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Ethical Standards for Human-to-Animal Chimera Experiments in Stem Cell Research

The purpose of this report is to offer investigators and members of SCRO and animal research committees well-grounded ethical standards for evaluating research involving the transfer of multipotent and pluripotent human stem cells and their direct derivatives into animal systems. This report is deliberately written in general terms so that its recommendations can apply to diverse institutions and international settings. Thus, investigators and reviewers should aspire to these proposed ethical standards while exercising appropriate judgment in individual situations.

Categories of Human-to-Animal Chimera Research

Chimeras are organisms containing cells from two or more zygotes or the imperfect equivalents thereof (such as parthenotes). Scientists widely consider chimera studies to be indispensable for answering fundamental questions in stem cell (Tam and Rossant, 2003) and developmental biology (McLaren, 1976). In stem cell research, human-to-animal chimera experiments typically involve the transfer of multipotent or pluripotent human stem cells into animals in embryonic, fetal, or postnatal stages of development to study stem cell behavior. Other forms of human-to-animal chimera studies involve transferring into animals human stem cell derivatives that are no longer pluripotent or multipotent for preclinical research. We set aside for discussion elsewhere the topic of nuclear-cytoplasmic hybrid research—e.g., the use of animal oocytes for human nuclear transfer experiments—although much of our analysis in this report is applicable to that form of research.

One common type of human-to-animal chimera study is the use of human embryonic stem cells (hES cells) to form teratomas in immunodeficient mice to assess stem cell quality and developmental potential. While this routine practice raises no ethical difficulties (McLaren, 2007) (see Lensch et al., 2007), other forms of chimera research may—such as preimplantation studies resulting in high but transient levels of human-to-animal chimerism in vitro (Karpowicz et al., 2004), and the transfer of differentiated human stem cells into the central nervous systems of higher-order animals (Greene et al., 2005).

These examples underscore the need to distinguish between two categories of chimera research: (1) in vitro studies using animal embryos before the development of the primitive streak; and (2) in vivo studies involving sentient animals. While both categories of research provoke intriguing questions, the latter raises additional issues of animal welfare and should be examined accordingly. We do this after first exploring two fundamental concerns that pertain to chimera research as such.

Commonly Held Concerns and Assumptions about Chimera Research

Much of the controversy with chimera research reflects uneasiness with crossing what are assumed to be morally inviolable species borders (Robert and Baylis, 2003). Thus an ethical framework on chimera research cannot ignore two central questions. First, is crossing species boundaries wrong? And second, does the generation of stem cell chimeras represent a particular instance of this crossing that raises special concerns?

History shows that humans and animals have always been exchanging bits of their biological matter, intentionally or by chance, naturally or through artificial aids of various sorts. Yet unlike stem cell chimera research, the majority of these encounters do not elicit fear or opposition.

Diet is a good example. With few exceptions, humans accept the entry of animal products into our daily metabolism. However, diet influences our bodies at both genetic and epigenetic levels. The effect of certain classes of nutrients on the methylation level of our DNA (one of the most meaningful types of epigenetic modification) is the best defined example of the enduring effect of diet on our genetic networks, an effect that might be even passed on to future generations (Cropley et al., 2006).

Vaccines and xenotransplantation are other more visible instances of animal-human mixing. For example, millions of patients worldwide live with heart valves harvested from pigs or cows. In these instances, resistance is normally based on specific dangers (for example, the risk of transmitting animal viruses to humans) rather than on general condemnation of human-animal mixing per se.

The different reactions elicited by these various modalities of human-animal mixing suggest that fears are directly related to the degree of deterministic biological agency that is attributed to the "fundamental units" that get mixed. Animal cells broken down to simple metabolites (as in the diet), or perceived as simple scaffolds (as in valve replacement), are considered largely unproblematic, whereas the mixing of stem cells or genes may induce deep-seated aversion. This is in turn the result of a tacit understanding of living systems that has percolated deeply into the public discourse and that grants genes and stem cells a degree of deterministic biological agency that other cells or metabolites do not possess. It is an ironic, although predictable, twist of fate that the more genes and stem cells are presented to the public as ultimate determinants of life, the more they are granted this status.

The different types of human-animal mixing considered above actually present a continuum of options, in which the lines to be drawn between the acceptable and the unacceptable do not align neatly with preexisting biological

categories (such as genes, cells, or metabolites) and their often inaccurate perception. The most recent understanding of gene and cell function from both molecular biology and philosophy of biology challenges the view of genes or cells as deterministic agents of biological phenotypes. For example, what does "animal or human gene" or "animal or human cell" actually mean? In the light of the evolutionary conservation of many signaling 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. In other words, what is at stake is what happens to developmental trajectories when signaling modules from different species are mixed (whether these modules come in the flavor of genes, cells, proteins, etc.).

