon, representatives from those who provide day-to-day animal care, project and personal licence holders, and one or more lay persons, and involve both establishment staff and others.[375]

Although the local ethical review process considers some ethical matters, we understand that there is variability between ethical review processes. Concern was expressed to us that some focus more on ensuring the practicalities of conducting proposed research within an establishment (e.g. funding and capacity), than considering societal or ethical implications in their broadest context. In this case, it will mainly fall to the Inspectorate to identify broad societal or ethical concerns relating to a particular research project, and to bring these to the attention of the APC or the Secretary of State.

Implementation of the European Directive on the protection of animals

The revised European Directive (2010/63/EU) on the protection of animals used for scientific purposes was adopted in October 2010 and is to be transposed into the national legislation of all Member States by 2013.[376] The influence of the Directive on the UK's current legislation (ASPA) and regulatory system will be explored during Government consultation. We anticipate three areas, of relevance to the current study, which may give rise to discussion and could potentially result in changes to ASPA:

- Regulation of fetal mammals. The Directive applies to 'fetal forms of mammals as from the last third of their normal development', whereas ASPA applies to 'mammals … from halfway through the gestation or incubation period'.[377]
- 'Animal welfare bodies'. The Directive requires that each breeder, supplier and user of research animals sets up an animal welfare body.[378]
- 'National committee for the protection of animals' used for scientific purposes. The Directive requires that each Member State establishes such a committee 'for the protection of animals used for scientific purposes'. Such committees should (among other things) provide advice and ensure the sharing of best practice both nationally and internationally.[379] The development of this committee was discussed in the 2009/10 review of the APC.[380] Proposed aspects of the roles of this committee are not part of the functions of the APC in its current form.[381]

374 See Guidance on the Operation of ASPA, Appendix J, 2.
375 See Guidance on the Operation of ASPA, Appendix J, 5.
376 Directive 2010/63/EU on the protection of animals used for scientific purposes is available at http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2010:276:0033:0079:EN:PDF
377 *Ibid* Article 1, 3a (ii).
378 *Ibid* Articles 26 and 27.
379 *Ibid* Article 49.
380 Omand D., (2010). *Report of the 2009/10 NDPB Review of the Animal Procedures Committee*. http://www.homeoffice.gov.uk/publications/apc/publications-2010/review-apc-0910?view=Binary Recommendation 22.
381 Functions of the 'national committee for the protection of animals' perhaps not clearly covered by the current APC include advising animal-welfare bodies on matters dealing with the acquisition, breeding, accommodation, care and use of animals in procedures and ensuring sharing of best practice; exchanging information on the operation of animal-welfare bodies and project evaluation; and sharing best practice within the European Union.

Box 6.1 Permitted purposes of research under ASPA and additional restrictions

A project licence will only be granted for one or more of the following scientific or experimental purposes:

- The prevention (whether by the testing of any product or otherwise) or the diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants.
- The assessment, detection, regulation or modification of physiological conditions in man, animals or plants.
- The protection of the natural environment in the interests of the health or welfare of man or animals.
- The advancement of knowledge in biological or behavioural sciences.
- Education or training other than in primary or secondary schools.
- Forensic enquiries.
- The breeding of animals for experimental or other scientific use. This generally refers to genetically modified animals or animals with harmful mutations.[382]

In line with guidance from the Home Secretary, licences will not be issued for programmes of work involving:

- The use of Great Apes (that is, chimpanzee, pygmy chimpanzee, gorilla and orang-utan).
- The use of protected animals for testing finished cosmetics products and substances intended primarily for use as cosmetics ingredients.
- The use of protected animals for the development or testing of alcohol or tobacco products (the use of tobacco or alcohol as research tools may, however, still be considered and licensed in the context of investigating disease or novel treatments).
- The use of protected animals for the development or testing of offensive weapons (licences may still be granted for the testing and development of means for protecting or treating UK servicemen and women, or the wider population).[383]

Box 6.2 Remit of APC in reviewing research licence applications[384]

By agreement with Ministers, the APC sees all applications for project licences that involve:

- The proposed use of wild-caught non-human primates.
- The proposed use of cats, dogs, equidae[385], or non-human primates in procedures of substantial severity.
- A substantial severity banding or major animal welfare or ethical implications, involving (a) xenotransplantation of whole organs or (b) chronic pain models or (c) study of the central nervous system.[386, 387]
- Applications of any kind raising novel or contentious issues, or giving rise to serious societal concerns.

'*Approximately 1% of applications for licences (e.g. those in the categories described … above) go to the APC for consideration. In practice therefore the APC is examining only the most substantial severity applications (usually involving non-human primates) … In the last three years the APC has given advice on 9 applications …*'[388]

382 See ASPA (1986) Section 5 (3).
383 See Guidance on the operation of ASPA, Section 5.23.
384 Omand D., (2010). *Report of the 2009/10 NDPB Review of the Animal Procedures Committee*. http://www.homeoffice.gov.uk/publications/apc/publications-2010/review-apc-0910?view=Binary (paragraph 8).
385 The Equidae family includes horses, asses and zebras.
386 Xenotransplantation is defined as the transplantation of cells, tissues or organs from one species to an animal of a different species.
387 For detail on the severity limits of experiments see Guidance on the operation of ASPA, Sections 5.40–5.49.
388 Omand D., (2010). *Report of the 2009/10 NDPB Review of the Animal Procedures Committee*. http://www.homeoffice.gov.uk/publications/apc/publications-2010/review-apc-0910?view=Binary. Para 15.

Box 6.3 The ethical review process under ASPA

The function of the (local) ethical review process is described in the guidance on the operation of ASPA.[389,390] The stated aims of ethical review process are:

- To provide independent ethical advice to the certificate holder, particularly with respect to project licence applications and standards of animal care and welfare.
- To provide support to named people and advice to licensees regarding animal welfare and ethical issues arising from their work.
- To promote the use of ethical analysis to increase awareness of animal welfare issues and to develop initiatives leading to the widest possible application of the 3Rs.

6.2.2 Human Fertilisation and Embryology Act 1990 (as amended)

Scope and purpose

The Human Fertilisation and Embryology Act 1990 (as amended by the Human Fertilisation and Embryology Act 2008) (HFE Act) regulates the creation, keeping and use of human embryos outside the human body, the storage and use of human gametes to create embryos, and the creation and use of human admixed embryos (see Box 6.4). The HFE Act defines, and places clear limits on the use of, human gametes, human embryos and human admixed embryos (see Boxes 6.5 and 6.6). Certain activities are prohibited other than when conducted under licence from the statutory regulator set up under the HFE Act, the Human Fertilisation and Embryology Authority (HFEA, see below).[391,392]

The creation and use of human embryos, and human admixed embryos *per se*, are the principal focus of the HFE Act, and outside the scope of this report (see 1.1). However, the Act is relevant to ACHM research in the following situations:

Application of the HFE Act to ACHM research involving human gametes

Animal models have been developed which involve the implantation of human oocytes and sperm, or immature germ-line cells, into animals (see 3.5). Technically, such research falls within the ambit of the HFE Act as it involves the use of human gametes outside the body. However, a research licence is not required from the HFEA to conduct such studies as they would not result in the production of a human or a human admixed embryo.[393] Research would require a licence under ASPA if it involved the use of a protected animal.

Application of HFE Act to ACHM research resulting in human admixed embryos: the predominance of human material and 'evolving' embryos

The HFE Act applies to embryos that are either entirely or predominantly human or equally human and animal. Human admixed embryos are mainly defined by reference to the scientific processes through which they are created (see Box 6.4).[394] However, there is a 5th sub-section of the definition, in which such embryos are defined by reference to the resulting creation (in which the human DNA predominates). It is easy to imagine situations in which it is far from clear whether a given embryo is more human or more animal, when the amounts of genetic mixture are extensive. Interpretation is complicated by lack of current knowledge of exactly which DNA sequences determine phenotypically critical features of species

389 Guidance on the operation of ASPA, Appendix J.
390 See also RSPCA/LASA (2010). *Guiding principles on good practice* for Ethical Review Processes. http://content.www.rspca.org.uk/cmsprd/Satellite?blobcol=urldata&blobheader=application%2Fpdf&blobkey=id&blobnocache=false&blobtable=MungoBlobs&blobwhere=1232992110664&ssbinary=true
391 The Human Fertilisation and Embryology Act (2008) Act is available at http://www.legislation.gov.uk/ukpga/2008/22/contents. For information on the HFEA see http://www.hfea.gov.uk/
392 At the time of publication, the structure and functions of several public bodies, including the HFEA and the HTA were subject to review under the provisions of the UK Public Bodies Bill [HL] 2010-11. See http://services.parliament.uk/bills/2010-11/publicbodieshl/documents.html
393 The HFE (Special Exemptions) Regulations (2009) provide an exemption from the requirement under the HFE Act for a licence to store gametes for research purposes. See http://www.legislation.gov.uk/uksi/2009/1918/contents/made
394 See HFE Act 1990 as amended, sub section 4A(6).

identity; and by the fact that the cellular composition of an embryo may change over time as some cell types expand faster than others – either by chance or by experimental design (as in the case of tetraploid complementation, see 2.2.2).[395]

These issues were discussed during the passage of the HFE Bill through parliament in 2008. Consideration was given to whether the concept 'predominantly human' in the 5th sub-section (sub-section (e)) implied dominance in purely quantitative terms, or whether the functional significance of the human contribution to the human admixed embryo should be determinative. The response elicited from the Minister was that the latter was the proper interpretation.[396] This clarification is helpful, but the difficulties of the assessment should not be underestimated given the current state of the science in this area (it will become easier as scientific knowledge increases).

What if a predominantly animal embryo containing human material were, during the course of an experiment, to alter in some way leading to human functionality becoming predominant? Under the current legislative framework, if such an outcome was possible it would be necessary to either:

- Hold licences for the research from both the Home Office under ASPA and the HFEA from the outset of the experiment.
- If the outcome was unexpected and the experiment was being conducted solely under a Home Office licence under ASPA, to ensure through close monitoring that the experiment was immediately halted once it became evident the threshold had been reached and to seek authorisation from the HFEA before resuming it.[397]

The difficulty of setting down a precise definition of when the HFE Act applies to embryos containing extensive mixtures of animal and human DNA inevitably means that some potential experiments may need consideration under both pieces of legislation. Part of the reason for the current study is to draw attention to the need to ensure that this process is as smooth and clear as possible, with a minimum of bureaucratic uncertainty and duplication in process while avoiding any chance that contentious experiments might escape suitable scrutiny.

Application of the HFE Act to ACHM research conducted using material from human embryos or human admixed embryos

Animal chimæras can be created by the engraftment of human embryonic cells, or embryonic cell lines into animals. For example, these approaches are used in pre-clinical studies to develop the methodologies for cell replacement therapies (see 3.3.2). A HFEA licence would only be required for the *in vitro* creation of a human embryo, or human admixed embryo, intended either as a source of cells for use in research, or for the subsequent derivation of cell lines.[398]

Human Fertilisation and Embryology Authority (HFEA)

The HFEA, which is constituted under the HFE Act, has responsibility for reviewing applications and issuing licences for licensable activities (including research involving human embryos and human admixed embryos). The HFEA also has responsibility for issuing both policy and clinical guidance within the scope of its remit, and monitoring scientific developments in the field. In contrast to ASPA (see above), the HFEA is fully empowered to make licensing decisions under the HFE Act, acting independently of its

395 Tetraploid complementation involves introducing cells from a donor organism into a recipient embryo at an early embryonic stage. Conditions are manipulated to give the donor cells a competitive advantage – donor cells then generate all the embryonic tissues, while the less favoured recipient cells produce only extra-embryonic (e.g. placental) tissues. The potential of such techniques is important, it illustrates that the proportion of cells and DNA from different origins within an organism can change through embryonic development; and secondly that embryos containing cells entirely derived from one organism could feasibly be generated within a recipient embryo (and maternal host) of another species.

396 See House of Lords Hansard (2008). 29 October, Column 1626. http://www.publications.parliament.uk/pa/ld200708/ldhansrd/text/81029-0009.htm

397 If the experiment was judged to involve the placement of a human admixed embryo into an animal, it would not be authorised by the HFEA.

398 In contrast HFE licences are not required for the research use of cells derived from ES cell lines or human ES cells derived from pre-implantation embryos (though the use of these to create chimæras should be reported to the UK Stem Cell Bank Steering Committee); or disaggregated human embryonic cells. Cells isolated from aborted human fetuses have also been investigated as the basis for cellular therapies; these are not subject to HFEA licensing.

sponsoring Government department (see Box 6.7).

The HFEA's code of practice provides guidance in relation to several aspects of the research use of human, and human admixed, embryos, including general requirements, information to be provided to embryo donors, consent and storage requirements.[399] Cell lines generated from human embryos created under an HFEA licence must be deposited in the UK Stem Cell Bank, at which point, the requirements of codes of practice of the bank will apply to the future use of the cell line. However, it is unlikely that a similar requirement would apply to a human admixed embryo or that the UK Stem Cell Bank would store cell lines from such embryos, or by extension apply its codes of practice to the use of such lines (see 6.2.7).

Box 6.4 Definitions within the HFE Act 1990 (as amended 2008)

Principal definitions

The HFE Act defines human gametes as including human germ-line cells at all stages of development, and human embryos as including human eggs in the process of fertilisation. The principal definitions are:

- '**Embryo**': refers to a live human embryo, and includes an egg that is in the process of fertilisation or is undergoing any other process capable of resulting in an embryo, but does not include a human admixed embryo.
- '**Gamete**': refers to a live human egg, including cells of the female germ line at any stage of maturity, but not including eggs that are in the process of fertilisation or are undergoing any other process capable of resulting in an embryo; or to a live human sperm, including cells of the male germ line at any stage of maturity.
- '**Permitted egg**': refers to an egg which has been produced by or extracted from the ovaries of a woman, and whose nuclear or mitochondrial DNA has not been altered.
- '**Permitted sperm**': refers to sperm which have been produced by or extracted from the testes of a man, and whose nuclear or mitochondrial DNA has not been altered.
- '**Permitted embryo**': refers to an embryo created by the fertilisation of a permitted egg by permitted sperm, where no nuclear or mitochondrial DNA of any cell of the embryo has been altered, and no cell has been added to it other than by division of the embryo's own cells.[400]

Definitions: human admixed embryos

The HFE Act defines five types of human admixed embryo each of which contains human and animal material in equal proportion or with human material in predominance. They can be summarised as:

- '**Cytoplasmic hybrids**': embryos created by techniques used in cloning, using human gametes or cells, and animal eggs. Such embryos are mostly human except for the presence of animal mitochondria.
- **Human–animal hybrid embryos**: embryos created using a human egg and the sperm of an animal, or an animal egg and a human sperm; or by combining a pronucleus of an animal with a human pronucleus.
- **Human transgenic embryos**: embryos created by introducing animal DNA into one or more cells of a human embryo.
- **Human–animal chimæras**: human embryos altered by the addition of one of more cells from an animal.
- Any embryo which does not fall within any of the categories above and which contains both human nuclear or mitochondrial DNA and nuclear or mitochondrial DNA of an animal, but where the animal DNA is not predominant.[401]

399 The HFEA Code of Practice is available at http://www.hfea.gov.uk/code.html
400 See HFE Act 1990 as amended, section 1; section 32A
401 See HFE Act 1990 as amended, sub-section 4A(6). See also Explanatory notes on the HFE Act (2008), Section 4, 31. http://www.legislation.gov.uk/ukpga/2008/22/notes/division/6/1/4

Box 6.5 Activities proscribed by the HFE Act 1990 (as amended 2008)

The following activities are specifically prohibited by the HFE Act:

- Placing any embryo or gametes, other than permitted embryos or gametes, into a woman.
- Placing a human embryo in any animal (where 'animal' means any animal other than man).
- Placing a human admixed embryo in an animal.
- Keeping or using a human embryo, or a human admixed embryo, after either the appearance of the primitive streak or 14 days of development.[402]

Box 6.6 Research involving human admixed embryos in the HFE Act 1990 (as amended 2008)

Research involving human admixed embryos

Licences for research may authorise:

- Mixing sperm with the egg of a hamster, or other animal specified in directions, for the purpose of developing more effective techniques for determining the fertility or normality of sperm, but only where anything which forms is destroyed when the research is complete and, in any event, no later than the two-cell stage.[403]
- Creation, keeping or using human admixed embryos *in vitro*, for the purposes of a project of research specified in the licence.

The principal purposes for which a research licence may be granted:

- Increasing knowledge about serious disease or other serious medical conditions.
- Developing treatments for serious disease or other serious medical conditions.
- Increasing knowledge about the causes of any congenital disease or congenital medical condition (that does not fall within paragraph (1).)
- Promoting advances in the treatment of infertility.
- Increasing knowledge about the causes of miscarriage.
- Developing more effective techniques of contraception.
- Developing methods for detecting the presence of gene, chromosome or mitochondrion abnormalities in embryos before implantation.
- Increasing knowledge about the development of embryos.[404]

402 See HFE Act 1990 as amended sub-section 3(2); sub-section 3(3)b; sub-section 4A(4); sub-section 4A(3)
403 There is a limit of 14 days for research use of all human admixed embryos. In HFE Act (2008) Schedule 2(6), a two-cell limit applies to forms created during the human sperm/hamster egg fertility test.
404 See HFE Act (2008). Schedule 2 (6).

Box 6.7 Comparison of regulatory mechanisms under HFE Act and ASPA

Regulator	HFE Authority	Secretary of State for Home Office (advised by Home Office Inspectorate and APC under ASPA)
What is regulated?	**Human embryos, human admixed embryos and human gametes**	**Protected animals**
Status	Independent authority with statutory licensing powers (independent of Government; of individuals with a professional interest (who under the requirements of the Act must not be in a majority on the HFEA)).	Office of Government with statutory powers licensing powers (and civil servants as agents for the Secretary of State); APC advisory only.
Composition	Group of individuals appointed to time-limited terms of office following an open process. Some rules about composition of authority in statute.	Office of state permanently appointed (Secretary of State acting through civil servants). Rules about composition in statute apply to APC only.
Statute	Set up under governing statute solely for purposes set out in the statute; powers and duties of regulator fully set out in the governing statute.	Regulatory powers under governing statute conferred on Secretary of State that exists separately from ASPA and has much broader functions; powers and duties in relation to its regulatory function under ASPA not fully set out in the governing statute. APC's limited powers and duties set out in statute.
Duty and power	Explicit duty to consider applications and issue licences that meet requirements.	Secretary of State has power but no explicit duty to consider applications and issue licences that meet requirements. APC has no decision making or licensing powers.
Guidance	Explicit duty and power to issue guidance under the Act.	No explicit power or duty on either Secretary of State or APC to issue guidance under the Act.

