

ISSCR guidelines for the transfer of human pluripotent stem cells and their direct derivatives into animal hosts

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SUMMARY

The newly revised 2021 *ISSCR Guidelines for Stem Cell Research and Clinical Translation* includes scientific and ethical guidance for the transfer of human pluripotent stem cells and their direct derivatives into animal models. In this white paper, the ISSCR subcommittee that drafted these guidelines for research involving the use of nonhuman embryos and postnatal animals explains and summarizes their recommendations.

The newly revised *ISSCR Guidelines for Stem Cell Research and Clinical Translation* includes scientific and ethical guidance for the transfer of human pluripotent stem cells and their direct derivatives into animal models (*ISSCR, 2021*). We are the members of the International Society for Stem Cell Research (ISSCR) Task Force subcommittee that drafted these guidelines for stem cell research involving the use of nonhuman embryos and animals, and we summarize and explain our recommendations in this white paper. This report should be read in conjunction with Section 2 of the guidelines, entitled “Laboratory-Based Human Embryonic Stem Cell Research, Embryo Research, and Related Research Activities,” and Appendix 1.

As noted in the beginning of the 2021 ISSCR guidelines: “The primary societal mission of basic biomedical research and its clinical translation is to alleviate and prevent human suffering caused by illness and injury.” To fulfill this mission, stem cell scientists, geneticists, developmental bi-

ologists, preclinical investigators, and others endeavor to understand basic human stem cell biology and differentiation potential and to generate compelling new animal models for understanding human disorders. Of equal importance, collective efforts are aimed to explore the safety and efficacy of new stem cell-based treatment modalities in laboratory animals prior to initiation of clinical trials. Together, these aims make research involving the transfer of human stem cells and their direct derivatives into animal hosts necessary for stem cell science to progress to clinical applications. While recognizing that some people have concerns about this research, the potential value of these scientific efforts led our subcommittee to consider not *whether* animal models ought to be used, but rather *how* and under what circumstances such research could be permissible.

To explain the scope of our recommendations, our subcommittee drafted our guidelines for stem cell-based animal research with three boundaries in mind.

The first is the legal boundary. Our recommendations were written in general terms so that they could be used by different institutions internationally with potentially differing local research restrictions. Investigators should use our guidelines while exercising appropriate judgment in individual cases and in consultation with research policy experts to ensure they are conforming to local and national laws, first and foremost.





The second boundary is the current scientific boundary. While there has been much learned from the use of animal hosts in stem cell research, more hard work remains, in particular the challenge of learning how to enable human cells to survive longer and to integrate more extensively in animal hosts. For years, public concerns have existed over the possibility of generating mixed human/nonhuman research animals through stem cell technology (Greene et al., 2005), especially within the central nervous system (CNS) of large animal hosts like nonhuman primates (NHPs). However, the simple truth is that the most anxiety-provoking scenarios—e.g., the generation of laboratory animals with human-like cognitive traits—are currently scientifically out of reach and/or might even fail to meet the professional standards for review we advocate in our guidelines. Given the reality of current technical limitations, our goals were (1) to offer research guidelines that are calibrated to what may be scientifically possible in the near future and (2) to help research proceed responsibly in an incremental fashion. Thus, our guidelines for the transfer of human stem cells and their direct derivatives into animal hosts are intended to provide guidance over the next 5–10 years. Our subcommittee did not want merely *imaginable* scenarios surrounding stem cell-based animal research to dictate what the professional standards ought to be for research conduct and oversight, especially before there was good scientific evidence to support imagined concerns. The incremental approach we advocate in our recommendations would allow researchers to obtain new scientific data and for the ISSCR to evaluate such data and revise future professional guidelines accordingly.