A clear reminder of this point comes from molecular biology in the 1990s, when scientists defined the genetic hierarchy underlying the development of the eye (Halder et al., 1995). A single gene, transplanted in tissues of the fly embryo such as the wings and the legs, was able to direct the formation of a whole eye, an ectopic eye. And yet, when the homolog human gene was transferred into a mouse to check for its ability to rescue the small eye mutation, the result remained compelling: again, an eye was formed, testifying to the remarkable evolutionary conservation of genes and developmental pathways. But, as expected, a human gene in the mouse rescues a mouse eye (Schedl et al., 1996). And although cells embody a clearly higher degree of agency than genes, they also are exquisitely dependent on their surroundings. Besides the obvious example of bone marrow transplantation, in which the hematopoietic stem cell is an "agent" in blood reconstitution only in a receptive host, local niches regulate key developmental transitions for several adult stem cell types in what has been aptly termed an "inseparable relationship" (Xie and Li, 2007). And well-characterized cases of transdifferentiation, from tail regeneration in axolotl to inflammation-induced lymphangiogenesis, highlight to what extent the microenvironment can shape cell function (Echeverri and Tanaka, 2002; Maruyama et al., 2005). Context, in other words, is just as essential as genes or cells.

It is revealing to juxtapose these experiments with perhaps the best known example of a human-to-animal chimera produced by genetic engineering: a mouse with a human ear growing on its back—which, to be sure, was neither a human-to-animal chimera, nor a product of genetic engineering. In 1997, tissue engineers grafted bovine knee cartilage cells onto a biodegradable scaffold molded into a small ear (Cao et al., 1997). They then implanted this scaffold on the back of the mouse so that the cartilage cells could be supplied by the blood vessels of the skin. The bovine ear on the back of the mouse is a relevant counterpart to the mouse eye rescued with a homolog human gene, highlighting the conceptual and practical difference between mixing tissues (a usually well-accepted practice) and mixing genes (the source of great fears). The iconic image of the human ear on the back of a mouse reveals that this notable so-called "chimera" owes much to an engineering of scaffolds and nothing to an engineering of genes.

We may now revisit the two questions posed earlier. First, human-animal crossing is not wrong per se and is in fact a persistent feature of human societies. Second, stem cell or gene chimeras do not represent an exceptional case, because neither stem cells nor genes can be handled a priori as biologically deterministic agents independent of their context. And to the extent that some cells may be, for all practical matters, more "context independent" than genes, a tenable ethical framework needs, on a case-by-case basis, a sound assessment of the developmental trajectories that are likely to be affected and that takes into account the epigenetic context of regulation in which the mixed genes or cells are going to be deployed. Such a process is set forth in the Recommendations section.

If the mixing of human and animal matter is neither new nor particularly problematic per se, are there other reasons to reject human-to-animal chimera research as morally wrong? Another major objection relies on the idea that human-animal mixing threatens human dignity. In particular, the concern is that transferring human stem cells and their derivatives may entail the emergence of uniquely human psychological functions in animal chimeras, thus undermining human dignity (Karpowicz et al., 2005).

However, the "threat to human dignity argument" is seriously flawed. The first problem is that the conclusion does not logically follow from the premises. Human dignity is a multifaceted and culturally relative notion that is characterized (at least minimally) by a family of nearly unique and valuable capacities generally found in human beings, such as the capacity for moral agency, self-awareness, empathy, and high-level emotional and cognitive functions. These mental properties are not unique to, but taken as a whole are intrinsic to, human beings, and as such cannot be diminished even if, hypothetically, animal chimeras were to emerge with complex psychological capacities associated with human dignity. Simply put, human dignity is not something that diminishes when it is ascribed to more individuals.

Second, human dignity is not a property of human cells. It is a property of human beings. While recognizing and valuing human dignity, it is important to avoid the mistaken reductionist view that would enshrine human dignity in stem cells and specialized tissues rather than human beings. Of course, humans are part of a vast evolutionary web including many species with "human-like" properties, including varying degrees of cognition, emotive capacity, and social interdependence. Therefore, while it is important to reiterate that chimera research, like all research on nonchimeric animal species, should be governed by animal welfare principles, it is also important that those principles are sufficiently developed to protect animal subjects of research of human-like mind, were such an outcome in fact to emerge regardless of the goal of the research (cf. Streiffer, 2005).

