Comment on NIH Proposal to Fund Human-Animal Chimeras

Date: 6 September 2016

To: Dr. Francis Collins, NIH Director

In response to: 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
Notice Number: NOT-OD-16-128

Dear Dr. Collins:

This comment is submitted in response to the above-captioned public notice in which the National Institutes of Health (NIH) has requested comment on proposed changes to research and funding guidelines. In summary, we oppose all the proposed changes to the Guidelines, for the numerous rationales detailed in the textual comment following this summation. Moreover, we strongly urge NIH not only to reject the proposed changes, but also to extend further the prohibitions on the types of chimeras disallowed and ineligible for NIH funding.

The proposed changes would allow, and approve taxpayer funding, for creation of human-animal chimeras that would produce human gametes within the chimera's body. The proposed changes would also allow, and approve taxpayer funding, for creation of human-animal chimeras that would have substantially or completely human cell-derived brains. The primary demarcations of species identity, especially in the case of humans, revolve around reproductive genetics and conscious thought. The types of chimeras proposed for creation in these proposals thus cross significant ethical lines directly related to questions of species boundaries, the most important of which is the question of what it means to be human.

Creation of chimeras as proposed may lead to substantial incentives for further human embryo destruction. Expanding federal funds to this chimera research using "pluripotent cells" or "pluripotent stem cells" could lead to additional efforts to create and destroy human embryos to obtain those cells for this chimera research. Further, construction of some chimeras under these proposals may actually lead to creation of human embryos, which would be a violation of federal statute.

The proposed internally-staffed review committee for oversight and advice regarding proposed chimera experiments is flawed from the outset due to its biased makeup.

There is no scientific or ethical necessity that validates NIH approval or taxpayer funding of experiments creating the proposed human-animal chimeras.

Comments on components of the proposed changes to the Guidelines.

Research in which human pluripotent stem cells are introduced into non-human primate embryos up through the end of the blastocyst stage, Not Eligible for NIH Funding.

The prohibition against adding human pluripotent stem cells to non-human primate embryos until the end of the blastocyst stage is insufficient to protect against ethical as well as scientific abuses. For one, the ambiguous phrase "up through the end of the blastocyst stage" implies that once a mature blastocyst is formed (Carnegie stage 3), experiments involving insertion of human cells into this pre-implantation embryonic stage are allowed and eligible for funding.[1] Any human pluripotent stem cells thus inserted into the inner cell mass of a non-human primate blastocyst could intermingle to become any cell type or tissue in the developing non-human primate embryo, including human gametes and significant numbers of human neuronal cells even to the point of predominant formation of the brain.

This chimera test is, not surprisingly, considered an ultimate standard for determination of cell pluripotency, and has been used repeatedly to test putative mouse pluripotent stem cells.[2] The injected cells, if pluripotent, contribute to all tissue and cell types in the developing embryo (as well as in the born animal). Use of such animal-human chimeras as testbeds for pluripotent stem cells has long been a stated goal of some scientists, without regard or consideration for ethical concerns. But in contrast to a non-controversial human-animal chimera creation, where pluripotent stem cells are injected into adult animals as tests of pluripotency,[3] via teratoma formation, the NIH-proposed human-animal chimeras instead create a new embryo of mixed genetic composition and uncertain species identity. Others claim that human-animal chimeras are the only way to investigate human development,[4] or can provide transplantable custom-grown human organs in large farm animals.[5] But these claims ignore the numerous, innovative alternatives, including use of organoids even for study of brain development,[6] as well as for functional organoid and organ growth for transplant, as well as other alternatives such as adult stem cells for repair of damaged organs. In point of fact, proponents have failed to provide substantive evidence for success of the human-animal chimera option, as well as evidence for necessity whether from a scientific or ethical basis.