The third boundary we considered is the boundary between prior regulatory experience with animal research oversight more generally and the novel aspects of stem cell-based research involving animal hosts. It is important to acknowledge that there are many well-articulated, institutionally embedded standards and regulations around the world for the use of animals in biomedical research. Stem cell research involving the use of animal hosts should be seen as part of these widely accepted animal research standards and, if necessary, should build upon these standards in practicable ways. Only when existing animal research standards are insufficient to capture unique aspects of stem cell science should additional guidelines be necessary. It is in the spirit of avoiding stem cell exceptionalism in the oversight of animal research that our subcommittee provides our recommendations. Indeed, the boundary between accepted practices in animal research and oversight on the one hand and new forms of stem cell research on the other is far less substantial than some may suppose, given the current state of the science. Therefore, our recommendations for stem cell research involving animal hosts will

seem familiar to biomedical researchers with experience in animal studies, as they should.

Both the 2021 ISSCR guidelines and this white paper are divided along two broad forms of stem cell research involving animals: (1) research involving the transfer of human pluripotent stem cells or their direct derivatives into nonhuman animal *embryos and prenatal animals* and (2) the transfer of human stem cells or their direct derivatives into *postnatal animals*. We begin with nonhuman embryo studies, followed by the use of postnatal animals, since these two forms of research involve different issues and oversight mechanisms, which deserve to be considered separately.

Guidelines for stem cell research using nonhuman embryos and prenatal animals

The 2021 ISSCR guidelines state that research involving the transfer of pluripotent human stem cells into nonhuman animal embryos may fall under different categories of review by a specialized research oversight process—what was previously called the EMRO process in the 2016 ISSCR guidelines (more information regarding each of these research categories is available in Clark et al. 2021, this issue). Just as there have been decades of regulatory experience around the world for animal research, stem cell research oversight has propagated globally and operated effectively since the early 2000s. Furthermore, human embryo research oversight has been successfully administered in the United Kingdom and many other locales for decades, long before the advent of human pluripotent stem cell research. Therefore, the categories of review published in the new ISSCR guidelines are not entirely new, but rather build upon these two types of research oversight experience. Given the fact that traditional animal research committees do not review animal embryo protocols per se, beyond ascertaining the welfare of any gestational surrogates or animal gamete donors used, our subcommittee believed it was appropriate for stem cell research involving animals and animal embryos to be incorporated into the following category system for embryo research proposed by the ISSCR. Here are the various categories of research presented in the 2021 guidelines, which together call for different levels of oversight:

Category 1A: research that is *exempt* from a specialized scientific and ethics oversight process after being assessed by the appropriate existing mandates and committees for laboratory research.

Category 1B: research that is *reportable* to the entity or body responsible for the specialized scientific and ethics oversight process, but not normally subject to further or ongoing review, at the discretion of the entity responsible for the oversight process and subject to regulations and policies in the jurisdiction.

Category 2: forms of research with embryos, certain chimeras, and stem cell-based embryo models that are



permissible only after review and approval through a specialized scientific and ethics review process.

Category 3A: *prohibited* research due to unresolved safety and ethics concerns.

Category 3B: *prohibited* research due to broad international consensus that such experiments lack a compelling scientific rationale and are widely considered to be unethical.

Depending on specific protocol details, stem cell research involving the use of nonhuman animals and embryos could span across any one of these categories.

Falling under category 1A is the transfer of human stem cells, their derivatives, or other human cells into postnatal animal hosts. Importantly, while this form of research is not relevant for specialized scientific and ethics review for *embryo* research, our subcommittee recommends that this type of work be reviewed by normal institutional animal research committees supplemented with stem cell-specific expertise in certain cases, as we explain in the subsequent section of this report entitled “guidelines for stem cell research using postnatal animals.”