In the remaining sections, we systematically build a proposal for evaluating the permissibility of human-to-animal chimera studies, setting in place the ethical guidelines that must be present should such research be considered.

Avoiding Unwarranted Stem Cell Exceptionalism

For decades, animal chimeras have served as a valuable research tool in diverse areas of biomedical research. Human primary tumor cell lines are commonly transplanted into experimental mice in the course of cancer research. And SCID-hu mouse models of the human immune system have been widely used since the 1980s. Considerations of fairness and justice dictate that one avoids unwarranted stem cell exceptionalism in assessing the permissibility of human-to-animal chimera studies in stem cell research. That is, one should use existing ethical standards for research, unless something specific to stem cell research drives a need for additional ethical standards. Accepting this basic principle of justice (treating like cases alike) means that one should adhere as much as possible to ethical analytic structures used in relevantly similar contexts.

For in vitro chimera studies, we recommend adherence to the ISSCR guidelines that no in vitro cultures of animal stem cells into human embryos be allowed to develop for longer than 14 days or until the formation of the primitive streak, and that no products of research involving transferred human cells be implanted into a human or nonhuman primate uterus (International Society of Stem Cell Research, 2006). These recommendations imply that the moral status of the research embryo up to 14 days of development is not affected by the possible degree of chimerism, in line with the rejection of unwarranted stem cell exceptionalism.

For chimera studies involving the transfer of human stem cells or their direct derivatives into gestated animals, we recommend building on existing animal welfare structures for animal research, adding stem cell-specific standards to address hypothetical developmental potential and trajectories.

Building on Ethical Standards for Animal Research

Over more than the last half-century, there has evolved a well-defined scientific and ethical tradition to guide the ethical use of animals in scientific research. This tradition is expressed through laws, international and national guidance documents, and international standards for voluntary accreditation (e.g., Association for Assessment and Accreditation of Laboratory Care International, http://www.aaalac.org), as well as in academic literature (National Research Council, 1998; Orlans, 1993; Rollin and Kesel, 1990).

Common principles include (but are not limited to) the following: (1) the research must have substantial scientific merit, and there must be no acceptable alternative method for answering the research question; (2) the research must be conducted in appropriate facilities by well-trained and supported staff; (3) the fewest possible animals must be used, without undue pain and stress, and with environmental enrichment appropriate to the species; (4) experimental endpoints must be clearly defined, and euthanasia must be conducted humanely according to approved methods; (5) an independent review body should provide ongoing review and monitoring, with the power to suspend or terminate studies.

A significant literature has developed that details standards of care, behavioral norms and indicators (e.g., Canadian Council on Animal Care, 1993; National Research Council, 1996), and species-specific tests used in neuroscience and behavioral research that are instrumental in assessing pain, anxiety, and suffering of sentient research animals (National Research Council, 2003). In using the term sentience here, we borrow from a literature that provides no single definitive term but points to qualities of mind that are related to awareness, cognition, and the suffering that could be occasioned by research. These are functionally relevant to animal welfare principles that physical and mental suffering should be minimized.

Many current animal welfare tests are primarily species specific; this raises special questions for chimeras, at least those in which the significant potential for a change in sentience or behavior is theoretically anticipated. These concerns are justified not by experience or practically grounded expectations but by reasonable caution. Similar issues arise with transgenic animals, in that both new norms of behavior, and new defects which require special care, may arise from the genetic alteration. Best practices in such cases have been well defined (e.g., Canadian Council on Animal Care, 1993; National Research Council, 1996) and include establishing baseline data for transgenic animals; vigorous data collection during research concerning any deviation from the norms of typical animals; use of pilot studies; and ongoing monitoring and reporting to oversight committees empowered to evaluate the need for prompt protocol changes and withdrawal of animal subjects. The application of these steps to chimeric animals is set forth in the next section.

Recommendations

Having considered the common arguments against human-to-animal chimera research and the need to avoid unwarranted stem cell exceptionalism, we offer the following recommendations.

(1) Chimera research presupposes an adequate scientific and ethical infrastructure for nonchimeric in vitro and animal research. Proposed research must have scientific merit, be directed to the increase of knowledge and potential public benefit, take place in appropriate facilities with properly trained and supported scientists and staff, and be peer reviewed. Chimera research should be subject to the general animal welfare principle that, to the extent practicable, research on nonsentient constructs should be employed before research on sentient animals, and research on less sentient animals should be preferred to research on more sentient ones.

(2) Chimera research involving human stem cells, to the extent that it involves creating, gestating, and raising an animal with significantly increased potential for sentience,

should be assessed based on a reasonable extension of the standards recognized for research with animal models. This means, for example, that it should be based on rational, practical, fact-based assessments of its effects on sentience, rather than merely speculative concerns about increases in intelligence contrary to what is known about brain functional requirements and known scientific facts.