The HFEA is an independent decision-making body, whose members are appointed by the Health Secretary by an open process for time-limited terms of office. Its composition is governed by the HFE Act itself, which provides that while the Chair cannot be a medical practitioner, or involved in commissioning, or undertaking research related to keeping or using gametes or embryos, this expertise must be represented in the HFEA membership. The APC is an independent advisory body, whose members are appointed by the Home Secretary for time-limited terms of office. The APC's composition is governed by ASPA, which provides that at least two-thirds of the membership must be a veterinary surgeon, medical practitioner or have expertise in a relevant biological science and at least one member must be a lawyer.[405]

405 See ASPA (1986), Section 19.

6.2.3 Human Tissue Act 2004

Scope and purpose

The Human Tissue Act 2004 (the HT Act) is the legal framework in England, Wales and Northern Ireland regulating the storage and use of human organs and tissue from the living, and the removal, storage and use of tissue and organs from the deceased, for health-related purposes and public display.[406] The Act is principally intended to ensure that appropriate consent is in place to enable the lawful retention and use of body parts, organs and tissue, for 'scheduled purposes', which include medical research. The Act also prohibits certain forms of DNA analysis without consent throughout the UK.

The HT Act applies to human bodies and human tissue that consist of, or contain, human cells *other than*: hair and nails from living people; human gametes and embryos; and other human material created outside the human body (e.g. human cell lines).[407] It prohibits the possession of 'bodily material' (from a living or deceased human body, consisting of or including human cells, including hair, nails and gametes) with the intention of analysing its DNA without consent.[408] Except to the extent of the prohibition above, DNA itself (extracted human DNA, where no whole cells remain) is not regulated by the Act.

Application of the HT Act to ACHM research

The requirements of the HT Act apply to the creation of *chimæric* animals using human tissue in some circumstances. For example, where human tissue is removed directly from the body of an identifiable living person, and inserted into an animal the HT Act requirements concerning consent and licences for any storage of such tissue would apply.[409,410] The HT Act would not apply to the creation of *transgenic* animals using 'human-like DNA sequence' (since extracted or artificially synthesised human DNA is not regulated by the HT Act), nor would it apply to the creation of chimæras using human cell lines (since cell lines are outside the scope of the Act).

Human Tissue Authority

The Human Tissue Authority (HTA), regulates and licences the use and storage of human tissue under the HT Act.[411] The HTA's remit does not include ethical approval, which is necessary for research involving human tissue in some circumstances and governed by the National Research Ethics Service (NRES).[412]

6.2.4 Health and safety law, including GM Regulations

ACHM research is subject to general health and safety requirements including the Health and Safety at Work Act (1974) and Carriage of Dangerous Goods legislation. Some types of ACHM research are also subject to the controls set out in the Genetically Modified Organisms (GMO) (Contained Use) Regulations and the GMO (Deliberate Release) Regulations, regulated by the Health and Safety Executive (HSE) and the Department for Environment Food and Rural Affairs (Defra) respectively.[413] The GM regulations are designed to control risks from GMOs to human health (both the contained use and deliberate release regulations) and the environment (the deliberate release regulations only). They apply to biological organisms, cellular (including animal cells in culture) and non-cellular material, other than humans and human embryos, which have been genetically altered other than as a result of a naturally occurring process and which are capable of replicating

406 The Human Tissue Act (2004) is available at http://www.legislation.gov.uk/ukpga/2004/30/contents. Removal of material from the living is regulated separately. The equivalent legislation in Scotland is the Human Tissue (Scotland) Act 2006 (which only applies to post not ante mortem tissue) in Scotland. http://www.legislation.gov.uk/asp/2006/4/contents

407 There is an exception in that the HT Act (2004) applies to stem cell lines intended for human application.

408 Unlike the rest of the HT Act, this provision extends to the whole of the UK, including Scotland.

409 In addition to any requirements under ASPA (1986).

410 Though there are various exceptions to requirements that may be relevant, including (a) a storage licence is not required (1) for tissue stored incidentally to transportation for less than a week or (2) for tissue stored solely for use in a NHS research ethics committee ('REC') approved project; (b) consent is not required (1) for use of tissue imported into England, Wales and Northern Ireland or (2) for use of tissue taken from a living person used in anonymised (to the researcher) form for a REC approved project.

411 For detail on the wider remit of the HTA see http://www.hta.gov.uk/

412 For detail on NRES see http://www.nres.npsa.nhs.uk/

413 For detail on the GMO regulations see http://www.hse.gov.uk/biosafety/gmo/law.htm and http://www.defra.gov.uk/environment/quality/gm/; Certain decisions are reserved to Scottish Ministers in Scotland.

or transferring genetic material. Thus, the regulations apply to transgenic ACHM.[414] We presume the application of the regulations to any particular chimæra will depend on whether their genetic material can be said to have been altered other than as a result of a naturally occurring process (since each cell in a chimæra contains an unmodified genome of one of the precursor animals) and whether any change is capable of transmission, (since the chimærism may not involve the germ cells).

Users of GM animals in contained facilities must notify their facilities to the Health and Safety Executive (HSE), carry out risk assessments addressing both risks to human health and to the wider environment, ensure necessary controls are in place to minimise such risks and notify or seek the consent of (depending on the risk level) the HSE in relation to GM activities. GMOs cannot be released from containment without the approval of Defra following assessment by official assessors to ensure there are no risks to human health or the environment. Deliberate release of GM animals governed by both the GM regulations and ASPA also requires the approval of the Home Secretary (though release of GM animals has never been so authorised). Accidental release must be notified to the HSE.[415]

6.2.5 Intellectual property rights

Intellectual property law does not regulate the conduct of research involving animals containing human material, but can strongly influence whether research takes place, and may impede (or create the conditions to enable) research and development activity. For example, a pharmaceutical company making a transgenic animal expressing a human protein is likely to seek to patent the animal. UK legislation makes provision for biological materials to be patented, including: 'inventions which concern plants or animals' [416] and 'an element isolated from the human body … including the sequence or partial sequence of a gene, even if the structure of that element is identical to that of a natural element'. However 'processes for modifying the genetic identity of animals which are likely to cause them suffering *without any substantial medical benefit* to man or animal', and also animals resulting from such processes cannot be patented. [417]

6.2.6 Data Protection Act 1998

The Data Protection Act 1998 (DPA) is the principal legislation relevant to the use of medical information in research in the UK.[418] It regulates the use of 'personal data', that is, data relating to an individual who can be uniquely 'identified from those data, or from a combination of those data and other information which is in the possession of, or is likely to come into the possession of a data controller'. Although the DPA is generally unlikely to apply to ACHM research, it would apply if a particular individual could be uniquely identified from a section of genetic code/ sequence, obtained through the sequencing of human DNA, when combined with other data in the possession of the same researcher. It seems likely that this could be the case in some circumstances, in which event the provisions of the Act including the requirements relating to consent, fair processing and right of access (by the individual concerned) would apply.

6.2.7 Non-legislative requirements in the UK

In addition to statutory legislation, scientific and medical research is subject to, and guided by, a complex raft of non-legislative guidance, which varies in some cases between the four different administrations within the UK.[419] Some touches on the creation of human admixed embryos, but beyond

414 See the Scientific Advisory Committee on Genetic Modification (SACGM) compendium of guidance. *Part 5 Genetic modification of animals*. http://www.hse.gov.uk/biosafety/gmo/acgm/acgmcomp/index.htm

415 As at March 2011, the HSE were not aware of any incidents of accidental release of GM animals posing any risk to human health.

416 If the technical feasibility of the invention is not confined to a particular plant or animal variety.

417 The relevant UK legislation is the Patents Act (1977), as amended by the Patents Regulations 2000 (SI 2000/2037) – which implemented the provisions of Articles 1 to 11 of the European Directive 98/44/EC on the legal protection of biotechnological inventions. Patent law is overseen by the Intellectual Property Office, an Executive Agency of the Department for Business Innovation and Skills. See http://www.ipo.gov.uk/

418 The Data Protection Act (1998) is available at http://www.legislation.gov.uk/ukpga/1998/29/contents

419 The pathway of regulation and governance of research involving human participants, their tissue or data is addressed in Academy of Medical Sciences (2008). *A new pathway for the regulation and governance of health research*. http://www.acmedsci.ac.uk/p47prid88.html

that, to our knowledge no guidance relates specifically to the creation or use of ACHM as such.[420] Guidance and other non-legislative requirements that have particular impact on ACHM research include the NHS research governance framework (RGF, below) and its equivalent in Scotland, HFEA and HTA codes of practice, professional codes, stem cell banks' codes of practice, guidance from funding bodies (both public and charitable), grant conditions, and publishing requirements. This guidance supports the formal legislation in the development and maintenance of good practice among the research community, including in relation to ACHM research. In many cases it is sufficiently flexible to enable ethical, societal and other issues relating to ACHM research to be identified and considered, notwithstanding that ACHM was not in the contemplation of the draftsmen.

NHS Research Governance Framework (RGF)
The RGF outlines the principles of good governance for research carried out in the NHS, including the different permissions required (e.g. those of the HFEA or the HTA), and more generic requirements which can apply to health research involving NHS facilities, patients, their tissue or data. Although the RGF does not apply to animal research as such, it does set out a regulatory framework, including the requirement for NHS research ethics approval, that is applicable to ACHM research insofar as it involves the use of human tissue or patient data.[421] A similar framework applies in Scotland.

Stem cell guidance
ACHM research that involves the introduction of human stem cells into an animal is guided by the general requirements of the Department of Health Code of Practice for the use of human stem cell lines and the codes of practice of the UK Stem Cell Bank. If the research involves the use of human embryonic stem cells generated under an HFEA licence and supplied by the UK Stem Cell Bank, the Stem Cell Bank Steering Committee must approve the release of the cell line from the bank, and the owner of the line would be required to license its use subject to the HFEA and UK Stem Cell Bank Codes of Practice, which set out general requirements concerning the use of human ESCs (though any resulting animal cell lines could not be deposited in the Stem Cell Bank) (see also 6.2.2). [422]

420 For example, see the HFEA code of practice http://www.hfea.gov.uk/3468.html
421 Department of Health (2005). *Research governance framework for health and social care: Second edition* http://www.dh.gov.uk/en/Publicationsandstatistics/Publications/PublicationsPolicyAndGuidance/DH_4108962; the equivalent in Scotland is the research governance framework for health and community care. http://www.cso.scot.nhs.uk/publications/ResGov/Framework/RGFEdTwo.pdf
422 For details on the UK Stem Cell Bank see http://www.ukstemcellbank.org.uk/; their Code of Practice is available at http://www.ukstemcellbank.org.uk/codesofpractice/codeofpracticefortheuseofhumanstemcelllines.cfm

Box 6.8 Public views on research regulation

Most dialogue participants were aware that medical research is regulated in the UK, though they had little knowledge of how regulation is brought about or the organisations involved. A majority of the workshop participants expressed confidence that, in the UK, the regulation of research involving animals containing human material would be adequate, properly enforced, and reflective of their concerns and principles. This finding was echoed in the survey data, in which 44% of participants agreed that they would trust regulation of such research in the UK (29% said they would distrust such regulation, the remainder were neutral or unsure). Participants' main concerns about the research regulation related to:

- The possibility that permitting some research of this type might lead scientists to seek to conduct unacceptable research in future (a 'slippery slope' argument).
- Knowledge of situations where regulatory errors were thought to have occurred (participants cited the release of foot-and-mouth disease at Pirbright in 2007).
- A suggestion that 'rogue' scientists would evade authorities and regulation.
- The view that research of this kind would not be adequately regulated beyond the UK and so 'malpractice' would take place elsewhere.

'You trust your doctor and your scientists. Not in other countries but the UK is fine'.

Participants indicated several factors which they felt were important for future regulation of research involving animals containing human material, these included:

- A general principle of transparency should be applied, in that information on research of this type should be available in the public domain.
- Regulation should be conducted by independent/impartial people, and a mixture of different interests should be represented (e.g. public members, independent scientists, specifically appointed regulators).
- Regulation should focus on animal welfare, ensuring that animal suffering and the numbers of animals used are minimised.
- Regulation should aim to eliminate risks, including the unintended release of environmental contaminants or disease-causing factors.
- Regulation should be enforced in a manner that prevents evasion by 'rogue' scientists.
- Regulation should be appropriate to the type of animal created and the human tissue and organs involved.

6.3 Summary

As we have noted, ACHM research conducted within the UK is principally regulated by ASPA, though the focus of ASPA and its implementation is on animal welfare and 3Rs, rather than wider ethical considerations.

Although there is some grading within the ASPA system in the form of the four categories of applications that have been identified as requiring review by the APC, the categorisation is principally designed around animal welfare issues rather than broader considerations, and in the case of the fourth category, which potentially addresses broader issues, lacks definition (see Box 6.2). Given the evolving nature of the science associated with ACHM research, **we see considerable benefit in further developing a graded approach to licensing and regulatory oversight, which is principle based and transparent, seeks to define different levels of sensitivity and differentiates the degrees of scrutiny required accordingly**. We propose a possible approach in Chapter 8 (see 8.2).

Recognising the specialist knowledge required to evaluate likely (and sometimes uncertain) outcomes in this complex field of science, as well as the socially sensitive nature of the judgements to be made, we would also consider that a national expert body, which includes the relevant expertise, is needed to advise on ACHM research (see 8.3). In order to build and maintain trust and ensure accountability to the public, the body needs to operate transparently, be outward facing and engage with the public and the scientific community. To ensure consistency and transparency, it needs to have the power to develop guidelines. There would also be considerable importance and value in it playing a broader function, including the role of sharing knowledge and best practice attributed to the national committee required under the 2010 EU Directive.

As we have set out, the regulatory environment is complex. There are several pieces of UK legislation relevant to the regulation of ACHM. In some cases, more than one regulatory regime applies to a specific piece of ACHM research, or the research is at the borders of specific regimes. Aside from complexity, this also creates the possibility of inconsistency between regulatory regimes. To manage this effectively, a key feature of the UK regulatory environment in the future needs to be that **all relevant stakeholders (Home Office, HFEA, HTA and others) develop a coordinated, consistent approach to regulating the field of research, work together under an agreed framework of operation to continue to monitor scientific developments and consider jointly how to address borderline cases (see 8.6)**. Borderline cases include experiments that involve animal embryos containing human cells or genes that are close to the boundary of human admixed embryos under the HFE Act as well as certain ACHM experiments that involve a degree of uncertainty as to outcome. Regulatory guidance is likely to be particularly helpful in such cases.

Table 6.1 Regulators and regulatory approvals relevant to research involving ACHM in the UK[423]

Regulated research activity	Scope	Regulatory approval required	Legislation	Jurisdiction	Regulator	Sponsor Dept
Use of 'protected' animals which may cause the animal pain, suffering, distress or lasting harm[424]	'Protected' animals include live vertebrates and octopuses and embryonic/ fetal/ larval forms from mid-point of gestation/ incubation/ from point of independent feeding	1. Personal licence 2. Project Licence 3. Certificate of Designation for premises 4. Local ethical review process	ASPA 1986	UK	Secretary of State supported by: ASPD&I, APC, local ethics panel	HO
Use of human gametes	–	None	HFE Act 1990 as amended	UK	Human Fertilisation and Embryology Authority (HFEA)	DH
Use of human embryos	Human embryos (including human eggs in the process of fertilisation)	1. Research licence				
Use of human admixed embryos	Human admixed embryos as defined in HFE Act	1. Research licence				
Storage of human tissue for research	Cellular material from the human body other than embryos, gametes, and hair and nail from the living	1. Storage licence 2. REC approval or use (within limits) of human tissue from licensed tissue bank	HT Act 2004	England Wales and & NI	Human Tissue Authority (HTA)	DH
Use of NHS patients, non-NHS patients and healthy volunteers, their tissue or their data		REC approval		UK	NHS Research Ethics Committees (RECS)	DH
Deliberate release of genetically modified organisms	Genetically modified (other than naturally) organisms capable of replicating or transferring genetic material		GM (Deliberate Release) Regulations		Defra	Defra
Contained use and accidental release of genetically modified organisms	Genetically modified (other than naturally) organisms, including animal cells in culture but excluding humans and human embryos, capable of replicating or transferring genetic material		GM (Contained Use) Regulations		Health and Safety Executive	DWP
Clinical trials	Medicines Devices Products	Marketing authorisation/ regulatory approval	Medicines for Human Use (Clinical Trials) Regulations 2004	UK	Medicines and Healthcare products Regulatory Agency (MHRA) (also EMEA)	DH

423 Abbreviations included in Table 6.1: HO, Home Office; DH, Department of Health; DWP, Department for Work and Pensions.
424 Disregarding the effect of any anaesthetic/other process rendering the animal insentient. Some ACHM research requires regulatory approval from more than one body – the approvals are not mutually exclusive.

7 International perspective

7.1 Introduction

In Chapter 6 we described the law and regulation applicable to the creation and use of ACHM in biomedical research in the UK. However, like much biomedical research, research involving ACHM is an international activity. It frequently involves international collaboration, takes place across national boundaries, and involves funders or researchers who are often free to choose the location in which their research is conducted. In this chapter, we outline the regulation of this research from an international perspective and consider some of the challenges this poses.

As far as we are aware, very few countries have specifically considered the regulation of research involving ACHM. As in the UK, to our knowledge there are no specific national laws, regulation or guidance documents addressing ACHM research, though a range of laws and regulatory frameworks, particularly those governing the use of animals, cover different aspects of this research.

A similar pattern of legislation is evident at European Union level; whilst there is no specific European legislation on ACHM, there are European equivalents of many (though not all) UK and other national European laws which are of relevance. Internationally, and within European states, broad principles that may be applied to ACHM research are addressed in legal instruments (largely in the context of human cloning).

Guidelines developed by international groups address aspects of ACHM research, particularly the use of human stem cells to create inter-species chimæras. Adoption of these guidelines, though largely voluntary, provides a basis for the development of international best practice in this field, which would be of particular value given the degree of diversity in national laws and regulation.

7.2 National regulation and international research

Research involving ACHM, like other forms of medical research, is principally governed by national law and regulation. Although these may derive from international instruments (such as European Directives), legislation relevant to research is, in the main, implemented and enforced at national level, and research is therefore predominantly governed solely by the laws of the country where it takes place. Occasionally, research is regulated extra-territorially; for example in some cases, researchers are subject to the laws of the country of which they are citizens, even when they conduct research elsewhere. However, this is relatively unusual (for example, none of the regulations discussed in Chapter 6 apply to research conducted solely outside the UK, even where conducted by UK citizens). It is considerably more likely that the conditions and requirements imposed by funding and professional bodies operate extra-territorially.[425]

Research involving ACHM conducted across different national locations needs to be designed to take into account legal and regulatory divergence, with research in each national area potentially being subject to different legal and regulatory limits and controls. Although, as this chapter will show, there is some harmonisation (for example, the recent European Directive was intended to promote greater standardisation around the use of animals in research) this is relatively limited. There remains considerable diversity across nations beyond the European Union, both in regulation of the research use of animals, and other aspects of ACHM research (e.g. the use of human tissue).