Further, even if the ambiguous phrase "up through the end of the blastocyst stage" is meant to imply introduction of human cells is allowed only at the beginning of the next phase of development, this still introduces human pluripotent stem cells early in embryonic development either just prior to or during (Carnegie stage 4 and stage 5) implantation (approximately seven days post-fertilization). At this stage of development, there is still significant opportunity for

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[1] NIH itself notes that the human blastocyst is formed five days post-fertilization, which is two days prior to implantation into the uterine wall; web page "From Fertilization to Blastocyst" accessed 5 Sept 2016 at: http://stemcells.nih.gov/info/pages/cellmovie.aspx

[2] Wernig M *et al.*, In vitro reprogramming of fibroblasts into a pluripotent ES-cell-like state, *Nature* 448, 318, 2007; doi: 10.1038/nature05944

[3] Lensch MW *et al.*, Teratoma Formation Assays with Human Embryonic Stem Cells: A Rationale for One Type of Human-Animal Chimera, *Cell Stem Cell* 1, 253, 2007; doi: 10.1016/j.stem.2007.07.019

[4] Sharma A *et al.*, Lift NIH restrictions on chimera research, *Science* 350, 640, 2015; doi: 10.1126/science.350.6261.640-a

[5] Rashid T *et al.*, Revisiting the Flight of Icarus: Making Human Organs from PSCs with Large Animal Chimeras, *Cell Stem Cell* 15, 406, 2014; doi: 10.1016/j.stem.2014.09.013

[6] See, *e.g.*, Otani T *et al*., 2D and 3D Stem Cell Models of Primate Cortical Development Identify Species-Specific Differences in Progenitor Behavior Contributing to Brain Size, *Cell Stem Cell* 18, 467, 2016; doi: 10.1016/j.stem.2016.03.003; *and* Lancaster MA *et al.*, Cerebral organoids model human brain development and microcephaly, *Nature* 501, 373, 2013; doi: 10.1038/nature12517

introduced human pluripotent stem cells to participate extensively in brain development or to form human gametes within the body of the non-human primate.

In all vertebrate species and many invertebrate species, the primordial germ cells originate at some distance from the forming gonadal (mesodermal) tissue, and must migrate to the site of the developing gonad; until the arrival of the PGC's, the site is often referred to as the "indifferent gonad," because formation into testis or ovary requires the presence of the primordial germ cells to stimulate sex-specific differentiation. For humans, the primordial germ cells arrive at the indifferent gonad at approximately 5-7 weeks post-fertilization (roughly Carnegie stages 15-19).[7] While this timing for arrival at the gonadal ridge varies somewhat even for other mammals, it is significantly later in development than "the end of the blastocyst stage" and leaves ample time for introduced human cells to participate in formation of gonadal tissue.[8]

Human cells introduced even after "the end of the blastocyst stage" still have significant opportunity and ability to form a substantially human brain in chimeric non-human primates as well. The initial formation of the primitive streak (Carnegie stage 6) and neurulation (the formation of the nervous system including the brain, commencing Carnegie stages 7-9) occur beyond blastocyst stage. This means that human pluripotent stem cells could be injected before or at the very beginning of nervous system formation, and contribute substantially to formation of the brain in the chimeric non-human primate.

This proposal is severely flawed and will not preempt formation of chimeric non-human primates that contain human gametes or a substantially human cell-derived brain. Introduction of human cells into non-human primates, which are already closely genetically related to humans, increases the likelihood of crossing and even blurring species boundaries, perhaps affecting the moral status of these human-animal chimeras. NIH should neither approve nor fund this dubious research which has such a propensity to create organisms with morally uncertain status.

Research involving the breeding of animals where the introduction of human cells may contribute to the germ line, Not Eligible for NIH Funding.