(3) Additional data collection and monitoring should be commensurate with the anticipated characteristics of the chimeric animal in the context of the proposed research. Creation of a teratoma to confirm stem cells' characteristics should require no additional or exceptional monitoring beyond the application of animal welfare principles, whereas a significant contribution to the central nervous system by hES cells or their direct derivatives will require additional considerations. One cannot assume that hES cell chimerism will always supplement the awareness and capacities of a chimera. The chimeric intervention may have no predicted or reasonably predictable effect on sentience, in which case review should be satisfied by application of traditional animal welfare principles. To be sure, even in such cases, precisely because a chimera is an interruption of an animal's known characteristic equilibrium, chimerism may create deficiencies and other issues that, as with transgenics and knockout animal models, require special scientific and ethical consideration of the effect of proposed research on the chimeric animal. These should be addressed through diligent application of ethical principles for the protection of animals in research, and primarily through regular mechanisms.

(4) Monitoring and data collection should be based upon a sound assessment of the developmental trajectories that are likely to be affected, and take into account the epigenetic context of regulation in which the mixed genes or cells are going to be deployed. It should be grounded in knowledge of such trajectories and reasonable scientific inferences concerning their potential, together with thorough reference to the tests and assessments currently available for the host species (e.g., Canadian Council on Animal Care, 1993; National Research Council, 1996). Review committees should avoid data collection for the sake of data collection, with the meaningfulness of the data to be determined later, or translating uncertainty into tests and additional procedures whose materiality is not well grounded in scientific knowledge and inference. Data collection should be linked to known functional links, or links to be evaluated in a scientifically legitimate manner. No single test, such as the percentage presence of human-derived cells in the brain, should be necessarily required, unless its functional link to pertinent physical or mental qualities is either demonstrated or is consistent with scientific knowledge or scientifically reasonable inferences concerning whether, in the context of other data, it will be a valid predictor of sentience. Neuroscientific and other research should further identify what tests meet this requirement, and it will be important that researchers and journals make research results rapidly available so that SCROs can take advantage of the rigor it provides. With that research, further guidelines will be possible based on well-grounded physical, behavioral, and other parameters.

(5) Chimera neuroscientific research involving human stem cells or their direct derivatives, in hypothetically approximating some aspects of human functioning, may thus demand accepted or new specialized cognitive and other assessments of the sort conducted in neuroscientific research. There may be an irreducible degree of uncertainty about the cognitive nature of the new chimeric animal, and how it would manifest distress, anxiety, or other factors relevant to one's assessment of animal welfare. In such cases, as with transgenic animals, researchers and institutions should familiarize themselves with these options, which may not be widely known to them, and consider requiring that a baseline of normal data be created before experimentation is permitted, keeping in mind the requirement above that data collection should be required only if its scientific and ethical materiality is either demonstrated or based on reasonable and rigorous scientific knowledge or inferences. These data could include behavioral and other data necessary for animal welfare, but may also include appropriate physiological (including endocrine data related to indicators of stress, anxiety, and pleasure), anatomic, morphological, and other data suggestive of the degree of integration with human cell types, if any, and the functioning of those cells and the functional potential of the resulting chimeric brain. As with transgenic animals, investigators and institutions should consider requiring the use of pilot studies to produce initial data on chimeric animals subject to experimental interventions, employ ongoing monitoring of deviations from normal behaviors, and prescribe reporting to pertinent animal welfare committees. Because new behaviors may be elusive, investigators and institutions should use objective evaluations when possible, and mitigate interobserver subjectivity when subjective tests are used.

(6) Investigators and institutions should also make appropriate adjustments to research protocols to take into account new data or unanticipated responses from animal subjects relating to the ethical permissibility of the animal's participation in the study, including novel signals demonstrating deterioration or enhancement of an animal's condition and other factors pertinent to withdrawing the animal from the study. Regular reassessment of animal welfare during the course of experimentation is strongly encouraged.

(7) Research with the known, intended, or well-grounded significant potential to create humanized cognition, awareness, or other mental attributes, while not absolutely prohibited, should be subject to close scrutiny, taking the most careful steps to collect data pertinent to ethical protection of animal subjects, and an extraordinary degree of justification.

(8) Protocols, to be eligible for review, must satisfy appropriate animal welfare standards and must in addition specifically address and propose an appropriate plan for the issues identified in recommendations 2–7.