Guidelines developed by international groups can encourage common standards and aid researchers working across national borders in navigating divergent governance systems.

425 For example, research involving stem cells funded by the US National Institutes of Health (NIH) must accord with their guidance even if conducted in the UK (see http://stemcells.nih.gov/policy/2009guidelines.htm). The UK's Medical Research Council has supplementary terms and conditions for research that it funds that involves human stem cells (see AC24 in http://www.mrc.ac.uk/Utilities/Documentrecord/index.htm?d=MRC001898).

However, these are currently limited in relation to ACHM research, and only address specific aspects or types of ACHM.[426]

National divergence poses a risk of researchers locating their research in certain countries in order to avoid particular national restrictions. The development of international standards backed by collaboration between national and international policymakers may help to reduce this risk, as well as facilitate cross-border research. It would also be beneficial for national regulators to collaborate, and to encourage international data-sharing, where evidence from incremental research studies has been acquired (see Box 3.8).

7.3 Europe

7.3.1 European Union

Some of the UK legislation governing research involving ACHM originates from European Union law (two notable exceptions are the Human Fertilisation and Embryology Act, which has no EU equivalent, and the Human Tissue Act, which only reflects EU law in certain limited respects).[427] The application of EU Directives on the protection of animals used for scientific purposes, the use of genetically modified organisms, data protection and patents to ACHM research, is summarised below.

EU legislation for the protection of animals

Directive 86/609/EEC on the protection of animals used for scientific purposes was revised in September 2010 by Directive 2010/63/EU, which is due to be implemented in all EU member states by 2013 (see also 6.2.1).[428,429] The revised Directive is the principal European legal framework relating to ACHM research. It applies to regulated scientific procedures involving non-human vertebrates, including larval forms capable of independent feeding and fetal forms of mammals in the last third of their development, and to cephalopods (a class of molluscs including octopi and squid). The Directive places clear limits on the scientific procedures that can be carried out using such animals, and places emphasis on the welfare principles of the 3Rs (reduction, refinement and replacement, see 4.1.1). A minority of ACHM research may be outside the scope of the Directive, as it does not apply to research involving vertebrate animals at less than two-thirds of gestation or invertebrate animals (see 6.2.1).[430]

Creation and use of genetically modified entities

ACHM research may also be within the remit of European Directives intended to safeguard against environmental, and health and safety risks associated with the use, storage and containment, and disposal or release of genetically modified organisms and microorganisms. Directive 2009/41/EC lays down measures for the contained use of genetically modified microorganisms (microbiological entities both cellular and non-cellular, in which genetic material has been altered other than naturally) (GMMs).[431] Directive 2001/18/EC (as amended in 2008) lays down requirements concerning the deliberate release into the environment of genetically modified organisms (biological entities other than human beings capable of replicating or transferring genetic material, in which the genetic material has been altered other than naturally) (GMOs).[432] In particular, it requires users to conduct risk assessments and

426 Examples of international guidelines include CURE Report China (http://www.mrc.ac.uk/Utilities/Documentrecord/index.htm?d=MRC006303); Swiss Commission for Research Partnership with Developing Countries KFPE (1998) (http://www.kfpe.ch/download/Guidelines_e.pdf); and further examples in 'The ethics of research related to healthcare in developing countries: a follow-up discussion paper' available at http://www.nuffieldbioethics.org/research-developing-countries-follow)

427 The European Tissue Directive 2004/23/EC applies only to human tissue for human application, and is not relevant to the use of human tissue for research (http://eur-lex.europa.eu/LexUriServ/site/en/oj/2004/l_102/l_10220040407en00480058.pdf).

428 Council Directive 86/609/EEC of 24 November 1986 on the approximation of laws, regulations and administrative provisions of the Member States regarding the protection of animals used for experimental and other scientific purposes is available at http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CELEX:31986L0609:en:HTML

429 Directive 2010/63/EU on the protection of animals used for scientific purposes is available at http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2010:276:0033:0079:EN:PDF

430 See 6.2.1 for a discussion of the implementation of Directive 2010/63/EU in the UK.

431 Directive 2009/41/EC on the contained use of genetically modified microorganisms is available at http://www.bmwf.gv.at/fileadmin/user_upload/forschung/gentechnik/2009-41-EC.pdf

432 Directive 2001/18/EC 2001 on the deliberate release into the environment of genetically modified organisms is available at http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CELEX:32001L0018:EN:HTML

to notify and seek the consent of the competent national authority prior to GMO release.[433]

Patent law

Directive 98/44/EC on the legal protection of biotechnological interventions limits the legal protection of inventions including biological material, and may therefore affect some ACHM research (see 6.2.5).[434] In addition to generic requirements, the Directive sets limits to patentability on moral grounds, specifically outlawing the patenting of inventions the exploitation of which would be contrary to *'ordre public or morality'* and *'processes, the use of which offend against human dignity …'.*[435,436] This definition includes processes for modifying the genetic identity of animals which are likely to cause them suffering without any substantial medical benefit to man or animal, and animals resulting from such processes.[437] Objections to patents on the grounds of these provisions have been raised in a number of cases. The issue was explored in relation to a challenge to the oncomouse patent for example, though the arguments were ultimately rejected by the European Patent Office and the patent upheld.[438,439]

Protection of personal data

ACHM research conducted within the European Union which involves human tissue or data from which a living individual can be identified, is subject to the requirements of the Data Protection Directive (Directive 95/46/EC).[440] In particular, the Directive includes a requirement that the individual concerned is made aware of and has, subject to limited exceptions, given their consent for the use of their tissue or data.

7.3.2 Council of Europe

Convention on Human Rights and Biomedicine (1999)

The Council of Europe Convention on Human Rights and Biomedicine sets out international standards concerning the protection of human rights in relation to biology and medicine.[441] The Convention does not specifically address ACHM research, but includes principles that may be considered of relevance. Notably, it places emphasis on the protection of '*the dignity and identity of all human beings*' and the importance of '*the need to respect the human being both as an individual and as a member of the human species*', recognising '*the importance of ensuring the dignity of the human being*' and '*that the misuse of biology and medicine may lead to acts endangering human dignity*'.[442] The absence of any definition of 'human being' has enabled considerable diversity of interpretation of the Convention across Europe.[443]

7.4 International

7.4.1 International legal instruments

A range of international legal instruments are of broad relevance to medical research, including that involving the use of human

433 In England and Wales the competent authority enforcing jurisdiction over the GMO (Contained Use Regulations) includes the Health and Safety Executive (HSE), the Secretary of State and the Department for Environment Food and Rural Affairs (Defra). In Scotland the competent authorities includes HSE, the Scottish Executive Environment and Rural Affairs Department and the Scottish Ministers. Northern Ireland has its own separate competent authority. The UK Government and Devolved Administrations have established joint arrangements for assessing applications for the deliberate release of GMOs. This involves consultation with the Advisory Committee on Releases to the Environment (ACRE), the HSE, the Food Standards Agency (FSA), and as appropriate, the statutory nature conservation bodies, such as English Nature. For the HSE's guidance see http://www.hse.gov.uk/biosafety/gmo/acgm/acgmcomp/part5.pdf

434 Directive 98/44/EC on the legal protection of biotechnological inventions is available at http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:1998:213:0013:0021:EN:PDF

435 Reference to 'ordre public' is made in Article 6 of Directive 98/44/EC (which is implemented through Article 53(a) of the European Patent Convention regulations).

436 Recital 38 of Directive 98/44/EC continues '…such as processes to produce chimæras from germ cells or totipotent cells of humans and animals, are obviously also excluded from patentability'.

437 Article 6 of Directive 98/44/EC defines as unpatentable processes for cloning human beings, processes for modifying the germ line genetic identity of human beings and uses of human embryos for industrial or commercial purposes.

438 EPO Case No. T0315/03 (transgenic animals/HARVARD); 6 July 2004 http://www.epo.org/law-practice/case-law-appeals/pdf/t030315ex1.pdf

439 A second case (Brüstle vs. Greenpeace EV, (Case no. C34/10) which had yet to be considered by the European Court of Justice at the time of writing) was to be the first case before the Court to involve consideration of Article 6(2)(c) of EU Directive 98/44 (non-patentability of use of human embryos for industrial or commercial purposes as being contrary to *ordre public*). The 'opinion of the attorney general' in the case was published on 10 March 2011.

440 Directive 95/46/EC on the protection of individuals with regard to the processing of personal data and on the free movement of such data is available at http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CELEX:31995L0046:EN:NOT

441 The Convention for the Protection of Human Rights and Dignity of the Human Being with regard to the Application of Biology and Medicine: Convention on Human Rights and Biomedicine is available at http://conventions.coe.int/Treaty/en/Treaties/html/164.htm. The UK has not acceded to this convention.

442 *Ibid* Preamble and Article 1.

443 For example, it leaves open whether a human embryo or fetus could be considered a human being and a rights holder under the Convention, and by extension the application of the Convention in relation to assisted reproduction and use of human embryos in research.

genetic or cellular material.[444] However, the only international legal instruments that might be applied to ACHM research of which we are aware are non-binding instruments, intended principally to address human cloning.

This UNESCO Universal Declaration on the human genome and human rights (1977) was the first international legal text to address the relationship of biotechnological development and human rights with the human genome.[445] The non-binding declaration states that '*No research, or its applications, should prevail over the respect for human rights, fundamental freedoms, and human dignity of individuals or groups of people*' and that '*Practices contrary to human dignity, such as the reproductive cloning of human beings, shall not be permitted.*'[446]

A second non-binding declaration, the UN Declaration on human cloning (2005), was adopted by a weak majority vote in which UN states were called on to '*adopt the measures necessary to prohibit the application of genetic engineering techniques that may be contrary to human dignity*'.[447] The application of these Declarations to the creation or use of ACHM is a matter of interpretation (e.g. it is disputable whether the creation of ACHM would be considered 'contrary to human dignity'). However, as UN declarations, they are likely to influence some states' national laws, policies and practices.

7.4.2 International guidance

At an international level, the instruments that are of most direct relevance to ACHM research are guidelines developed by funding bodies, scientists and other groups. These include guidance on the use of human ES cells, and other human stem cell types. In these areas the development of international guidelines is relatively mature.

International Society for Stem Cell Research (ISSCR) Guidelines for the Conduct of Human Embryonic Stem Cell Research (2006)

The ISSCR guidelines specify rigorous ethical standards for scientists working with human ES cells and seek to promote responsible, transparent and uniform practices worldwide.[448] They set out a categorisation of research involving stem cells and prescribe the required nature of regulatory review and oversight for each category of research (see Box 7.1). Research involving the incorporation of human ES cells and other human stem cells into animals (i.e. the creation of human–animal chimæras) is addressed, and specific forms of research which should not be pursued at present are identified (see Box 7.1). The guidelines also:

- Encourage the deposition of derived human stem cell lines in national or international depositories that allow open distribution, to facilitate the wider dissemination of these valuable research tools.
- Set out guidance for procurement of tissue for human ES cell research, and specify minimum requirements for obtaining informed consent of donors.
- Indicate that funding organisations should pledge to comply with the guidelines, and that publishers should require a statement of compliance with them.

Hinxton Group

In early 2004, members of the Stem Cell Policy and Ethics Program (SCOPE) at the Johns Hopkins Berman Institute of Bioethics brought together an international and interdisciplinary group to explore the ethical and policy challenges of transnational scientific collaboration raised by variations in national regulations governing embryo research and stem cell science. Drawn from delegates at an

444 For example, the Declaration of Helsinki of the World Medical Association on Ethical Principles for Medical Research Involving Human Subjects, adopted in 1964 (as amended), the International Ethical Guidelines for Biomedical Research Involving Human Subjects of the Council for International Organizations of Medical Sciences, adopted in 1982 (as amended) and the UNESCO Universal Declaration on Bioethics and Human Rights adopted in 2005.

445 The Universal Declaration on the Human Genome and Human Rights is available at http://portal.unesco.org/en/ev.php-URL_ID=13177&URL_DO=DO_TOPIC&URL_SECTION=201.html

446 *Ibid* Articles 10–11.

447 The United Nations Declaration on Human Cloning (2005) is available at http://daccess-dds-ny.un.org/doc/UNDOC/GEN/N04/493/06/PDF/N0449306.pdf?OpenElement. For the associated press release, see http://www.un.org/News/Press/docs/2005/ga10333.doc.htm

448 International Society for Stem Cell Research Guidelines (2006) are available at http://www.isscr.org/guidelines/ISSCRhESCguidelines2006.pdf

Box 7.1 Categorisation of experiments, from the ISSCR guidelines for the conduct of human embryonic stem cell research[450]

Category 1: Experiments that are permissible after review under existing mandates and by existing local committees, and are determined to be exempt from full Stem Cell Research Oversight (SCRO) review.[451]

Category 2: Forms of research that are permissible only after additional and comprehensive review by a specialised mechanism or body established to address the issues pertinent to stem cell research (i.e. the SCRO function). This category includes:

- Forms of research that generate chimæric animals using human cells. Examples of such forms of research include, but are not limited to introducing totipotent or pluripotent human stem cells into non-human animals at any stage of post-fertilisation, fetal, or postnatal development.
- In general, chimærism of the cerebral cortex or the germ-line are of greatest concern.[452]

Category 3: Research that should not be pursued at this time because of broad international consensus that such experiments lack a compelling scientific rationale or raise strong ethical concerns. Such forms of research include:

- Research in which any products of research involving human totipotent or pluripotent cells are implanted into a human or NHP uterus.
- Research in which animal chimæras incorporating human cells with the potential to form gametes are bred to each other.[453]

initial meeting in 2004, the Hinxton Group is an informal collection of individuals interested in ethical and well-regulated science, coordinated by a US/UK steering committee.[449]

National Institutes of Health Guidelines on the use of Human Stem Cells (2009)

These guidelines apply to research involving human embryonic stem cells and certain uses of human induced pluripotent stem cells.[454] Although designed in relation to research funded by the US National Institutes of Health (NIH), they have wider influence and place clear practical limits on certain categories of ACHM research. In the US, the guidelines limit the use of NIH funding for research involving human ES cell lines to those approved lines listed on the NIH Registry and prohibit NIH funding of:

- Reserach in which human ES cells or human iPS cells are introduced into NHP blastocysts.
- Research involving the breeding of animals where the introduction of human ES cells or human iPS cells may contribute to the germ line.[455]

Final Report of The National Academies' Human Embryonic Stem Cell Research Advisory Committee and 2010 Amendments to The National Academies' Guidelines for Human Embryonic Stem Cell Research.

Guidance from the US National Academies intercalates with, and extends, the NIH

449 The three meetings of the Hinxton Group (www.hinxtongroup.org) have dealt with: 'Transnational cooperation in stem cell research', 'Science, ethics and policy challenges of pluripotent stem cell-derived gametes' and 'Policies and practices governing data and materials sharing and intellectual property in stem cell science'.
450 The International Society for Stem Cell Research Guidelines (2006) are available at http://www.isscr.org/guidelines/ISSCRhESCguidelines2006.pdf
451 *Ibid* Extracts: Section10.1. In accordance with the ISSCR guidelines, 'Each institution, academic or commercial, that engages in human stem cell research shall determine an appropriate Stem Cell Research Oversight (SCRO) procedure, either internal or external, by which their researchers will be subject to review, approval, and monitoring of their human stem cell research activities.' The requirements for this procedure are detailed in the guidelines (Sections 8–9).
452 *Ibid* Section 10.2e.
453 *Ibid* Section 10.3b and 10.3c.
454 National Institutes of Health Guidelines on Human Stem Cell Research (2009) are available at http://stemcells.nih.gov/policy/2009guidelines.htm
455 *Ibid* Section IV.

guidelines to provide guidelines for non-federally funded research involving human ES cells and other human stem cell types.[456] The National Academy of Sciences (NAS) guidance acts as the principal reference on the limits of permissible research uses of embryonic stem cell lines (only briefly addressed in the NIH guidelines) and sets out specific recommendations applicable to research using inter-species chimæras involving human embryonic stem cells (Box 7.2) and other stem cell types (Box 7.3). These guidelines established a categorisation for certain types of ACHM; we suggest a similar approach would be of value in the UK (see 8.2).

A number of other reports have considered aspects of research involving ACHM at national or European level (Box 7.4).

Box 7.2 Extracts from NAS guidance on the research use of human ES cells

The US National Academy of Sciences (NAS) guidance sets out requirements in relation to particular uses of human ES cells. These include:

- All protocols involving the combination of human ES cells with non-human embryos, fetuses, or adult vertebrate animals must be submitted to the local Institutional Animal Care and Use Committee (IACUC) for review of animal welfare issues and to the Embryonic Stem Cell Research Oversight (ESCRO) committee for consideration of the consequences of the human contributions to the resulting chimæras.
- Transplantation of differentiated derivatives of human ES cells or even human ES cells themselves into adult animals will not require extensive ESCRO committee review. If there is a possibility that the human cells could contribute in a major organised way to the brain of the recipient animal, however, the scientific justification for the experiments must be strong, and proof of principle using non-human (preferably primate) cells, is desirable.
- Experiments in which human ES cells, their derivatives, or other pluripotent cells are introduced into non-human fetuses and allowed to develop into adult chimæras need more careful consideration because the extent of human contribution to the resulting animal may be higher. Consideration of any major functional contributions to the brain should be a main focus of review.
- Introduction of human ES cells into non-human mammalian blastocysts should be considered only under circumstances in which no other experiment can provide the information needed.[457]

Defined categories of human ES cell research, include:

- **Permissible after ESCRO committee review:**
  - Research involving the introduction of human ES cells into non-human animals other than humans or primates at any stage of embryonic, fetal, or postnatal development.
  - Research involving the introduction of human ES cell into NHPs at any stage of fetal or postnatal development.
- **Currently prohibited:**
  - Research in which human ES cells are introduced into NHP blastocysts or in which any embryonic stem cells are introduced into human blastocysts.
  - No animal into which human ES cells have been introduced such that they could contribute to the germ line should be allowed to breed.

Guidance indicates that particular attention should be paid to at least three factors: the extent to which the implanted cells colonise and integrate into the animal tissue; the degree of differentiation of the implanted cells; and the possible effects of the implanted cells on the function of the animal tissue.[458]

456 National Academies (2010). *Final report of the National Academies' human embryonic stem cell research advisory committee and 2010 amendments to the National Academies' guidelines for human embryonic stem cell research. Appendix C: National Academies' guidelines for human embryonic stem cell research amended as of May 2010.* Available at http://books.nap.edu/openbook.php?record_id=12923&page=19

457 *Ibid* Sections 6.4–6.7.