Falling under category 1B is research in which human pluripotent stem cells are transferred into nonhuman mammalian embryos and cultured *in vitro* for the minimum time necessary to achieve the scientific objective without gestation. We contend that animal embryos containing transferred human cells are not themselves human embryos. For this reason, they are not ethically equivalent to *in vitro* fertilization-derived human embryos maintained in culture and permitted for research use. To date, studies involving so-called “chimeric embryo” cultures have yet to show a significant human contribution to host animal embryos (Tan et al., 2021). Nevertheless, researchers are encouraged to report their planned *in vitro* experiments to their committee or body responsible for the specialized scientific and ethics oversight process to help identify cases that may warrant full review. To this end, scientists pursuing human-to-nonhuman chimeric *in vitro* embryo research (without gestation in animal surrogates) should consult with institutional review committees or the body responsible for the specialized scientific and ethics review process to ensure that their proposed research does not require specialized review and approval.

Falling under category 2 is research in which human pluripotent stem cells or their derivatives with broad potential are introduced into (1) a nonhuman embryo or fetus *in utero* or (2) a nonhuman embryo *in vitro* followed by transfer into a nonhuman uterus. Such experiments—if they are scientifically justified for the use of NHPs above all other laboratory species—must exclude great and lesser ape species hosts (i.e., chimpanzees, gorillas, orangutans, bonobos, gibbons, and siamangs), as apes are prohibited

from being used for invasive research in most parts of the world.

This form of chimeric embryo research is permissible only after it has been reviewed through a process of specialized scientific and ethics review. Unlike *in vitro* studies, the developmental potential of chimeric embryos might be significantly greater if they are gestated for a period of time in a nonhuman uterus, since at our current level of technology, *in vitro* culture conditions are not as permissive as the natural uterine environment for advanced maturation. Due to the possibility of greater degrees of human cell integration and development within animal host embryos or fetuses, the gestation of chimeric embryos thus warrants close scientific and ethical review. This review should take into account the following points:

1. Any proposed chimeric embryo study involving uterine transfer or gestation must have a compelling scientific rationale and necessitate the use of these approaches rather than alternative models, while also using the minimum number of chimeric embryos necessary to achieve the scientific objective.
2. Researchers must justify why a particular species of host embryo is necessary. We recommend that scientific studies of chimeric embryo gestation are potentially permissible and thus require review for all laboratory animal species and NHP host species, except great apes and lesser apes (i.e., except chimpanzees, gorillas, orangutans, bonobos, gibbons, and siamangs). We explicitly exclude the use of great and lesser apes, first because the procedures necessary to derive oocytes for *in vitro* host embryo creation and/or to remove chimeric embryos from ape surrogates are themselves impermissibly invasive for these species. It is a commonplace research restriction that apes cannot be used for invasive biomedical research (Institute of Medicine, 2011). Second, the use of great and lesser apes for chimeric embryo research purposes could cross into the ISSCR’s impermissible categories of research as defined by categories 3A and 3B, especially in light of the fact that other alternatives—notably, NHP species that are more evolutionarily distant from humans—are available and routinely used for similarly invasive studies in reproductive medicine.
3. The length of chimeric embryo gestation must be scientifically well justified and minimally necessary to achieve the scientific aim. Investigators must proceed step by step, stopping at well-defined incremental time points to assess the degree and scope of chimerism during development before going all the way to full gestation (if full gestation is among the well-justified goals of the research).



4. To avoid uncontrolled and widespread chimerism, researchers should endeavor to target chimerism to a particular organ system or region of the gestating chimeric animal (Kobayashi et al., 2015; Hashimoto et al., 2019). Techniques such as blastocyst complementation—whereby a specific cell type or organ is effectively deleted as the host embryo develops and replaced by engrafted pluripotent human stem cells or their derivatives—can lead to a specific organ being replaced entirely by derivatives from the donor-derived stem cells. By itself this targeted chimerism may not prevent contributions elsewhere in the chimera, thus the need for an incremental approach. Nonetheless, if the host cells have an advantage over the donor cells, such as even a slightly faster rate of cell replication, then the donor cells will be disadvantaged and effectively selected against, leading to little or no contribution outside the organ of choice.
5. For targeted chimeric embryo studies that aim to chimerize the CNS of the host species and then permit full gestation and live birth, we refer decision-makers to our subsection below dealing with the chimerism of postnatal animals.