(9) Through retained advisors or committee diversity, review committees should ensure that they have sufficient scientific expertise to make appropriate judgments concerning the matters discussed in these recommendations. There should be flexibility in the precise allocation of responsibility for implementation of these principles between SCROs and institutional or local animal welfare committees. Ultimately, however, it is the responsibility of the SCRO to ensure that all elements have been met, and to receive, review, and act on ongoing notices of any concerns or unanticipated issues. It is the responsibility of the institution in which the research occurs to work with the SCRO, and with other review bodies, to address local social and ethical concerns.

These recommendations build on, and are consistent with, current ethical standards for biomedical and basic science research. We offer them to clarify the ethical standards for evaluating human-to-animal chimera experiments in stem cell research, and to identify those areas for further scientific research and publication that will be ethically material as the research continues.

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REFERENCES

Canadian Council on Animal Care (1993). Guide to the care and use of experimental animals. http://www.ccac.ca/en/CCAC_Programs/Guidelines_Policies/GUIDES/ENGLISH/toc_v1.htm.

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. Plast. Reconstr. Surg. *100*, 297–302.

Cropley, J.E., Suter, C.M., Beckman, K.B., and Martin, D.I. (2006). Germ-line epigenetic modification of the murine A vy allele by nutritional supplementation. Proc. Natl. Acad. Sci. USA *103*, 17308–17312.

Echeverri, K., and Tanaka, E.M. (2002). Ectoderm to mesoderm lineage switching during axolotl tail regeneration. Science *298*, 1993–1996.

Greene, M., Schill, K., Takahashi, S., Bateman-House, A., Beauchamp, T., Bok, H., Cheney, D., Coyle, J., Deacon, T., Dennett, D., et al. (2005). Ethics: moral issues of human-non-human primate neural grafting. Science *309*, 385–386.

Halder, G., Callaerts, P., and Gehring, W.J. (1995). Induction of ectopic eyes by targeted expression of the eyeless gene in Drosophila. Science *267*, 1788–1792.

International Society of Stem Cell Research (2006). Guidelines for the conduct of human embryonic stem cell research. http://www.isscr.org/guidelines/ISSCRhESCguidelines2006.pdf

Karpowicz, P., Cohen, C.B., and van der Kooy, D. (2004). It is ethical to transplant human stem cells into nonhuman embryos. Nat. Med. *10*, 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 Inst. Ethics J. *15*, 107–134.

Lensch, M.W., Schlaeger, T.M., Zon, L.I., and Daley, G.Q. (2007). Teratoma formation assays with human embryonic stem cells: a rationale for one type of human-animal chimera. Cell Stem Cell, in press. Published online August 16, 2007. 10.1016/j.stem.2007.07.019.

Maruyama, K., Ii, M., Cursiefen, C., Jackson, D.G., Keino, H., Tomita, M., Van Rooijen, N., Takenaka, H., D'Amore, P.A., Stein-Streilein, J., et al. (2005). Inflammation-induced lymphangiogenesis in the cornea arises from CD11b-positive macrophages. J. Clin. Invest. *115*, 2363–2372.

McLaren, A. (1976). Mammalian Chimeras (New York: Cambridge University Press).

McLaren, A. (2007). A scientist's view of the ethics of human embryonic stem cell research. Cell Stem Cell *1*, 23–26.

National Research Council: Committee on Guidelines for the Use of Animals in Neuroscience and Behavioral Research (2003). Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research (Washington, D.C.: National Academies Press).

National Research Council: Committee on Well-Being of Nonhuman Primates (1998). The psychological well-being of nonhuman primates (Washington, D.C.: National Academies Press).

National Research Council: Institute of Laboratory Animal Research, Commission on Life Sciences (1996). Guide for the Care and Use of Laboratory Animals (Washington, D.C.: National Academies Press).

Orlans, F.B. (1993). In the name of science: issues in responsible animal experimentation (New York: Oxford University Press).

Robert, J.S., and Baylis, F. (2003). Crossing species boundaries. Am. J. Bioeth. *3*, 1–13.

Rollin, B.E., and Kesel, M.L. (1990). The experimental animal in biomedical research (Boston: CRC Press).

Schedl, A., Ross, A., Lee, M., Engelkamp, D., Rashbass, P., van Heyningen, V., and Hastie, N.D. (1996). Influence of PAX6 gene dosage on development: overexpression causes severe eye abnormalities. Cell *86*, 71–82.

Streiffer, R. (2005). At the edge of humanity: human stem cells, chimeras, and moral status. Kennedy Inst. Ethics J. *15*, 347–370.

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

Xie, T., and Li, L. (2007). Stem cells and their niche: an inseparable relationship. Development *134*, 2001–2006.

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