458 *Ibid* Sections 1.3a–1.3c.

Box 7.3 NAS guidance on the use of non-embryo-derived human pluripotent stem cells and multipotent neural stem cells

Proposals for use of human pluripotent stem cells in animals should be considered in one of the following categories:

- **Permissible after currently mandated reviews and proper documentation.**
  Experiments that involve only transplantation into postnatal animals with no likelihood of contributing to the central nervous system or germ line.
- **Permissible after additional review by an ESCRO committee.**
  Experiments in which there is a significant possibility that the implanted human pluripotent stem cells could give rise to neural or gametic cells and tissues. Such experiments would include generation of all preimplantation chimæras as well as neural transplantation into embryos or perinatal animals.
- **Should not be conducted at this time:**
  (1) Experiments that involve transplantation of human pluripotent stem cells into human blastocysts.
  (2) Research in which human pluripotent stem cells are introduced into NHP embryos, pending further research that will clarify the potential of such introduced cells to contribute to neural tissue or to the germ line.
- **Prohibition on Breeding:**
  No animal into which human pluripotent stem cells have been introduced such that they could contribute to the germ line should be allowed to breed.[459]

Multipotent neural stem cells

'It is also relevant to note that neural stem cells, although not pluripotent, are multipotent and may have the potential to contribute to neural tissue in chimeric animals. ESCRO committees should decide whether they wish to review and monitor such experiments with neural stem cells in a similar fashion.' [460]

459 *Ibid* Section 7.5.
460 *Ibid* Section 7.4.

Box 7.4 Other initiatives

- **Human–animal combinations in stem cell research.** In 2010 a working group of the Singapore Bioethics Advisory Committee reviewed national ethical, legal and social issues related to research involving cytoplasmic hybrids and human–animal chimæras involving human stem cells. Ethical issues and regulatory policies in other major scientific jurisdictions were also examined. The group recommended a prohibition on breeding animals into which human pluripotent stem cells had been introduced, and emphasised the need, where research involves the introduction of pluripotent human stem cells into animals, to avoid the creation of entities in which human sentience or consciousness might occur.[461]
- **German Ethics Council Opinion on human–animal mixed-species entities.** The German Ethics Council's opinion is under consideration following a public survey and meeting of international experts on 'human–animal mixed-species entities' in 2010.[462]
- **Chimbrids.** The 'Chimæras and hybrids in comparative European and International research' study involved researchers from 15 European states and six further nations in 2005–2007. Scientific, ethical, philosophical and legal aspects of research involving inter-species mixtures were addressed. It was recommended that 'chimbrid' research proposals should be independently examined by an interdisciplinary body; and particular experiments to be subject to prohibition or special consideration were identified.[463]
- **ESTOOLS Ethics Workshop 2.** This multi-national group of European stem cell researchers held a workshop in Lund, Sweden, in October 2008 which considered 'ethical aspects of research on inter-species embryos and iPS cells', including ethical and regulatory aspects of inter-species embryo research.[464]
- **Man or mouse? Ethical aspects of chimæra research.** In 2006–7 the Danish Ethical Council for Animals and Danish Council of Ethics conducted a joint study which included ethical discussion of research involving human–animal chimæras. Modification of Danish regulation was recommended to ensure that chimæras 'difficult to place biologically, ethically and legally' would not be created; however, these recommendations have not yet been enacted.[465]
- **The Cultural, Ethical and Spiritual Dimensions of the Use of Human Genes in Other Organisms.** The New Zealand Bioethics Council's 2003–4 study included a programme of public consultation across broad demographics. Its recommendations included that genetic manipulations, intended to produce social or mental capacities in animals that are recognisably human-like, or produce significant morphological changes in life forms to make them more similar to human life forms, should not be pursued.[466,467]

461 The Bioethics Advisory Committee Singapore (2010). *Human–animal combinations in stem cell research.* http://www.bioethics-singapore.org/uploadfile/62913%20PMFull%20HAC%20Report.pdf

462 The German Ethics Council (2010). http://www.ethikrat.org/press/press-releases/2010/press-release-02-2010

463 The Coordination Action Chimbrids (Chimæras and Hybrids in Comparative European and International Research: scientific, ethical, philosophical and legal aspects) (2009). Taupitz J., & Weschka M, Springer. See http://www.jura.uni-mannheim.de/imgbchimbrids/index.php?option=com_content&task=view&id=12&Itemid=31

464 ESTOOLS is the largest grouping of human embryonic and induced pluripotent stem cell researchers in Europe. Spanning 10 countries, the project brings together the combined expertise of 21 academic and commercial research teams. For the report of the Ethics Workshop 2 see http://www.estools.eu/assets/files/Uploaded_Files_1/Lund%20workshop/ESTOOLS%202nd%20Ethics%20workshop%20October%202008%20Lund%20Report.pdf

465 The Danish Council of Ethics (2008) *Man or mouse? Ethical aspects of chimæra research* http://etiskraad.dk/upload/publications-en/stem-cell-research/man-or-mouse/index.htm.

466 The Bioethics Council of New Zealand (2004). *The Cultural, Ethical and Spiritual Dimensions of the Use of Human Genes in Other Organisms* http://ndhadeliver.natlib.govt.nz/ArcAggregator/frameView/IE1074184/http://www.bioethics.org.nz/. The Council disbanded in 2009.

467 For completion we note that a draft US Senate Human Chimæra Prohibition Act was introduced in 2005 following a recommendation in a report by the President's Council on Bioethics (President's Council on Bioethics (2004). Reproduction and Responsibility: The Regulation of New Biotechnologies; http://bioethics.gov/reports/reproductionandresponsibility/chapter10.html). The Bill sought to prohibit the creation human chimæras, including attempts to create, transfer or receive them. The Bill was endorsed in the 2006 State of the Union Address but did not become law. A draft Human–animal Hybrid Prohibition Act, introduced in 2009, did not become law.

As in many fields of science, much research involving ACHM depends on collaborative working between groups of scientists working in different legal jurisdictions. Whilst intra- and international scientific collaboration is vital to the success of such research, this can create regulatory challenges where work is conducted under different legislative frameworks.

We endorse the views of bodies such as the Hinxton group in encouraging international coordination, which stated in relation to human ES cell research: '*Steps should be taken to develop consensus in ethical standards and practices in hESC research for international collaboration to proceed with confidence and for research from anywhere in the world that adheres to these standards and practices to be accepted as valid and valuable by the scientific community and academic journals. To achieve this goal, it will be necessary to specify what these standards and practices should be through the international efforts of scientists, philosophers, bioethicists, lawyers, clinicians, journal editors and regulators involved in this field, in collaboration and consultation with the public. This process of identification of international ethical standards and practices should include concerted efforts to engage people throughout the world in honest and realistic conversations about the science and ethics of stem cell research and its emerging applications.*'[468]

We believe this statement has equal validity in relation to ACHM research, particularly given the diversity across national regulation and practice outlined in this Chapter. **We therefore strongly encourage initiatives to raise awareness and promote consistency in research practice at an international level, which could be led by regulators, policy-makers, national and international bioethics bodies, medical research councils or the research community itself. The UK is well placed to take a lead in encouraging such dialogue** (see 8.7).

468 Hinxton Group Consensus statement 2006: http://www.hinxtongroup.org/au_trans_cs.html

8 Conclusions and recommendations

8.1 Overview

We have reviewed the types of research conducted using animals incorporating human gene sequences or human cells. The overall purposes of such work are to study the function of human genes and cells, to create improved animal models of human disease, and to develop, produce and test novel therapeutic products. Not all such experiments are successful, as in all types of science, but this research has yielded important new knowledge and significant insights with promise for the future, as well as methods and products that have considerable clinical value.

8.1.1 ACHM and animal research

Consideration of the research use of ACHM must always be set in the general context of animal research, which is tightly regulated in the UK under the Animal (Scientific Procedures) Act (ASPA), such that any suffering inflicted on a protected animal must be justified by the potential value of the research, and animal welfare principles, as commonly embodied in 3Rs, must be applied.[469] Comparable national regulation exists in many scientifically advanced countries, and is incorporated in the European Directive (2010/63/EU). We see no reason to either relax or tighten UK standards in the case of ACHM. However, we have considered whether any additional scrutiny might be required for ACHM research.

8.1.2 ACHM history and prospects

Research involving ACHM has a long history. No specific safety or regulatory concerns have emerged from such research to date, although a few issues have prompted ethical debate (see 8.5 for discussion of safety issues). Developments in transgenesis and particularly in stem cell research lead us to anticipate a major increase in the use of these techniques to investigate the biological effects of normal and abnormal human genes and cells in animals: to study their roles in development, normal function and human disease processes; to test the safety and efficacy of novel therapeutics (particularly biological therapeutics); and to produce clinically useful proteins, cells and tissues.

These approaches hold promise for advancing biomedical and biological research but, as with virtually all scientific developments, we repeat our caution that not all avenues explored will prove fruitful; and that the timescales between initial research and applicable health interventions are long (up to decades), variable and impossible to predict with confidence. The use of ACHM can also offer approaches which may advance the 3Rs principles, improving the effectiveness of animal use by making individual experiments more informative about human biology.[470]

8.1.3 ACHM ethical and societal aspects

The great majority of experiments that we can currently anticipate do not present novel ethical issues and should continue to be satisfactorily regulated under the existing framework governing all animal research. They include familiar experiments such as the creation of transgenic rodents containing relatively small numbers of human genes, tissue grafting, and the transfer of tissue-specific stem cells to humanise individual organs.

Evidence we received, the public dialogue, the published literature and our own deliberations, identify a limited number of research areas which may require greater scrutiny. These include research that may raise issues of ethical and social acceptability or have unusual implications for the animals involved. Experiments that approach these sensitive areas may, however, be of substantial medical and scientific importance. We therefore propose that such research projects should remain eligible for consideration for licensing by the appropriate regulatory authorities (see sections 8.3 and 8.6), but subject to additional expert scrutiny.

469 The 3Rs principles are that experiments involving animals can be licensed only if there are no scientifically suitable alternatives that *replace* animal use, *reduce* the number of animals needed or *refine* the procedures used to cause less suffering (see 4.1 and 6.2.1).

470 This is not to imply that we expect *overall use* of animals in medical research to diminish in the short term as a result of research involving ACHM, in part because their development will open up new avenues of research involving animal experimentation.

8.2 Categorisation of ACHM

We propose that experiments involving ACHM could be usefully classified into three categories:[471]

8.2.1 Category 1

The great majority of ACHM experiments, as outlined in section 8.1.3 above, which do not present issues beyond those of the general use of animals in research, should be subject to the same oversight and regulation under ASPA as other animal research.

8.2.2 Category 2

A limited number of types of ACHM research, outlined below in this section (8.2.2), should be permissible subject to additional specialist scrutiny by the national expert body we propose in section 8.3. Such experiments should be approached with caution. Strong scientific justification should be provided to the national expert body, who should closely consider the ethical and any safety issues in addition to the potential value of the research. Authorisation may require studies to adopt an incremental (graduated) approach as described in section 8.2.4 and Box 3.8. Proposed studies should be assessed on a case-by-case basis, at least until experience allows the formulation of guidelines. Although we would expect this list to evolve over time as knowledge advances, the major types of research that we would currently include in this category are:

- Substantial modification of an animal's brain that may make the brain function potentially more 'human-like', particularly in large animals.
- Experiments that may lead to the generation or propagation of functional human germ cells in animals.
- Experiments that could be expected to significantly alter the appearance or behaviour of animals, affecting those characteristics that are perceived to contribute most to distinguishing our species from our close evolutionary relatives.
- Experiments involving the addition of human genes or cells to NHPs. We recognise that research on NHPs is appropriate, and in some types of research probably essential if it is to lead to clinical benefit, but such research should remain under a high degree of regulatory scrutiny.[472]

8.2.3 Category 3

A very narrow range of experiments should not, for now, be licensed because they either lack compelling scientific justification or raise very strong ethical concerns. The list of such experiments should be kept under regular review by the proposed national expert body, but should at present include:

- Allowing the development of an embryo, formed by pre-implantation mixing of NHP and human embryonic or pluripotent stem cells, beyond 14 days of development or the first signs of primitive streak development, (whichever occurs first), unless there is persuasive evidence that the fate of the implanted (human) cells will not lead to 'sensitive' phenotypic changes in the developing fetus.[473,474] This supplements the 14 day provision applied to human admixed embryos under the HFE Act, so that mixed embryos that are judged to not quite meet the criteria for being 'predominantly human', should nevertheless be regulated on the basis of the likely phenotypic effect on the embryos created. Currently, any mixed origin embryo judged to be 'predominantly human' is regulated by HFEA and cannot be kept beyond the 14 day stage, whereas an embryo judged to be predominantly animal is unregulated until the mid-point of gestation (likely to be increased to two-thirds on implementation of the European Directive 2010/63/EU) and can in principle be kept indefinitely. As to whether or not an admixed embryo is predominantly 'human' is an expert judgement, including an assessment of likely phenotype, but neither

471 A graded approach already operates to some degree under ASPA. Project licenses including certain types of experiment, including those that raise 'novel or contentious' issues, must be referred to the Animal Procedures Committee for review (see Box 6.2). The principle of a graded approach has also been enunciated by the International Society for Stem Cell Research (see 7.4.2), the US National Academy of Sciences (Box 7.2-3), and in reference to the 'human neuron mouse' by Greely *et al.* (see 3.4).

472 For example, stem cell therapeutic approaches may need to be tested on NHPs because their greater similarity (cell cycle time, brain structure, molecular homology) to humans will provide better assessment of colonisation and neural contact development.

473 This applies whether the embryo is implanted within an animal uterus or maintained as an intact embryo *in vitro.*

474 Equivalent statutory restrictions are applicable to human and human admixed embryos under the HFE Act (see 6.2.2).

the precise eventual composition of an individual embryo nor the phenotypic effect of the admixture will be easily predictable in the current state of knowledge.
- Transplantation of sufficient human-derived neural cells into an NHP as to make it possible, in the judgement of the national expert body, that there could be substantial functional modification of the NHP brain, such as to engender 'human-like' behaviour. Assessing the likely phenotypic effect of such experiments will be informed by prior work on other species (possibly including stem cell transfer between NHPs) or by data on the effects of 'graded' transplantation of human cells into NHPs.
- Breeding of animals that have, or may develop, human-derived germ cells in their gonads where this could lead to the production of human embryos or true hybrid embryos within an animal.[475]

8.2.4 Graduated licensing

Since the outcome of many of the experiments outlined in category 2 (8.2.2) will be somewhat unpredictable until initial studies have been conducted, we recommend consideration of graduated licensing. By this we mean licensing limited initial experiments, involving small numbers of animals, starting with those species considered least likely to experience pain, suffering, or long-lasting harm, and with careful monitoring of the outcomes according to agreed measurable criteria, before further work is permitted.[476] Given the exploratory nature of the work, there should be active dialogue between investigator and the national expert body, and the results of such experiments should in turn inform the future regulatory process for similar experiments. In Chapter 3 (Box 3.8) we outline an example of this approach in neuroscience, but the principles are generic.

8.2.5 Flexibility of regulation

The types of experiment in these categories, and the boundaries which are set, are virtually certain to evolve with time, new knowledge and changing social norms. Regulators should monitor and respond to changes in societal views and scientific knowledge, and regulatory mechanisms should be sufficiently flexible to accommodate such change.

8.3 National expert body

The limited number of such experiments, the specialist knowledge required to evaluate their likely outcomes and the socially sensitive nature of the judgements to be made, dictate that oversight of research involving ACHM should be carried out by a single, national, expert, review body. **We recommend that the Home Office ensures that a national expert body with a duty to advise on the use of ACHM in research is put in place.**

We recommend that this national expert body should:

- **Be multidisciplinary, involving people with knowledge of ethics, the humanities, social sciences, law and the biological sciences as well as people without specific expertise in these fields, and be able to co-opt additional expertise when relevant.[477]**
- **Be transparent, making its proceedings, deliberations, reasoning, conclusions and recommendations available for public scrutiny.**
- **Be outward facing so that interested persons are aware of its function and feel able to input into its work programme.**
- **Be actively involved in public engagement and consultation; and maintain regular forward-looking dialogue with the scientific community.** This will enable it to anticipate future scientific directions. A major strength of this approach would be the ability to ensure that scientific work in this area proceeds with reasonable

475 Placement of human embryos into animals is prohibited by the HFE Act, and this seems likely to be interpreted to include placement of human embryos into animals modified to contain human uterine tissue.
476 We do not intend this to lead to the duplication of animal experiments. Where there is satisfactory evidence from previous experiments this should be taken into account and not repeated.
477 Given the special issues associated with experiments on NHPs, we recommend that the national expert body should include, either in its membership or as an advisor, an independent scientist with experience in NHP research who should be present to advise the group when such issues are discussed.

public understanding and support, and is not unduly influenced by extreme views. Responses from public participants in our dialogue indicated that the UK public would be receptive to such an approach.

- **Have the power to develop guidelines to promote consistency and transparency in the regulatory process.**

To ensure a consistent approach in ethical and animal welfare matters (see Chapters 4 and 5), we consider it desirable that research involving ACHM is considered by the same body that advises Government on other aspects of animal research. We are aware that, in implementing the EU Directive 2010/63/EU, the UK is required to establish a 'national committee for the protection of animals used for scientific purposes'.[478] We anticipate this body will succeed the currently constituted Animal Procedures Committee. **We recommend that the Home Office ensures that the body which meets the requirement of the 'national committee for the protection of animals used for scientific purposes' in the UK has within its remit and competence the function of the national expert body for ACHM.**

8.4 Welfare

We have commented that research involving ACHM does not have a generally increased potential for causing animal suffering compared with other experiments permitted under existing regulation, and that the development and use of ACHM could contribute to 3Rs principles. There may, however, be a few specific situations in which modification of the appearance or behaviour of a normally social animal may cause it to experience distress, including as a result of the actions of others of its own species, or of its human carers. Such effects can also occur in other experimental situations. This type of harm should be taken into account in the overall assessment of potential animal suffering in ACHM experiments, as it would with similar changes induced by other experimental procedures. We emphasise that research involving ACHM should be subject to scrutiny, and advancement from the perspective of animal welfare, in a manner no different from other animal research.