The prohibition against allowing breeding of chimeras that produce human gametes presupposes NIH willingly allowing production of such human-animal chimeras in the first place, by techniques and at developmental stages as already discussed. Creation of such chimeras should not be allowed, as this crosses an important ethical line regarding species identity and human dignity. Moreover, any such promises for prohibition on chimera breeding will be wholly ineffective. Even if NIH grantees meet or exceed the highest standards of animal care and use -- AAALAC and USDA[9] -- there is no way to guarantee with 100% certainty that incidents of breeding with these chimeras can be prevented. Once created and mature, it will be nearly impossible to prevent breeding of such chimeras. The only surety will be preventing creation of

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[7] See, *e.g.*, Schöni-Affolter F *et al.*, Human Embryology, Differentiation of the Gonads; embryology.ch; accessed at http://www.embryology.ch/anglais/ugenital/diffmorpho01.html for a visual representation; also see *e.g.*, Carlson BM, Patten's Foundations of Embryology, 6th edition (McGraw-Hill, NY, NY), 1996

[8] See, *e.g.*, Hayashi K *et al.*, Germ Cell Specification in Mice, *Science* 316, 394, 2007; doi: 10.1126/science.1137545; Richardson BE and Lehmann R, Mechanisms guiding primordial germ cell migration: strategies from different organisms, *Nature Reviews Molecular Cell Biology* 11, 37, 2010; doi: 10.1038/nrm2815; Guo F *et al.*, The Transcriptome and DNA Methylome Landscapes of Human Primordial Germ Cells, *Cell* 161, 1437, 2015; doi: 10.1016/j.cell.2015.05.015

[9] http://www.aaalac.org/ and https://www.aphis.usda.gov/aphis/ourfocus/animalwelfare

such human-animal chimeras at all. Again, there has been no valid ethical or scientific rationale for the necessity of creating this type of human-animal chimera.

The proposed steering committee to provide programmatic input on scope of research

The request for comment notes that this review committee will be composed of Federal employees, and be an intramural committee to advise on research scope and funding decisions. The proposal for this oversight committee is almost laughable (the phrase "fox guarding the henhouse" comes to mind), and is not in the least reassuring that there will be transparent, unbiased assessment of the ethics or scientific merit of any chimera experiments. Indeed, the 2015 NIH prohibition on funding human-animal chimeras raised ethical issues as a basis for the funding moratorium. Despite this, the current proposal does not address the multitude of ethical concerns with creation of human-animal chimeras, and the steering committee is nowhere tasked with considering the ethical issues or prohibitions based on ethics.

Scope to include research in which: a. human pluripotent cells are introduced into non-human vertebrate embryos, up through the end of the gastrulation stage.

Inclusion of this scope of research allows virtually any experiment in creation of human-animal chimeras, and should be rejected. The phrasing used – "up through the end of the gastrulation stage" – necessarily implies that all prior stages of any vertebrate embryo may be used for creation of human-animal chimeras. In essence, what is proposed is no limit whatsoever on the possible combinations of animal and human for chimera creation. We flatly reject this proposal, as it completely tramples any sense of ethical or scientific restraint.

Another problem with this proposal is that it could potentially lead to creation of a fully-human or substantially-human embryo, which would violate the Dickey-Wicker amendment. Because there is no lower limit on the stage of the non-human vertebrate embryo used, a very early non-human embryo could be combined with one or more human pluripotent cells. Even though the human cells would not be totipotent,[10] the pluripotent human cells could potentially grow faster than the animal cells, outcompeting them and resulting in an embryo solely or substantially composed of human cells. This outcome could also take place if the human cells are added prior to blastulation and end up as innermost cells in the morula; the inner position determines those cells that become inner cell mass (the embryo) in a mammalian blastocyst.