8.5 Safety

We have considered a variety of safety issues that could arise from experiments involving ACHM. There are some hazards that are specific to the purpose and nature of individual research protocols, such as those altering an animal's susceptibility to human infections, which must be appropriately regulated and managed according to established procedures. We have also considered more generic issues, predominantly relating to the risk of activating endogenous viruses or altering the host range of infectious agents. The risk levels are thought to be very low, but not zero.[479] Any manipulation which is known to, or could, alter viral or other pathogen recognition sites, or in any other way affect susceptibility to pathogens, or which deliberately involves the activation of human and animal proviruses within the same ACHM (such that they could recombine) should be carefully risk-assessed and appropriate control mechanisms put in place. It is critical that the provenance of human material to be used clinically is known and considered during the risk assessment.

The nature of the risks, and ways of mitigating them, are similar to those regularly used for other research involving potentially infectious materials. **We recommend that, for those classes of ACHM where it is relevant, a risk assessment should be undertaken and appropriate containment levels specified. The risk assessment is the responsibility of investigators, research institutions, and regulators; and should where relevant take the advice of an independent virologist.**

478 Article 49, Directive 2010/63/EU on the protection of animals used for scientific purposes. Available at http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CELEX:31986L0609:en:HTML

479 Notably when human cells are isolated from ACHM and then maintained in culture or introduced into humans.

8.6 Interfaces between regulatory authorities

Research involving human embryos is regulated by the HFEA under the HFE Act (see 6.2.2). As was recognised during the passage of this Act, there are situations in which this regulation of human embryo research and the matters discussed in the current report interface very closely, and may partly overlap. Chimæric embryos containing both human and animal stem cells are examples, because whether they are considered 'human' for the purposes of regulation depends on the proportion of human cells, their distribution and, most importantly, their expected effect on the phenotype of the resultant embryo. The proportions and distribution of cells of different species in a single structure may evolve over time; such change may be unanticipated or result from experimental design; and the state of current knowledge is such that predicting phenotypic effects may be difficult. In each case, an expert judgement will have to be made, as to whether and how to proceed. The technical potential to create transgenic animals containing ever larger amounts of human DNA sequence raises similar issues.

The existing UK legislative structure is such that some awkward cases may fall at the boundary of jurisdiction. **We recommend that the Home Office and the Department of Health work closely together to ensure that there are no regulatory gaps, overlaps, or inconsistencies, between the two regulatory systems.** They should bear in mind that animal embryos are not regulated until the middle of gestation (likely to be increased to two-thirds of gestation under the new European Directive), although we recognise that maternal animals carrying these embryos may be regulated under ASPA.

We consider it essential that the Home Office and the HFEA (or, as appropriate, the Department of Health) work together to develop and maintain a smooth, functionally integrated operational interface at the boundaries of their areas of responsibility. This should be supported by clear guidance to the research community, to ensure the timely and appropriate adjudication of innovative scientific projects without undue bureaucracy. Such an interface may well involve the expert advisory bodies in the two systems, as well as officials acting for the agencies concerned.

The Home Office (and, where relevant, the Department of Health) should consult, as appropriate, with other bodies who may sometimes have a role in the regulation of ACHM, namely, the Human Tissue Authority, the Health and Safety Executive, the Department for the Environment, Food and Rural Affairs and the Steering Committee of the National Stem Cell Bank.

8.7 International regulation

We have considered other recent (non-UK) national and international studies which have examined aspects of the use of ACHM in research (Chapter 7). To date, consideration of ACHM research from policy, societal, ethical and regulatory perspectives is limited. We have also noted that this field of science, like so many, could take place across several jurisdictions with differing regulatory requirements, allowing funders and researchers to exercise choice about the location of their research. **We recommend raising international awareness of ACHM, promoting international consistency in research practice involving their use, and exploring the development of international standards or guidance. This might be achieved through international collaboration amongst regulators, policy-makers, national and international bioethics bodies and medical research councils, or initiatives within the research community. This is an area in which the UK should provide leadership.**

8.8 Summary

In short, we advocate a tiered approach to regulation such that the great majority of uncontentious experiments proceed as under current ASPA regulation, while a small number of categories of experiment are referred for more expert scrutiny, with graduated licensing allowing progress to be made under regular review. A very limited number of experiments should not be licensed at the current time. The graduated licensing process should be interfaced with the corresponding processes that regulate human embryos so that the regulators are aware of each other's activities and so that there is no gap or unnecessary overlap between their jurisdictions.

Annex I Report preparation

Working group membership

This report was prepared by a working group of the Academy of Medical Sciences. Members participated in a personal capacity, not as representatives of the organisations listed. A summary of working group members' interests is given below.

Chair

Professor Martin Bobrow CBE FRS FMedSci is Professor Emeritus of Medical Genetics at the University of Cambridge. His research interests are primarily in genetic disease and molecular diagnostics, with clinical specialism in genetics, including genetic diagnosis and genetic counselling. Currently a non-executive Director of the Cambridge University Hospitals NHS Foundation Trust, and Chair of the Muscular Dystrophy Campaign, Professor Bobrow has been Chairman of Unrelated Living Donor Regulating Authority (ULTRA) and the Advisory Committee on Radiation in the Environment (COMARE), Deputy Chairman of Wellcome Trust and the Nuffield Council on Bioethics, and a member of the MRC Council, Human Genetics Advisory Commission and NHS Central R&D Committee. In continuity with the current study, Professor Bobrow also chaired the Academy of Medical Sciences' *'Inter-species embryos'* study, which informed revision of medical research aspects of the UK Human Fertilisation and Embryology Act (2008).

Members

Professor Thomas Baldwin is a Professor of Philosophy at the University of York; he is also the Editor of Mind, the leading UK philosophy journal. His research focuses on 20th century philosophy, both analytical and continental (he has just completed a critical edition of some unpublished writings by G E Moore and is now writing a book about J-P Sartre). He currently teaches metaphysics, political philosophy and ethics. He is a member of the Human Genetics Commission and of the Government's Expert Advisory Committee on obesity. He has been Deputy Chairman of the Human Fertilisation and Embryology Authority, and a member of the UK Stem Cell Bank Steering Committee and of the Nuffield Council on Bioethics.

Reverend Dr Michael Banner is Dean and Fellow of Trinity College, Cambridge. He was previously Professor of Public Policy and Ethics in the Life Sciences at the University of Edinburgh, and of Moral and Social Theology at King's College London. He currently Chairs the Cambridge University (Animal Procedures) Licence Review Committee, and is a member of the Human Tissue Authority. He has been Chairman of the Home Office Animal Procedures Committee, the Shell Panel on Animal Testing, the Government Committee of Enquiry on the Ethics of Emerging Technologies in Breeding Farm Animals, and the Department of Health CJD Incidents Panel, Director of the UK Economic and Social Research Council's Genomics Research Forum, and a member of the Royal Commission on Environmental Pollution, and the Agriculture and Environment Biotechnology Commission. He is currently writing a book on animals and ethics for Oxford University Press.

Professor Peter Brophy FRSE FMedSci is Director of the Centre for Neuroregeneration and Professor of Anatomy at the University of Edinburgh. His research specialties are in the molecular and cell biology of myelination and demyelination, particularly axon–glia interaction and the genetics of inherited peripheral neuropathy using transgenesis and gene targeting in mice. Professor Brophy chaired the 2008 International Gordon Conference on Myelin and the Committee on Stokes Professorship Awards, Science Foundation Ireland, and currently chairs

the Scientific Advisory Board for the INSERM 'Institut du Fer à Moulin', Paris. He has also been a member of Wellcome Trust's Neurosciences and Mental Health Panel, the French 'Agence Nationale de Recherche', the Canadian Government's Foundation for Innovation Neuroscience Panel and a research panel member for bodies including Action Research and the Multiple Sclerosis Society.

Ms Tara Camm is a UK qualified solicitor, who has spent over 15 years in the not-for-profit sector, bringing her legal training and expertise to bear on a diverse range of strategic, policy and operational issues affecting not-for-profit organisations in the UK and internationally. She is currently General Counsel and Company Secretary for Plan International, one of the largest non-governmental organisations in the world promoting child rights to relieve child poverty. Previously Principal Solicitor for Wellcome Trust, Ms Camm has significant interest and experience in biomedical science law. She led the legal work for the creation of the UK Biobank, including its Ethics and Governance Framework and Council, and has had extensive involvement in the development of UK and international guidance and legislation affecting biomedical science, including in the UK, the Human Tissue Act (2004), the Mental Capacity Act (2005) and the Human Fertilisation and Embryology Act (2008).

Professor Dame Kay Davies DBE CBE FRS FMedSci is Head of Department of Physiology, Anatomy and Genetics, and Director of the MRC Functional Genomics Unit at the University of Oxford. Her research interests centre on the molecular genetic analysis of human muscular and neurological diseases, particularly muscular dystrophy, motor neuron disease and ataxia. She also has an active interest in the ethical implications of genetics research and the public understanding of science, and considerable experience of the use of biotechnology companies as a conduit for translating the results of experimental science into new therapeutics and diagnostics. Professor Davies is Executive Editor of the journal Human Molecular Genetics, and a member of Wellcome Trust Board of Governors.

Professor John Harris FMedSci is Lord Alliance Professor of Bioethics, and Director of the Institute for Science, Ethics and Innovation, at The University of Manchester. His specialities are in the ethics of scientific and technological innovation, including areas of genetics, transplantation, human enhancement and reproduction; he leads the Wellcome Strategic Programme in 'The Human Body, its Scope Limits and Future'. Currently joint Editor-in-Chief of The Journal of Medical Ethics, Professor Harris is a member of several editorial boards including that of the Cambridge Quarterly of Healthcare Ethics. A member of the Human Genetics Commission, he was formerly a member of the Medical Ethics Committee of the British Medical Association, and the Government Advisory Committee on Genetic Testing. Professor Harris was a Founder Director of the International Association of Bioethics, and has been consultant to bodies including the European Parliament and the World Health Organization.

Professor Roger Lemon FMedSci is Sobell Chair of Neurophysiology and Head of the Sobell Department of Motor Neuroscience and Movement Disorders at the Institute of Neurology, University College London. His main research interest is in the control of skilled hand movements by the brain, including the impacts on these movements of damage to the cortex, for example as a result of stroke or in cerebral palsy. He sits on the Ethical Review Panel of the UK Centre for Macaques, is a member of the Council of Understanding Animal Research, Chairs the Expert Group of the EU Animals Directive of the European Science Foundation, and is Associate Editor at the Journal of Neuroscience, Guarantor and Associate Editor at Brain and a Receiving Editor of Neuroscience Research.

Dr Robin Lovell-Badge FRS FMedSci is Head of Division of Stem Cell Biology and Developmental Genetics, at the MRC's National Institute for Medical Research. His research specialties are in genetics, early embryonic development, sex determination and the development of the mammalian nervous system, as well as the biology and use of stem cells. Dr Lovell-Badge's work in the wider communication of science has included school lectures, National Institute for Medical Research programmes, media interviews, and parliamentary and public debates on embryo and stem cell research and genetics. Dr Lovell-Badge is President of the Institute of Animal Technologists, a Visiting Professor at the University of Hong Kong and an honorary professor at University College London. He is a member of the Academy of Medical Sciences' Communications Group and has advisory board membership including the Scientific and Clinical Advances Advisory Committee of the Human Fertilisation and Embryology Authority, and the Science Media Centre. He is also a member of the organising committee of the Hinxton Group.

Professor Jack Price is Professor of Developmental Neurobiology at King's College London. He got his first degree with the Open University, then a PhD in Neurobiology from University College London. Following post-doctoral training at Massachusetts Institute of Technology, he ran a research group at the National Institute for Medical Research for eight years. He was then Director of Molecular Neuroscience at SmithKline Beecham Pharmaceuticals, until taking up his present position of Professor of Developmental Neurobiology in 1998. He became Head of the newly formed Centre for the Cellular Basis of Behaviour in 2006. He has worked on neural stem cells in various guises for about twenty years and has more recently been pursuing an interest in the development of psychiatric disorders. He is also currently Consultant and Director of Cell Biology for ReNeuron Ltd., a UK biotechnology company developing stem cells for therapeutic and drug-discovery applications.

Professor Terence Rabbitts FRS FMedSci works at the Leeds Institute of Molecular Medicine where he was Scientific Director until 2010. His research interests centre on the molecular analysis and modelling of chromosome abnormalities in human cancer, immunogenetics and the development of cancer biotherapies. Professor Rabbitts was formerly the joint Head of the Division of Protein and Nucleic Acid Chemistry at the Medical Research Council Laboratory of Molecular Biology, Cambridge. He chaired the Scientific Advisory Boards of Cambridge Antibody Technology and Quadrant HealthCare, and was a Domantis scientific advisory board member. He is currently a member of the scientific advisory boards of Oryzon, DiThera and the Institute of Genetics and Molecular Medicine, Edinburgh. He is a member of the Academy of Medical Sciences' Council, the European Molecular Biology Organization and has been awarded the Colworth Medal of the Biochemical Society and the CIBA Prize.

Professor Martin Raff CBE FRS FMedSci is Emeritus Professor of Biology at the Medical Research Council Laboratory for Molecular Cell Biology, University College London. His research interests were in cell biology, with focus on developmental neurobiology and mammalian cell proliferation and differentiation. A Fellow of the Academia Europaea, foreign member of the American Academy of Arts and Sciences and the National Academy of Sciences, and a member of the Lasker Awards jury, Professor Raff is also co-author of 'Molecular biology of the cell'. He is a Director of the Company of Biologists, and is a member of scientific advisory boards in America and Europe, including Wellcome Trust Centre for Human Genetics, the Weatherall Institute of Molecular Medicine, and the Medical Research Council Clinical Sciences Centre within the UK. Professor Raff has been President of the British Society of Cell Biology and Chairman of the UK Life Sciences Committee.

Professor Trevor Robbins FRS FMedSci was elected to the Chair of Experimental Psychology (and Head of Department) at the University of Cambridge in October 2002. He is a Fellow of the British Psychological Society, the Academy of Medical Sciences, and the Royal Society. He has been President of the British Association for Psychopharmacology (1994–1996) and the European Behavioural Pharmacology Society (1992–1994), winning the latter Society's inaugural Distinguished Scientist Award in 2001. He was the F. Kavli Distinguished International Lecturer at the Society for Neuroscience meeting in 2005 and he gave the Staglin Mental Health Music Festival Keynote address in 2008. He was recently jointly given the prestigious Distinguished Scientific Achievement Award for 2011 by the American Psychological Association. He has been a member of the MRC Council and chaired the Neuroscience and Mental Health Board from 1996 until 1999. Currently, he directs the MRC/Wellcome Trust-funded 'Behavioural and Clinical Neuroscience Institute', the mission of which is to enhance translation from basic to clinical neuroscience.

Professor Nikolas Rose is the Martin White Professor of Sociology and Director of the BIOS Centre for the study of Bioscience, Biomedicine, Biotechnology and Society at the London School of Economics and Political Science. His current research concerns the social, ethical, cultural and legal implications of biological and genetic psychiatry and behavioural neuroscience, examining in particular the emergence of novel ways of governing human mental life and conduct, and their consequences. He is also working with colleagues at Imperial College London in the joint Imperial–LSE Centre for Synthetic Biology and Innovation. He has published on areas including the social and political history of the human sciences, the history of empirical thought in sociology, and changing rationalities and techniques of political power. He is a member of the Nuffield Council on Bioethics, Chair of the European Neuroscience and Society Network, Editor of BioSocieties and a Visiting Professor at the Institute of Psychiatry, King's College, London.

Professor Christopher Shaw FMedSci is Professor of Neurology and Neurogenetics at the Institute of Psychiatry, King's College London. He is also Head of the Department of Clinical Neurosciences and Director of the MRC Centre for Neurodegeneration Research and Director of the Maurice Wohl Clinical Neurosciences Institute. He is also an Honorary Consultant Neurologist at King's College and Guy's Hospitals. His early training in General Medicine and Clinical Neurology was conducted in New Zealand. He was awarded a Wellcome Trust New Zealand Health Research Council Fellowship to come to the UK and study Neurobiology in the Neurology Unit of Cambridge University from 1992 to 1995. From that time he was a Neurologist at King's College Hospital and running a research laboratory in the Institute of Psychiatry. His major area of clinical and research interest is in the genetic and molecular basis of motor neuron disease. He runs a clinic at King's College Hospital for people with motor neuron disease.

Professor Veronica van Heyningen CBE FRS FRSE FMedSci is a Group Leader and joint Section Head of the Medical and Developmental Genetics Section at the MRC Human Genetics Unit in Edinburgh. Her research focuses on human eye anomalies such as aniridia and anophthalmia/microphthalmia to define gene networks implicated in disease and normal development. Her broader interests include exploration of the mechanisms of mutation, long-range regulation of gene expression and phenotype modulation. Professor van Heyningen has been a member of UK Human Genetics Commission, and is the current President of the UK Genetics Society.

Observers

Representatives from the study sponsors and Government stakeholders were invited to join working group meetings as observers to clarify factual points. They were not present for the discussions of the study's conclusions and recommendations. The observers were:
Dr Joseph Chan, Animals (Scientific Procedures) Inspectorate, Home Office
Dr John Connolly, Head of Advanced Therapies, Department of Health
Mr Andrew Earnshaw, Senior Policy Manager, Advanced Therapies, Department of Health
Ms Eve Jacques, Corporate Affairs Group, Medical Research Council
Ms Nancy Lee, Senior Policy Adviser, Wellcome Trust
Dr Frances Rawle, Head of Corporate Governance and Policy, Medical Research Council
Mr Carl Reynolds, Dialogue and Engagement Specialist, Department of for Business, Innovation and Skills Sciencewise Expert Resource Centre
Dr Neil Watt, Animals (Scientific Procedures) Inspectorate, Home Office

Secretariat

Dr Laura Boothman (Lead Secretariat), Policy Officer, Academy of Medical Sciences
Ms Catherine Luckin, Policy Officer, Academy of Medical Sciences
Dr Rachel Quinn, Director, Medical Science Policy, Academy of Medical Sciences

Review group membership

The report was reviewed by a group on behalf of the Academy's Council. Reviewers were asked to consider whether the report met the terms of reference and whether the evidence and arguments presented in the report were sound and supported the conclusions. Reviewers were not asked to endorse the report or its findings. Review group members were:
Professor Ronald Laskey CBE FRS FMedSci (Chair)
Emeritus Professor of Embryology, University of Cambridge
Professor Sir Richard Gardner FRS
Emeritus Professor, Department of Biology, University of York
Lord Richard Harries of Pentregarth FMedSci
Former Bishop of Oxford
Dr Stephen Inglis
Director of the National Institute for Biological Standards and Control (NIBSC)
Professor Ian Kimber
Chair, Board of National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs)
Professor Ian McConnell FRSE FMedSci
Emeritus Professor of Veterinary Science, Department of Veterinary Medicine, University of Cambridge
Dr Paul Whiting
Head of Molecular and Cellular Biology and Site Head, Regenerative Medicine, Pfizer

Annex II Consultation and evidence gathering

Call for evidence

The Academy issued an open call for evidence to inform the study. Those who submitted written evidence are listed below.

Organisations

Animal Procedures Committee (APC)
The Anscombe Bioethics Centre
AstraZeneca
Biotechnology and Biological Sciences Research Council
British Pharmacological Society Animal Welfare and Integrative Pharmacology Committee
British Union for the Abolition of Vivisection
Church of England Mission and Public Affairs Council
Department of Health
Fund for the Replacement of Animals in Medical Experiments
Genetic Interest Group
Human Fertilisation and Embryology Authority
Human Tissue Authority
Institute of Animal Technology
Medical Research Council (MRC)
National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs)
Northeast England Stem Cell Institute
Nuffield Council on Bioethics
Royal Society for the Prevention of Cruelty to Animals (RSPCA)
Safer Medicines Trust
Scottish Council on Human Bioethics
Wellcome Trust Sanger Institute

Individuals

Professor Richard Anderson, University of Edinburgh
Dr Sian Beynon-Jones, University of York
Elio Caccavale, University of Dundee; Professor Richard Ashcroft, Queen Mary University of London; and Professor Michael Reiss, Institute of Education (joint submission)
Ms J Deeks
Professor Robert Dingwall, Ms Michelle Hudson and Ms Kathleen Job, University of Nottingham (joint submission)
Professor Hank Greely, Stanford University
Dr Mark Greene, University of Delaware
Dr Gill Haddow, INNOGEN, University of Edinburgh
Dr Alison Harvey, King’s College London
Dr D Jones
Dr Jonathan Kelley, University of Nevada

Dr Edward Moore OStJ
Dr Barbara Nicholas, formerly secretariat to New Zealand Bioethics Council
Miss J M Pick
Sir Robert Worcester KBE DL, Ipsos MORI

Additional evidence gathering

The following individuals provided oral evidence to the working group:
Sir Patrick Bateson FRS, Emeritus Professor of Ethnology, Department of Zoology, University of Cambridge
Professor Allan Bradley FRS FMedSci, Director Emeritus, Wellcome Trust Sanger Institute
Mr Phil Banks, APC Secretariat
Dr John Connolly, Head of Advanced Therapies, Department of Health
Professor Elizabeth Fisher FMedSci, Professor of Molecular Genetics, Department of Neurodegenerative Disease, University College London
Dr Simon Glendinning, APC member
Dr Maggie Jennings, Head of Research Animals Department, RSPCA
Professor Keith Kendrick, APC member
Dr Sophie Petit-Zeman, Head of External Relations, Association of Medical Research Charities
Dr Vicky Robinson, Chief Executive, NC3Rs
Dr Victor Tybulewicz FMedSci, Head of Division of Immune Cell Biology, MRC National Institute for Medical Research
Mr Martin Walsh, Head of Policy, Animals (Scientific Procedures) Division, Home Office

We are particularly grateful for the advice and assistance of:
Professor Robin Weiss FRS FMedSci, University College London
Dr Jonathan Stoye, MRC National Institute for Medical Research

The following individuals submitted evidence, information or relevant publications through correspondence with the working group and the secretariat:
Professor Robin Ali FMedSci, University College London
Professor Jeffrey Almond FMedSci, Sanofi Pasteur
Professor Peter Andrews, University of Sheffield
Dr Roger Barker, University of Cambridge
Antony Blackburn-Starza
Dr Gary Burns MBE, AstraZeneca
Professor Hilary Critchley FMedSci, University of Edinburgh
Anne Lykkeskov, The Danish Council of Ethics
Dr John Dick, University of Toronto
Professor Stephen Dunnett FMedSci, Cardiff University
Dr Kristina Elvidge, Muscular Dystrophy Campaign
Dr Maurizio Salvi, European Group on Ethics
Professor Sir Martin Evans FRS, Cardiff University
Dr Simon Fisher, University of Oxford
Professor Richard Flavell FRS, Yale School of Medicine
Professor Robin Franklin, University of Cambridge
Dr Carrie Friese, London School of Economics

Dr Jonathan Gawn, Health and Safety Laboratory
Professor Daniel Geschwind, University of California, Los Angeles
Professor Roger Gosden
Professor Melvyn Greaves FRS FMedSci, Institute of Cancer Research
Dr Christine Hauskeller, University of Exeter
Professor Douglas Higgs FRS FMedSci, University of Oxford
Julian Hitchcock, Field Fisher Waterhouse
Calvin WL Ho, Bioethics Advisory Committee Singapore
David Jones, Medicines and Healthcare products Regulatory Agency
Mary Kirwan, Chase Paymentech
Professor Andrew Lever FMedSci, University of Cambridge
Professor Alison Murdoch, Newcastle University
Professor Trevor Owens, University of Southern Denmark
Professor Andrew Parker, University of Oxford
Professor David Rubinsztein FMedSci, University of Cambridge
Dr Sebastian Sethe, Lawford Davies Denoon
Professor Richard Sharpe, MRC Centre for Reproductive Health
Professor Pamela Shaw FMedSci, University of Sheffield
Dr William C Skarnes, Wellcome Trust Sanger Institute
Professor Peter St George-Hyslop FRS FMedSci, University of Cambridge
Dr Glyn Stacey, UK Stem Cell Bank
Professor Francis Stewart, Biotechnology Center, Technische Universität, Dresden
Professor Jerome Strauss, Virginia Commonwealth University
Professor Swee Lay Thein FMedSci, King's College London
Professor Adrian Thrasher FMedSci, University College London
Professor John Todd FRS FMedSci, University of Cambridge
Professor Arthur Toga, University of California, Los Angeles
Dr Irving Weissmann Institute for Stem Cell Biology and Regenerative Medicine, Stanford Cancer Center and Ludwig Center, Stamford
Professor Charles Weissmann ForMemRS FMedSci, The Scripps Research Institute
Professor Bruce Whitelaw, University of Edinburgh

We are very grateful to all those who have contributed information to the study, including those who submitted evidence anonymously and anyone that we have inadvertently omitted from this list.

Annex III Overview of dialogue methodology and evaluation

Background and objectives

To ensure that the working group's discussions and recommendations were informed by public concerns and aspirations alongside the scientific evidence and the social, ethical and legal perspectives, the Academy commissioned a programme of public dialogue. 'Exploring the boundaries' was designed and managed by a consortium led by Ipsos MORI and including Dialogue by Design and the British Science Association. It was supported by the Sciencewise Expert Resource Centre programme, funded by the Department for Business, Innovation and Skills. Oversight was provided by a group consisting of members of the working group, the Department of Health and Sciencewise Expert Resource Centre. A comprehensive report of the dialogue methodology and findings has been published separately.[480]

The dialogue focused specifically on public awareness of, and attitudes towards, research using ACHM, distinguishing this from more general use of animals in research. The purpose of the 'Exploring the boundaries' dialogue was to provide a forum in which individuals could explore their concerns and aspirations around this unfamiliar topic. In introducing the subject, the dialogue set out to identify areas of consensus, disagreement or uncertainty on a broad range of issues raised by current and possible future uses of ACHM. It was designed to actively seek the views of a range of different audiences, including patients and carers, those with strong views on animal welfare and individuals for whom religious faith is important. The dialogue sought to provide an in-depth assessment of the attitudes towards research using ACHM of these varied audiences, but also to indicate the views of the public overall towards such work.

The dialogue programme was a core aspect of the study's evidence-gathering process. The working group considered its findings alongside the other evidence throughout their discussions and in considering their recommendations. The working group members were involved in providing oversight, including contributing towards the development of the dialogue materials, and attending the events.

Aims and objectives

The overall aim of the dialogue was to engage members of the public on the issues raised by the current and future uses of research involving ACHM. The objectives of the dialogue were to:

- Provide opportunities for members of the public to discuss and explore their aspirations and concerns relating to the scientific, social, ethical, safety or regulatory aspects of research involving ACHM.
- Identify areas of consensus, disagreement or uncertainty on a broad range of issues raised by current and possible future scientific developments, and explore both initial views and changes in opinion.
- Inform the final recommendations made by the Academy for public policy and research needs.

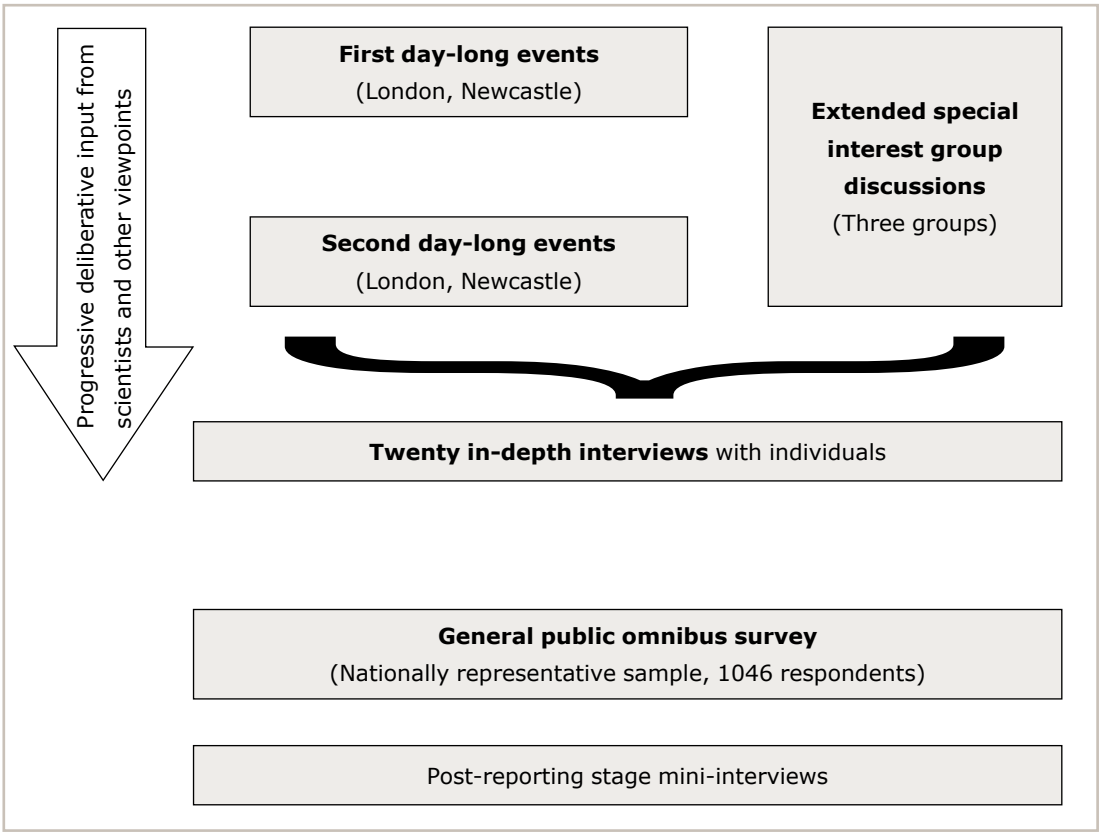
A secondary objective was to enable the Academy and the wider science community to build on previous experience in public dialogue, to pioneer innovative approaches in public engagement where appropriate, and to develop knowledge and understanding of public dialogue and its potential for future applications.

480 Ipsos MORI (2010). *Exploring the Boundaries: report on a public dialogue into animals containing human material* http://www.acmedsci.ac.uk/download.php?file=/images/page/128619890736.pdf

Methodology

The dialogue consisted of qualitative and quantitative elements, as outlined in the summary diagram below. In total, over 1100 individuals were involved. Following an initial literature review, a process of stakeholder engagement, including a workshop, was undertaken to agree the detailed aims of the dialogue and to inform the development of its themes and the stimulus materials. The stakeholders involved included members of the dialogue oversight group and representatives from non-governmental organisations, industry, religious organisations and animal welfare organisations.[481] The qualitative work then took place and its emerging findings were used to develop the questions for the quantitative study.

Summary of the dialogue methodology



Qualitative work

Seventy participants took part in the qualitative dialogue, the most substantial element of which was the general public dialogues that consisted of two groups in Newcastle and London. These groups met twice each in May and June 2010, for one day each time. Additional discussions were held with special interest groups. These were patients or carers of people with serious illnesses; people with religious faith and who stated that their beliefs were directly and practically important to them; and those who attached importance to animal welfare. Structuring the dialogue in this way enabled the views of a range of different audiences to be sought and explored thoroughly, provided an environment in which participants felt able to express their views and ensured that those with particularly powerful or emotive experiences or views did not unduly influence the dialogue as a whole.

Participants were recruited face-to-face by experienced recruitment professionals to

481 Ipsos MORI (2010). *Exploring the Boundaries: report on a public dialogue into animals containing human material.* http://www.acmedsci.ac.uk/download.php?file=/images/page/128619890736.pdf

ensure that a mixed and broadly representative group of people took part. A modest cash incentive was paid to encourage a diverse range of participants.

The dialogue process was designed to reveal how participants responded to information about research involving ACHM. The emphasis was on encouraging participants to reflect on information that was provided and on their interactions with each other and with the scientists and facilitators present. 'Focus group'-style sessions were used, in which participants could share their views, first spontaneously, then after reflection. Their eventual conclusions were the focus of analysis.

A sub-sample of 20 participants from both the general public dialogue and special interest groups were interviewed individually by telephone during July and August 2010. Interviewees for this stage were selected in part because the views they expressed during the day were distinct, which enabled issues raised by preliminary analysis of the qualitative data to be more thoroughly investigated and to confirm the validity of these views, for example that individuals were not unduly influenced by others in the group.

Data analysis

Notes were taken throughout each dialogue session and insights that each facilitator gained were then shared during analysis meetings at the end of each day and at the end of the dialogue process as a whole. During the general public dialogue events, an observational researcher also made notes on body language, facial expressions and evidence of behaviours, without taking part in the facilitation, as well as carrying out *ad hoc* interviews to explore participants' thoughts and feelings. The purpose of this was to understand more subtle and unspoken reactions alongside the main discussions and to help build hypotheses as to participants' thoughts and feelings throughout the process as their views developed.

Quantitative work

The quantitative findings in the report provide an indication of the views of the wider British public. The findings of the qualitative dialogue informed the survey questions. The findings were collected through the Ipsos MORI weekly computer-assisted personal interviewing (CAPI) survey, which is a cross-sectional representative survey of individuals aged over 15 years across Great Britain, performed face-to-face by trained interviewers. 1046 participants completed the 'Exploring the boundaries' survey in July 2010.

Findings

The findings of the public dialogue are highlighted throughout this report and are outlined and discussed in detail in the full report produced by Ipsos MORI.[482]

Evaluation

The Academy commissioned Laura Grant Associates to conduct an independent evaluation of the dialogue. The evaluation aimed to provide an independent assessment of the dialogue programme's credibility, effectiveness and success against its deliverables and objectives, throughout the programme and at its conclusion; and to assess its contribution to the overall Sciencewise Expert Resource Centre aim of creating excellence in public dialogue to inspire and inform better policy in science and technology. A comprehensive report of the full evaluation methodology and findings produced by Laura Grant Associates is available online.[483]

482 Ipsos MORI (2010). *Exploring the Boundaries: report on a public dialogue into animals containing human material.* http://www.acmedsci.ac.uk/download.php?file=/images/page/128619890736.pdf

483 Laura Grant Associates (2010). *Exploring the Boundaries: A dialogue on Animals Containing Human Material Evaluation Report.* http://www.acmedsci.ac.uk/download.php?file=/images/page/129111803577.pdf

Annex IV Glossary of terms and abbreviations

This glossary is intended to assist readers with the terminology and abbreviations used in this report; it is not presented as a definitive list of terms. Cross-references (e.g. see 2.2) refer to the sections of this report.[484]

ACHM: Animals containing human material. See 2.2, page 18.

Admixed: 'Admixture' is the process of mingling one substance with another. The Human Fertilisation and Embryology Act (as amended in 2008) defines five classes of 'human admixed embryos' containing both human and animal material, with the human contribution predominating. See Box 6.4, page 90.

Adult stem cell: Another term for 'tissue-specific' stem cell. See Box 3.3, page 38.

Amino acid: One of a group of chemical compounds that are the basic units of proteins.

Amniocentesis: A prenatal diagnostic technique in which a sample of amniotic fluid is withdrawn and examined for information about the fetus. (The 'amnion' is the innermost membrane enclosing the fetus.)

Amniotic stem cell: See Box 3.3, page 38.

Animal: In this text 'animal' (rather than 'non-human animal') is used to refer to animals of all species in the animal kingdom except humans. (In correct scientific taxonomy, humans are both primates and animals.)

Animal model: A living animal in which normal and abnormal biological processes can be studied, to gain insight into human health and disease. The more closely the process being modelled resembles the process in humans, the more scientifically valuable the model is likely to be.

Antibody: An antibody is a large protein found in blood and tissues, used by the immune system to identify and neutralise foreign material such as cells, bacteria and viruses. The antibody recognises a unique part of the foreign target, termed an antigen. Antibodies are produced as part of the immune response.

Antigen: A foreign substance that, when introduced into a living organism, stimulates the production of an antibody.

Aneuploid: Used to describe cells, tissues or organisms in which the number of chromosomes is abnormal in that it differs from the euploid. 'Euploid' entities are those in which each of the chromosomes of the set is represented in equal number (e.g. two copies of each chromosome is termed 'diploid'; one of each is 'haploid'). See also haploid and diploid.

ASPA: Animals (Scientific Procedures) Act (1986). See 6.2.1, page 83.

APC: Animal Procedures Committee. See 6.2.1, page 83.

484 Terms are drawn from sources including Department of Health (2010). *Code of Practice for the use of Human Stem Cell Lines*. http://workspace.imperial.ac.uk/clinicalresearchgovernanceoffice/Public/Code%20of%20practice%20for%20stem%20cell%20lines.pdf; Nuffield Council on Bioethics (2005). *The ethics of research involving animals*. Nuffield Council on Bioethics, London; The National Institutes of Health resource for stem cell research glossary, http://stemcells.nih.gov/info/glossary.asp; The Standard Oxford English Dictionary; Understanding Animal Research, http://www.understandinganimalresearch.org.uk/

ASPD, ASPI: Animals Scientific Procedures Division (ASPD) and Inspectorate (ASPI) of the Home Office.

Autologous: Derived from the same individual.

Autophagy: Literally, 'feeding upon oneself'. A biological process by which a cell digests internal components, for example to break down and recycle cellular components or to rid itself of toxins.

BIS: Department for Business, Innovation and Skills.

B-lymphocyte, B cell: A type of lymphocyte (a form of white blood cell), which forms part of the immune system and produces antibodies in response to antigens.