Not only is the lower limit for the stage of the non-human vertebrate embryo not defined, likewise the composition and genetics of the embryo are not defined. Thus, if a tetraploid non-human vertebrate embryo were combined with human pluripotent stem cells, a human embryo would result through the technique of tetraploid complementation. This technique has already shown success in producing specific mouse embryos and subsequently born individuals;[11] again,

[10] Condic ML, Totipotency: What It Is and What It Is Not, *Stem Cells And Development* 23, 796, 2014; doi: 10.1089/scd.2013.0364; *and* Condic ML, The Role of Maternal-Effect Genes in Mammalian Development: Are Mammalian Embryos Really an Exception?, *Stem Cell Reviews and Reports* 12, 276, 2016; doi: 10.1007/s12015-016-9648-6

[11] Kang L *et al.*, iPS Cells Can Support Full-Term Development of Tetraploid Blastocyst-Complemented Embryos, *Cell Stem Cell* 5, 135, 2009; doi: 10.1016/j.stem.2009.07.001; *and* Zhao XY *et al.*, iPS cells produce viable mice through tetraploid complementation, *Nature* 461, 86, 2009; doi: 10.1038/nature08267

this has been used as a test for pluripotency,[12] a stated aim of proponents of human-animal chimeras.

Production of human-nonhuman chimeric embryos early in development increases the potential for mutation as well as systemic xenotropic viral transfer. When chimerization occurs so early in development as proposed, there is a significant chance of endogenous retroviral activation. These changes could obscure any scientific information, as well as render useless any potential organs for transplant, whether later in development or from the born organism.[13]

Scope to include research in which: b. human cells are introduced into post-gastrulation non-human mammals (excluding rodents), such that there could be either a substantial contribution or a substantial functional modification to the animal brain by the human cells.

As already discussed in our comments, even limiting introduction of human cells to post-gastrulation non-human mammal embryos is unlikely to preclude the potential of substantial contribution to the neural structure of the developing animal. Neurulation actually commences at the end of gastrulation, so waiting until this point to add human cells poses no barrier and no assurance, whether scientific or ethical. This consideration of the scope of research actually entertains and encourages the possibility for substantial human neural development in the human-animal chimera. The proposal (not to mention the NIH workshop held November 2015, from which the proposal flows) also makes no effort to address the question of the meaning of "substantial" especially as it relates to "functional modification," or the means to determine behavioral qualities of the chimera that might raise concern. The proposal lacks any attempt to address the significant ethical issues of such chimera experiments.

We also note that a recent survey found a majority of the general public in Japan remain opposed to creation of the types of human-animal chimeras proposed by NIH, even over a period of years.[14] This is despite a general approval for various types of stem cell research, embryo research, and regenerative medicine. It would be prudent for NIH to listen to these same concerns expressed by the United States public in opposition to such human-animal chimeras, and reject the proposed changes in the Guidelines. We recommend in its place more definitive prohibitions on approval and funding of human-animal chimera creation to meet the concerns and reassure the public as well as policymakers that NIH is focused on scientifically worthy and ethically unimpeachable goals. We propose that the Guidelines be modified to prohibit creation of a human-animal chimera:
(a) such that human gametes develop within the body of a nonhuman life form, or
(b) such that it contains a human brain or a brain derived wholly or substantially from human cells.

Scientifically valid, ethically framed alternatives exist to assist the development of basic science as well as clinical medicine. We strongly support such ethical research and encourage you to reconsider the proposed changes in the Guidelines related to chimeras.

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[12] Zhou C *et al.*, Tetraploid complementation proves pluripotency of induced pluripotent stem cells derived from adipose tissue, *Cell Proliferation* 48, 39, 2015; doi: 10.1111/cpr.12152
[13] Ogle BM *et al.*, Spontaneous fusion of cells between species yields transdifferentiation and retroviral transfer in vivo, *FASEB J* 18, 548, 2004; doi: 10.1096/fj.03-0962fje
[14] Inoue Y *et al.*, Current Public Support for Human-Animal Chimera Research in Japan Is Limited, Despite High Levels of Scientific Approval, *Cell Stem Cell* 19, 152, 2016; doi: 10.1016/j.stem.2016.07.011

Submitted on behalf of the Charlotte Lozier Institute and the Family Research Council.

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