Bioluminesence: The emission of light by living organisms such as fireflies and deep-sea creatures. In biomedicine, cells or tissues can be made to emit light in this way as a form of marker (see GFP).

Blastocyst: An early embryo consisting of a hollow ball of 50–100 cells reached after about 4 or 5 days of embryonic development (depending on species), just prior to implantation in the uterus. The outer 'trophectoderm' cells give rise to part of the placenta, while a distinct group of about 15–20 'inner cell mass' cells give rise to the embryo proper and other extraembryonic tissues (e.g. placenta, yolk sac).

cDNA: Complementary deoxyribonucleic acid. A DNA strand that has been produced by 'reverse copying' a messenger ribonucleic acid (mRNA) (either by a laboratory technique or by a retrovirus). cDNA has the same sequence as DNA, except that it lacks introns.

Cell: The fundamental, usually microscopic, structural and functional unit of all living organisms, which consists of a small quantity of cytoplasm enclosed within a membrane, typically contains a nucleus and other organelles and internal compartments, and is capable of utilising energy, synthesising proteins and other biomolecules, and (usually) of self-replicating.

CNS: Central nervous system. The largest part of the nervous system, including the brain and spinal cord.

Cephalopod: An animal within the most highly organised class of molluscs. Cephalopods are characterised by a distinct head with 'arms' or tentacles attached. Examples are Cuttlefish and Octopuses.

Chimæra, chimæric animal: An animal comprised of whole cells from two different organisms. See 2.2.2, page 18.

Chloride channel: A protein 'pore' that enables the transit of chloride ions into and out of cells, across the cell membrane.

Chromosome: One of the threadlike structures containing identical sister strands of DNA, protected by proteins and carrying genetic information, that can be microscopically visible within a cell.

Cleavage stage embryo: An embryo prior to formation of a blastocyst, undergoing 'cleavage' divisions where there is little or no cell growth; thus during cleavage each cell of the one, two, four, and then eight cell embryo becomes progressively smaller.

Cognition, cognitive capacity: In its broadest sense, human 'cognition' can be defined as the 'faculty of knowing', to include aspects such as knowledge, reason, intelligence, understanding, sensation and perception (as distinguished from feeling and volition). See 3.4, page 46.

Complement: A protein complex found in blood and other body fluids, which forms part of the adaptive immune system. When combined with an antigen–antibody complex, complement produces a series of reactions (the 'complement cascade') to bring about cell lysis.

Congenital: Present from the time of (and often before) birth.

Cord blood stem cell: See Box 3.3, page 38.

Cytoplasm: The gel-like substance enclosed by the cell membrane. It contains many important molecules and organelles concerned with cell metabolism and movement.

Cytoplasmic hybrid: Cytoplasmic hybrid cells (cybrids) are those created by combining the nucleus (with a minimal amount of cytoplasm and mitochondria) of a cell of one species with the cytoplasm (including the mitochondria) of another species. Cytoplasmic hybrid embryos are those created by transferring a somatic cell nucleus from one species into the enucleated oocyte of another species.

Deontological: Relating to an ethical approach based on rules and duties. (Deontology is the study of duty, a branch of knowledge that deals with moral obligations.)

Defra: Department for Environment, Food and Rural Affairs.

DH: Department of Health.

Differentiation: The process by which cells become progressively more specialised towards their final function, both during development and adult maintenance.

Diploid: The state in which each type of chromosome (except the sex chromosomes) is represented twice. This is the normal state of all cells of the body, except the germ cells (sperm and eggs), which have only a single (haploid) set of chromosomes.

DNA: Deoxyribonucleic acid. A type of double-stranded nucleic acid molecule that encodes the genetic instructions used by almost all living organisms.

DNA base pair: Two nucleotides (the structural units of which DNA is composed), on opposite complementary DNA strands, which are connected by a hydrogen bond.

DNA regulatory region: A section of DNA that functions as a 'switch' to control gene expression. They may lie either side of the gene or even within its introns, and they are often highly conserved in evolution. Regulatory proteins (such as transcription factors) bind to regulatory regions. See Box 2.2, page 21.

DPA: Data Protection Act (1998).

Double-blind: A clinical trial or experiment, conducted by one person on another, in which information (such as whether a substance being administered is active or placebo) that may lead to bias in the results is concealed from both the tester and the subject. This method is used to eliminate subjective bias.

Ectoderm: The outermost of the three primary germ layers. It gives rise to the epidermis (outer part of the skin, including hair, nails, and sebaceous glands) the central nervous system (brain and spinal cord) and to sensory systems, such as the eye, olfactory system and inner ear. See also endoderm and mesoderm.

Ectopic: The location of cells or tissues at an abnormal site in the body. For example, ectopic pregnancy involves the implantation of a fertilised egg outside the uterus.
EG cell: Embryonic germ cell. See Box 3.3, page 38.

EMEA: European Medicines Agency.

Embryo: the first stages in the development of an animal, usually the result of fertilising an egg with a sperm. In humans, the embryo is usually referred to as a fetus from about the eighth week of fertilisation. In other mammals, 'fetus' may be used to refer to older embryos, but there is no strict definition of when fetal stages begin.

Embryonic stem cell: See Box 3.3, page 38.

Emphysema: A long-term, progressive disease of the lungs that primarily causes shortness of breath.

Endoderm: There are two types of endoderm; 'primitive' or 'extra-embryonic' endoderm (also sometimes called the 'hypoblast') forms the lower or outer layer of the early embryo (around the time of implantation in mammals) and contributes to the yolk sac, but contributes little to the embryo proper. 'Definitive' or 'embryonic' endoderm develops during gastrulation where it displaces the extra-embryonic endoderm and gives rise to the larynx, lungs, gut and associated organs, such as the thyroid and liver. See also ectoderm and mesoderm.

Endogenous: Having a cause (or origin) inside the body or self, not attributable to any external or environmental factor.

Endometriosis: a condition resulting from the development of endometrial (womb lining) tissue in an abnormal location outside the uterus.

Engraft: To insert a piece of material (e.g. cells, tissues or an organ) into an organism as a graft. (Autologous grafts involve movement of material from one location to other within the same body. Secondary chimæras are created by grafting cells tissue or organs from one animal into another.)

Enucleate: A cell lacking a nucleus; or the process of removing the nucleus of a cell.

Enteric nervous system: The part of the nervous system that directly controls the gastrointestinal system (gut).

Enzyme: A protein that catalyses (increases the speed of) a specific biochemical reaction.

Epiblast: The upper layer of cells present in the embryo just prior to gastrulation. These cells are pluripotent, and give rise to all three primary germ layers (ectoderm, mesoderm and endoderm) as well as to germ cells and to extra-embryonic mesoderm.

Epigenetic: ('Over' or 'above' genetics). Epigenetic factors are heritable changes in phenotype or gene expression, which result from mechanisms other than changes to the underlying DNA sequence. For example, characteristics resulting from alterations in DNA methylation or changes in chromosomal proteins.

Epitope: Part of an antigen, to which a particular antibody binds with a high degree of specificity.

EPO: European Patent Office.

ERP: Ethical review process. See 6.2, page 83.

ESC, ES cell: Embryonic stem cell. See Box 3.3, page 38.

ESCRO: Embryonic Stem Cell Research Oversight Committee. See Box 7.2, page 104.

Exogenous: Originating outside the body.

Exon: See intron.

Express, expression: Gene expression is the process by which information from a gene is used to synthesise a functional gene product. This involves transcription to produce an RNA molecule called messenger RNA (mRNA). mRNA is exported from the cell nucleus to the cytoplasm where its code is translated into proteins by assembling amino acids in the right order. The polypeptide chains produced are ultimately folded into proteins. Some genes only produce RNA products that fulfill different important functions in cells. See Box 2.2, page 21.

Extra-embryonic tissue: Tissue that contributes to the growth or development of an embryo without forming part of the embryo itself. Placental and yolk sac tissues are extra-embryonic.

Extra-embryonic stem cell: See Box 3.3, page 38.

Fetus: See embryo.

Fibroblast: A type of cell ubiquitously found in connective (supporting) tissues in most organs. Fibroblasts play a role during wound healing or tissue repair. They are the type of cell that most commonly grows in tissue culture, emerging from explants of pieces of most body tissues.

Fetal stem cell: See Box 3.3, page 38.

Fluorescence: Coloured light emitted by some chemicals, including some proteins, in response to the action of light (especially violet and ultraviolet rays) upon them.

Gamete: A mature haploid sexual reproductive cell, for example a sperm or egg, which can unite with another gamete to form a new organism. See germ cell.

Gastrulation: A phase early in the embryonic development of most animals, during which the single layer of cells called the blastula (or in higher vertebrates the epiblast), is reorganised into a three-layered patterned structure that will go on to form the three primary tissues of the embryo proper (ectoderm, mesoderm, endoderm). In human embryonic development it begins at around 14 days after fertilisation, in the mouse at about 7 days.

Gene: The basic unit of heredity in living organisms, now known to consist of a sequence of DNA (or RNA in certain viruses) containing a code for an RNA molecule that in many cases encodes a protein. The gene also includes any associated regulatory sequences. See Box 2.3, page 23.

Gene product: A substance produced by the expression of a gene, for example a protein molecule.

Gene replacement therapy: The insertion of gene copies within some of an individual's cells for the purpose of treating disease. See Box 2.3, page 23.

Genotype: The genetic constitution of an individual.

Genetic sequence: The order of nucleotide bases (the individual units of which DNA is composed) in a section of a DNA molecule.

Genetically altered: A cell, or organism, in which the DNA sequence has been modified.
See 2.2.1, page 18.

Genome: The complete DNA sequence of an individual, or a representative sequence for a species.

Germ cell: A sex cell or gamete (e.g. an egg or sperm); a reproductive cell that fuses with one from the opposite sex in fertilisation to form a single-celled zygote. The term is also used to refer to the progenitors of eggs and sperm during development.

Germ-line: The lineage of special cells set aside early in development that eventually differentiate into mature germ cells. (Cells that are not part of the germ-line are referred to as somatic cells.)

Gestation: The process of carrying young in the womb.

GFP: Green fluorescent protein. A protein that occurs naturally in some marine organisms. GFP fluoresces bright green under blue light, and is widely use as a marker in biomedical research. Its gene can be readily transfected into cells of many species, and confers the fluorescent property on the cells, which make the fluorescent protein and can then be easily visualised under appropriate illumination.

Glia: The supportive non-neuronal tissue of the nervous system, composed of different types of glial cell.

GM, GMO: Genetically modified, genetically modified organism.

Gonad: Any organ in an animal that produces gametes (e.g. a testis or an ovary).

Haematopoietic: A haematopoietic cell is one that is able to produce blood cells.

Haematopoietic stem cell: A stem cell that can give rise to all types of blood cell.

Haploid: A cell in which each type of chromosome is represented once (half the diploid number).

Hepatocyte: A type of cell that makes up the majority of liver tissue.

HFE Act: UK Human Fertilisation and Embryology Act. Unless specified, this term is used to refer to the 1990 Act as amended in 2008.

HFEA: Human Fertilisation and Embryology Authority.

HLA: Human leukocyte antigen. The HLA genes are the human versions of the major histocompatibility complex genes (MHC) found in most vertebrates. These genes encode cell-surface antigen-presenting proteins, antigens and other proteins. The major HLA antigens are the most important determinants of 'tissue type', which must be matched for optimal organ transplantation.

Homologous: Sharing a common ancestral origin; entities that are homologous have a similar structure. This term is used to describe genes that have a similar DNA sequence, or proteins that have the same (or very similar) structure. It is often used in describing genes or proteins of common evolutionary origin, found in different species.

Human (man): Individuals of the species *Homo sapiens*; human beings. Although in correct taxonomy, humans are both primates and animals, in this text 'animal' (rather than 'non-human animal') is used to refer to animals of all species *except* humans; and non-human primate (NHP) is used to refer to primates *except* humans.

HSC: Haematopoietic stem cell. See Box 3.3, page 38.

hESC, hES cell: Human embryonic stem cell. See stem cell.

hiPSC, hIPS cell: Human induced pluripotent stem cell. See stem cell.

HSE: Health and Safety Executive.

HTA: Human Tissue Authority.

HT Act: Human Tissue Act (2004).

Human lineage-specific sequence: A section of the genome that is unique to humans, or to humans and their near ancestors. See 3.2, page 32.

Humanised: An aspect of the biology of an animal (including for example a gene, protein, organ, element of external appearance or behavioural characteristic) that has been modified so that it more closely resembles that of the human.

Humanised antibody: An antibody produced by an animal (typically a mouse) whose antibody-producing genes have been replaced by human DNA sequence, causing it to produce antibody molecules that resemble those of the human. See 2.3.2, page 25.

Hybrid: An animal or plant that is the offspring of individuals of different kinds (usually, different species) (see 2.2.3); hybrid embryos (see Box 6.4); inter-species cell hybrids are cells created by the *in vitro* fusion of (usually somatic) cells from two different species (see 2.2.3), page 20.

Hyperacute response: A type of immune response that can occur rapidly after the transplantation of cells or tissues from one species into another (e.g. xenotransplantation of organs from pigs into humans). It is mediated by the binding of host antibodies to the donor graft causing damage to, and rejection of, the transplanted tissue.

ICSI: Intra-cytoplasmic sperm injection. The injection of a sperm directly into an egg.

Immature germ-line cell: A cell of the germ-line that has not fully differentiated into a mature gamete.

Immortalised cell line: A cell line that has the ability to grow through an indefinite number of divisions in cell culture. Some stem cell types and many cancer cell lines are immortal; such cell lines can also be produced deliberately in the laboratory, usually as the result of genetic manipulation. See 3.3.1, page 34.

Immune system (adaptive): The adaptive (or specific) immune system, found in vertebrates, is composed of highly specialised cells and processes that recognise and destroy foreign proteins, cells or micro-organisms entering the body. This is the body's first defence against infections. The adaptive immune response provides the ability to recognise and remember specific antigens and so to generate immunity.

Immunodeficiency: A lack, or deficiency, of a functional immune system. The term 'immuno-compromised' is used similarly. See 2.3.3, page 27.

Immuno-suppressive drug: A drug that prevents or inhibits immune system function.

In vitro: Literally, 'in glass'; an experiment performed in a test tube, culture dish, or other non-living environment.

***In vivo*:** An experiment conducted within a living organism.

iPSC, iPS cell: Induced pluripotent stem cell. See Box 3.3, page 38.

Intron: A segment of a DNA molecule, which separates the exons (protein coding sections) of a gene. An intron does not code for protein. See Box 2.3, page 23.

Ion channel: A protein 'pore' that enables the transit of ions into and out of cells across the cell membrane.

IVF: *In vitro* fertilisation. The fertilisation of an egg by a sperm outside the body.

ISSCR: International Society for Stem Cell Research.

kb: Kilobase. A unit of length for measuring DNA or RNA sequences, equivalent to the length of 1000 bases.

Latent: Latent diseases are those in which the usual symptoms are not yet manifest. For infectious disease, this may be because the causative microorganisms are lying dormant within the body until circumstances are suitable for the development of overt disease.

Limbal stem cell: A stem cell found towards the edge of the cornea of the eye.

Longitudinal: Refers to a study that involves repeated observations on the same subject over a long period of time.

Lymphatic system: A network of vessels through which lymph (a colourless fluid containing white blood cells, vital in immune system function) drains from the tissues into the blood.

Lysis: The disintegration, for example of a cell, brought about by the breakdown of the containing wall or membrane.

Lytic: Relating to, or causing, lysis. For example, in lytic viral infection a virus replicates within a cell and, in the process of its release, destroys the cell.

Macular degeneration: A condition that results in a loss of vision in the centre of the visual field, owing to the deterioration of the macula (an area near the centre of the retina in the eye).

Matrigel™: A gelatinous protein mixture that resembles the extracellular environment found in many tissues and is used by cell biologists as a substrate for cell culture.

Meiosis: Part of the process of gamete formation, involving two cell divisions, in the course of which the diploid chromosome number becomes reduced to the haploid.

Mesenchyme: Undifferentiated loose connective tissue that is derived mostly from embryonic mesoderm. It contains cells capable of developing into various tissues such as bone, cartilage and blood vessels.

Mesenchymal stem cell: see Box 3.3, page 38.

Mesoderm: The middle of the primary germ layers, which gives rise to many of the internal tissues, such as bone, cartilage, muscle, blood, dermis and connective tissues. See also ectoderm and endoderm.

Mesodermal stem cell: A stem cell of a type found in or derived from the mesoderm (the middle layer of cells or tissues of the embryo).

Metabolism: The chemical processes that occur within a living organism to maintain life, including both the synthesis of substances and their breakdown to produce energy.

Mitosis: The normal form of cell division in body tissues, resulting in two diploid daughter cells. See also meiosis.

Molecular phylogenetics: The study of similarities of DNA sequence, to gain information on the evolutionary relationships between organisms and species. See Box 2.1, page 17.

Monoclonal antibody: An antibody produced in the laboratory from a single clone (a genetically identical population) of cells. Monoclonal antibodies from the same clone are identical, so they recognise the same epitope on the antigen.

Monoclonal antibody therapy: A medical treatment that makes use of the highly specific binding of a monoclonal antibody to a specific biological target. The antibody itself can act as a therapeutic agent (e.g. by blocking receptors) or can carry with it an active drug molecule.

Mosaic animal: An animal containing two or more genetically distinct cell types that have arisen from the same zygote. Mosaic animals can occur as a result of naturally occurring mutations, or manipulations such as retroviral transfer. They are distinct from chimæras. See Box 2.3, page 23.

MHRA: Medicines and Healthcare products Regulatory Agency.

mRNA: Messenger ribonucleic acid. The molecule that carries the information from DNA to act as a template for protein synthesis (some mRNAs are non-protein-coding and have other functions). See Box 2.2, page 21.

MRI: Magnetic resonance imaging. A technique for producing images of bodily organs by measuring the response to high-frequency radiowaves in a strong magnetic field.

MSC: Mesenchymal stem cell. See Box 3.3, page 38.

Multipotent: See potency and Box 3.3, page 38.

Mutation: The modification of a DNA sequence that has the potential to lead to a change in the function of a gene. Mutations may be caused by mistakes in copying of DNA during cell division, or by exposure to DNA-damaging agents in the environment. Mutations can be harmful, beneficial or, most commonly, of no consequence. They can be caused by the alteration of single base units in DNA, or the deletion, insertion or rearrangement of larger sections of genes or chromosomes. Mutations can only be passed on to offspring if they occur in cells that make eggs or sperm.

NAS: National Academy of Sciences. One of the four United States National Academies.

Neuron: A specialised cell that transmits nerve impulses; a nerve cell.

Neural stem cell: A stem cell of a type found in the brain, from early development to adulthood.

Niche: The cellular microenvironment providing support and stimuli necessary to sustain stem cell self-renewal and to control stem cell differentiation. See 3.3, page 38.

NHP: Non-human primate. In this text 'NHP' is used to refer to species of primates *except* humans. 'Great Apes' is used to refer to chimpanzee, pygmy chimpanzee, gorilla and orang-utan (see Box 6.1, page 83), and 'monkey' to refer to NHPs *other than* humans and Great Apes (e.g. 5.6.2), page 78.

NRES: UK National Research Ethics Service, which provides ethical assessment of proposed medical research involving human subjects.

Nucleotide: The structural unit of nucleic acids, DNA and RNA.

Nucleus (cell nucleus): A membrane-bound structure, often spherical, present in most living cells, which contains the DNA in the form of chromosomes.

Oocyte: A cell of the female germ-line that may undergo division to form an ovum (a mature female reproductive cell, egg). However, the term oocyte is often loosely used in place of ovum or egg.

Olfactory: Relating to the sense of smell.

Oligodendrocyte precursor: A cell that can develop into an oligodendrocyte, a kind of glial cell that produces myelin (a substance that provides an insulating sheath around many nerve fibres) in the central nervous system.

Oncogene: A gene that in certain circumstances can transform a cell into a tumour cell.

Oncology: The study and treatment of cancer.

Open-label: Describes a clinical trial in which both the researchers and participants know the treatment that is being administered. This contrasts with the single blind method where participants are not aware of what treatment they are receiving, and the double-blind trial where neither experimenter nor the subject know whether active treatment or placebo is being administered.

Organism: An individual animal, plant or single-celled life form.

Ovariectomy: Surgical removal of one or both ovaries.

Perinatal: Relating to the time immediately before and after birth. In humans this is usually a period of several weeks; in rodents, a few days.

PET: Positron emission tomography. A medical imaging technique that produces a three-dimensional image of internal body structures. During a PET scan, the recording system detects rays emitted by a radioactive substance that is introduced into the body on a biologically active molecule.

Phenotype: The physical manifestation of an organism, which results from the expression of the genotype together with non-genetic influences.

Plasticity: The ability of a cell or organism to adapt to changes in its environment.

Pluripotent: See potency and Box 3.3, page 38.

Polypeptide: A molecule made up of several amino acids joined together in a chain. Proteins consist of one or more polypeptides.

Potency (or potential): Generic terms to denote the range of specialised cells that a stem cell may/can give rise to. Stem cell potency can be more specifically described as unipotent, multipotent or pluripotent. See Box 3.3, page 38. (A totipotent cell is one that can give rise to all cell types in an animal or human, including extra-embryonic tissues, and (according to a commonly accepted definition), carries the information required to organise development of the embryo correctly. The only cells that are totipotent are therefore the fertilised egg and those of early cleavage stage embryos. These do not self-renew, therefore they are not stem cells.)

Pre-clinical: Denotes research, or the stages of the drug development process, conducted before that in the clinic. It may include approaches such as *in vitro* research, computer simulation and research using animals.

Primitive streak: A structure that forms during the early stages of mammalian embryogenesis; its appearance is one of the first signs of gastrulation.

Prion: A misfolded form of protein believed to act as an infectious agent. Diseases including bovine spongiform encephalopathy (BSE, in cattle), scrapie (in sheep) and Creutzfeldt–Jakob disease (CJD, in humans) are thought to be prion-mediated.

Progenitor cell type: A generic term for any dividing cell with the capacity to differentiate. It includes putative stem cells in which self-renewal has not yet been demonstrated.

Pronucleus: Either of a pair of nuclei from gametes (in the haploid state following meiosis) in the egg after fertilisation (or activation) but before they come together to form the (diploid) chromosome complement of the zygote.

Protein: Large molecules composed of one or more long chains of amino acids (polypeptides). Proteins are an essential part of living organisms, as both structural and functional components of all body tissues.

Provirus: The genetic material of a virus as incorporated into the genome of a host cell.

Quiescent: In a state or period of inactivity or dormancy.

Receptor: A molecule within a cell (frequently in a cell membrane), which binds and responds specifically to a particular transmitter (cell signalling molecule), hormone, antigen or other biologically active molecule.

Recombinant DNA: DNA formed artificially by combining sections of DNA, often from different organisms.

Regenerative medicine: Approaches aimed at creating living, functional tissues to repair or replace the function of cells, tissues or organs lost because of damage or congenital defects. Many such approaches involve the use of stem cells.

Restricted: Limitation of the potency of a stem cell, meaning that it cannot give rise to some types of specialised cells in the body.

Retrovirus: An RNA virus that inserts a DNA copy of its genome into the host cell to replicate, for example human immuodeficiency virus (HIV).

RGF: Research Governance Framework.

SCRO: Stem Cell Research Oversight Committee. See Box 7.2, page 104.

Self-renewal: Cycles of division that generate at least one daughter cell equivalent to the mother cell, with latent capacity for differentiation. The defining property of stem cells. See 3.3, page 34.

Somatic cell: Any cell that forms part of the body of an organism, not including germ cells.

SCNT: Somatic cell nuclear transfer. The transfer of the nucleus from a somatic (e.g. fetal or adult body) cell into an oocyte from which the nucleus (or the nuclear DNA) has been removed. The basis of the technique used to clone mammals, famously including Dolly the sheep.

Species: A species is one of the basic units of biological classification and a taxonomic rank. A species is often defined as a group of organisms capable of interbreeding and producing fertile offspring. Although in many cases this definition is adequate, more precise or differing measures are often used, such as similarity of DNA or morphology. See Box 2.1, page 17.

Sperm: An abbreviated term used to denote a male sex cell of an animal. In scientific terminology, the developing male sex cells are named at different stages (e.g. 'spermatogonium', 'spermatocyte', 'spermatid'), and a mature, motile male sex cell is referred to as a 'spermatozoon'.

Spermatagonial stem cell: See Box 3.3, page 38.

Splicing: The modification of a primary messenger RNA (mRNA) transcript to remove the non-coding 'introns' and join up the coding 'exons' to make a functional mRNA, usually one able to be translated to form proteins.

Stem cell: A stem cell is a cell that can continuously produce unaltered daughters and has the ability to produce daughter cells that have different, more restricted properties. In this text, the term 'stem cell' is sometimes used to encompass other progenitor cells types. Human stem cells are abbreviated 'h', e.g; human ES cell (human embryonic stem cell); human iPS cell (human induced pluripotent stem cell).

Telomere: Repetitive nucleotide sequences at the ends of chromosomes that serve as a 'capping' structure. Telomeres are shortened with each cell division. Short telomeres are consequently considered a sign of ageing.

Teratocarcinomas: A form of malignant teratoma occurring especially in the testis.

Teratoma: A type of tumour that contains several different tissue types. See 3.6.1, page 56.

Tetraploid: A cell or nucleus in which four homologous sets of chromosomes are represented, in contrast to the two sets (diploid) normally found in somatic cells.

Tissue: A distinct type of material of which the body is composed, consisting of specialised cells and their products, for example connective tissue, muscle.

Tissue-specific (or adult) stem cell: See Box 3.3, page 38.

Toxicity testing: A stage in the development of therapeutic products, in which they are tested for their potential to cause unanticipated or harmful effects to the body. Toxicity tests are often conducted on animals to establish dose–toxicity relationships and maximum safe dosage levels before clinical trials are conducted in man.

Transfection: A process in which DNA is introduced into a cell containing a nucleus, and integrates into the recipient cell's nuclear DNA.

Transgenic: A cell, embryo or animal created by the insertion of some additional genetic material from another genome. See Box 2.3, page 23.

Transgene: A sequence of genetic material taken one organism (or artifically synthesised) and inserted into the genome of another cell, embryo, or animal. See Box 2.3, page 23.

Translation: The process by which a sequence of nucleotides in a mRNA molecule is read and 'translated' by cellular machinery to a specific sequence of amino acids, during synthesis of a protein.

Trophectoderm, trophoblast: A layer of tissue on the outside of a mammalian blastocyst, which supplies the embryo with nourishment and later forms the major part of the placenta.

Tumour: A swelling of a part of the body, generally without inflammation, caused by an abnormal growth of tissue.

Tumourigenic: Tumour-causing.

UNESCO: United Nations Educational, Scientific and Cultural Organization.

Unipotent: See potency and Box 3.3, page 38.

Vaccine: An antigenic substance prepared from the causative agent of a disease or a synthetic substitute, used to produce immunity against disease.

Vascularisation: The process of developing blood vessels.

Vector: A biological construct (e.g. a plasmid) used as a vehicle for transferring genetic material into a cell. See Box 2.2, page 21.

Vertebrate: An animal possessing a backbone or spinal column, such as a mammal, bird, reptile, amphibian or fish.

Xenograft: A tissue graft or organ transplant from a donor of one species into a recipient of another. ('Xeno-' from Greek meaning 'stranger'.)

X-ray: An electromagnetic wave of high energy, which is able to pass through many materials opaque to light; a photographic or digital image of the internal composition of something, especially a part of the body, produced by X-rays being passed through it.

Zygote: The diploid cell resulting from the union of haploid male and female gametes.

Recent reports from the Academy of Medical Sciences

A new pathway for the regulation and governance of health research
http://www.acmedsci.ac.uk/p99puid209.html

Biomedical research - a platform for increasing health and wealth in the UK
http://www.acmedsci.ac.uk/p99puid212.html

Academia, industry and the NHS: collaboration and innovation
http://www.acmedsci.ac.uk/p101puid202.html

Redressing the balance: the status and valuation of teaching in academic careers
http://www.acmedsci.ac.uk/p99puid181.html

Reaping the rewards: a vision for UK medical science
http://www.acmedsci.ac.uk/p99puid172.html

Rejuvenating ageing research
http://www.acmedsci.ac.uk/p99puid161.html

Building clinical academic capacity and the allocation of resources across academic specialties
http://www.acmedsci.ac.uk/p99puid150.html

Research in general practice: bringing innovation into patient care
http://www.acmedsci.ac.uk/p101puid163.html

The Bologna Process: will it affect UK biomedicine and clinical science
http://www.acmedsci.ac.uk/p101puid179.html

Academy of Medical Sciences
41 Portland Place
London, W1B 1QH
Tel: +44 (0)20 3176 2150

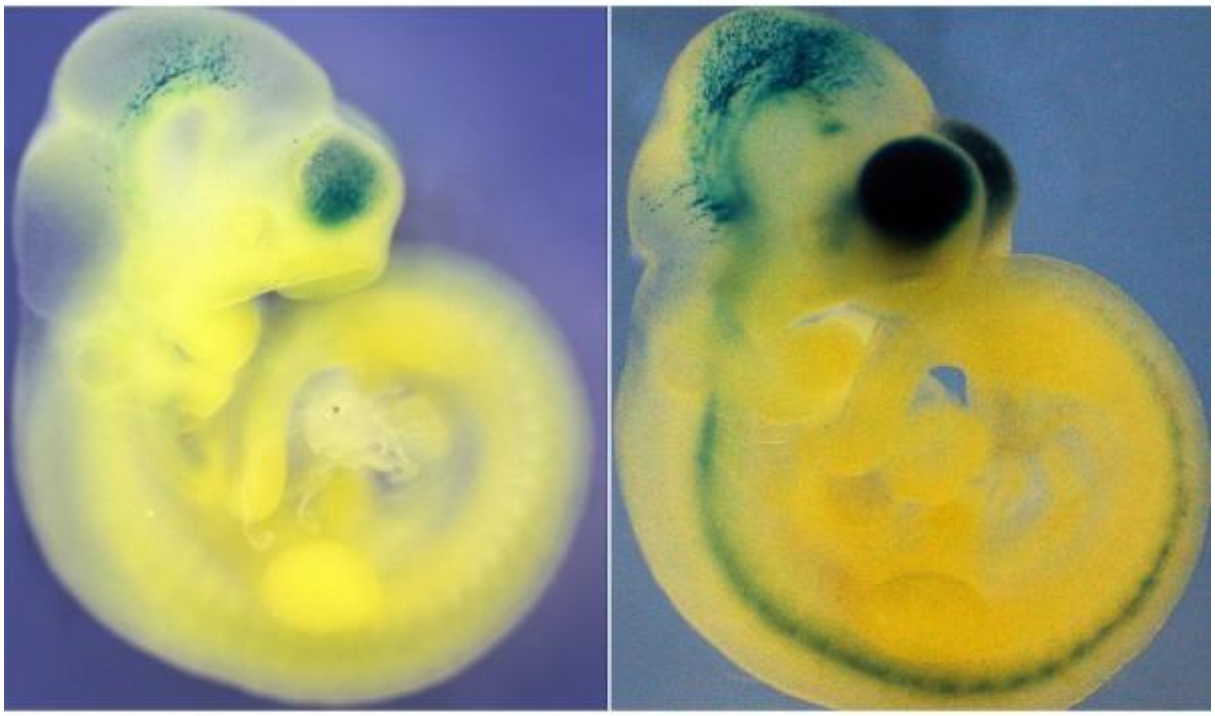
info@acmedsci.ac.uk
www.acmedsci.ac.uk

ATTACHMENT F

Pennisi,

Human DNA enlarges mouse brains,

SCIENCE (Feb. 19, 2015)



SILVER LAB

The blue stains in these developing mice embryos show that the human DNA inserted into the rodents turns on sooner and is more widespread (right) than the chimp version of the same DNA, promoting a bigger brain.

Human DNA enlarges mouse brains

By Elizabeth Pennisi (/author/elizabeth-pennisi)
19 February 2015 12:15 pm | 64 Comments (/biology/2015/02/human-dna-enlarges-mouse-brains#disqus_thread)

Researchers have increased the size of mouse brains by giving the rodents a piece of human DNA that controls gene activity. The work provides some of the strongest genetic evidence yet for how the human intellect surpassed those of all other apes.

"[The DNA] could easily be a huge component in how the human brain

expanded," says Mary Ann Raghanti, a biological anthropologist at Kent State University in Ohio, who was not involved with the work. "It opens up a whole world of possibilities about brain evolution."

For centuries, biologists have wondered what made humans human. Once the human and chimp genomes were deciphered about a decade ago, they realized they could now begin to pinpoint the molecular underpinnings of our big brain, bipedalism, varied diet, and other traits that have made our species so successful. By 2008, almost two dozen computerized comparisons of human and ape genomes had come up with hundreds of pieces of DNA that might be important. But rarely have researchers taken the next steps to try to prove that a piece of DNA really made a difference in human evolution. "You could imagine [their roles], but they were just sort of 'just so' stories," says Greg Wray, an evolutionary biologist at Duke University in Durham, North Carolina.

Wray is particularly interested in DNA segments called enhancers, which control the activity of genes nearby. He and Duke graduate student Lomax Boyd scanned the genomic databases and combed the scientific literature for enhancers that were different between humans and chimps and that were near genes that play a role in the brain. Out of more than 100 candidates, they and Duke developmental neurobiologist Debra Silver tested a half-dozen. They first inserted each enhancer into embryonic mice to learn whether it really did turn genes on. Then for HARE5, the most active enhancer in an area of the brain called the cortex, they made minigenes containing either the chimp or human version of the enhancer linked to a "reporter" gene that caused the developing mouse embryo to turn blue wherever the enhancer turned the gene on. Embryos' developing brains turned blue sooner and over a broader expanse if they carried the human version of the enhancer, Silver, Wray, and their colleagues report online today in *Current Biology*.

The researchers determined that HARE5 likely controls a gene called Frizzled 8, which is part of a molecular pathway important in brain development. Their further studies showed that the human version of the enhancer causes cells that are destined to become nerve cells to divide more frequently, thereby providing a larger of pool of cells that become part of the cortex. As a result, the embryos carrying human HARE5 have brains that are 12% larger (http://www.cell.com/current-biology/abstract/S0960-

9822%2815%2900073-1) than the brains of mice carrying the chimp version of the enhancer. Silver and Wray plan to test these mice to see if the bigger brains made them any smarter.

"They have found a smoking gun in the human genome that connects a regulatory element with a proposed pathway for increasing brain size," says Todd Preuss, a neuroanatomist at the Yerkes National Primate Research Center in Atlanta, who was not involved with the work.

But he; geneticist Evan Eichler of the University of Washington, Seattle; and others point out that there's more to be done. Several researchers worry that more extensive studies are needed to nail down that the HARE5 effects are not by chance. They'd like to see Silver and her colleagues replace the mouse HARE5 with the human and chimp HARE5—a feat the Duke group has yet to succeed in doing.

Even so, Eichler is pleased with just how much the Duke team has learned so far. And, Wray says, given the growing ability of researchers to study enhancers and other DNA in mice, "my guess is there are probably other stories like this in the works."

Posted in Biology (/category/biology) , Brain & Behavior (/category/brain-behavior) , Evolution (/category/evolution) , Plants & Animals (/category/plants-animals)

Science | DOI: 10.1126/science.aaa7878

ENCLOSURE 3

HHS'S 2015 CORRESPONDENCE WITH ALDF

DEPARTMENT OF HEALTH & HUMAN SERVICES

Office of the Secretary
Office of the Assistant Secretary for Health

Office for Human Research Protections
Rockville, Maryland 20852

MAY 19 2015

Christopher A. Berry, Esq.
Staff Attorney
Animal Legal Defense Fund
170 East Cotati Avenue
Cotati, California 94931

Dear Mr. Berry:

Secretary Burwell has asked me to thank you for your April 9, 2015 letter regarding the citizen petition for rulemaking to protect humanized chimeras under the Public Health Services Act, and to respond to you directly. The Office for Human Research Protections (OHRP) is the Department of Health and Human Services (HHS) office charged with administering the HHS regulations for the protection of human subjects in research (45 CFR part 46). In your letter, the Animal Legal Defense Fund (ALDF) asked HHS to promulgate regulations requiring Institutional Review Board (IRB) review of research involving human-animal chimeras with a "substantial possibility" of resulting in humanized high-level cognitive capacity. ALDF further asked HHS to recognize that chimeras actually acquiring high-level cognitive capacity qualify as individual research subjects in accordance with the Public Health Service Act and the Common Rule (45 CFR part 46, subpart A).

As ALDF has noted, such research is not covered under the scope of 45 CFR part 46. OHRP appreciates the suggestion that a new regulatory framework may be necessary to accommodate such research, and will take this request under consideration as it faces the challenges posed by such emerging science.

OHRP recognizes that issues are raised by the progression of science in the sphere of human-animal chimeras and human-animal transgenic research. Thank you for bringing such research to the attention of OHRP.

Sincerely,

Jerry Menikoff, M.D., J.D
Director
Office for Human Research Protections