Finally, our subcommittee placed under category 3B (i.e., that which lacks compelling scientific rationale and is widely considered to be unethical) any research involving (1) the transfer of chimeric embryos mixing animal and human cells—whether predominantly animal or human—to the uterus of a human or great or lesser ape and (2) the breeding of animal chimeras incorporating human cells with the potential to form human gametes. We suggest that research that might result in the presence of human gametes and their precursors in the gonads of laboratory animals is not of significant ethical concern per se, as long as the animals are not allowed to breed.

Guidelines for stem cell research using postnatal animals

In addition to endorsing the categories of scientific and ethics review outlined above, our subcommittee determined that nonembryonic studies involving the transfer of human stem cells or their direct derivatives into postnatal animals should continue to be reviewed by the usual animal research committees utilized by research institutions. Research involving animals should also comply with the principles of the 3Rs (replacement, reduction, and refinement; see www.nc3rs.org.uk) and follow the “ARRIVE guidelines” (Percie du Sert et al., 2020).

That said, our subcommittee strongly recommends that research involving the transfer of human stem cells or their direct neural and/or glial derivatives into the CNS of animal hosts requires review by animal research oversight

committees supplemented by reviewer expertise in stem cell or developmental biology (ISSCR, 2006; Hyun et al., 2007; Academy of Medical Sciences, 2011). This call for stem cell-specific review by an institutional animal research committee is justified by the novelty of modifying research animals in this manner. The potential CNS effects of human stem cells and their derivatives on postnatal animals have yet to be fully ascertained, due to a general lack of experience in this area of stem cell science. Furthermore, research aimed at integrating human neural cells into the brains of laboratory animals has raised concerns about the moral status of animals resulting from such human-to-animal stem cell transplantation research (Greely, 2021; Wu et al., 2016).

Some in our subcommittee believe these concerns run too far ahead of the actual science, and erroneously conflate higher degrees of *biological* structural humanization with greater *moral* humanization, the latter comprising unique human-like cognitive capacities, such as the emergence of higher-order intellectual processing capabilities and thought, and self-consciousness. But such complex mental traits are not biologically assured even in infant brains that are 100% human, absent the experience of social and nurturing conditions of child-rearing over a time span of years (Hyun, 2016). Furthermore, the behavioral repertoires of chimeras will necessarily be narrower still for biological reasons. Even in cases in which the contribution of human cells to the CNS in a laboratory animal is extensive, in addition to fundamental differences in size and early regional patterning from the host embryo, the primary sensory and motor output systems will be host derived. Nevertheless, in light of potential concerns around the possibility of significant or meaningful enhancement of animal cognition by human cells—to a degree that some might find disconcerting or of frank ethical concern—our subcommittee has provided the following research guidelines for investigators and regulators dealing with stem cell protocols that might alter animals’ neurological functions. This approach tries to avoid giving undue influence to unsupported, imagined possibilities and strives to be grounded in observable behaviors and reasonable inferences.

We recommend that supplemented animal research oversight in this area should build upon common review standards with an emphasis on animal welfare. As with all modified animal models used in bioscience, reviewers should weigh the potential benefits of the research and, in particular, the potential clinical implications and benefits thereof. Reviewers should utilize available baseline nonhuman animal data grounded in rigorous scientific knowledge or reasonable inferences, while applying a diligent application of animal welfare principles. Past experiences with genetically altered laboratory animals have



shown that reasonable caution might be warranted if changes carry the potential to produce new defects and deficits. Current best practices dictate that research involving modified animals must involve (1) the establishment of baseline animal data, (2) ongoing data collection during research concerning any deviation from the norms of species-typical animals, (3) the use of small pilot studies to ascertain any welfare changes in modified animals, and (4) ongoing monitoring and reporting to animal research oversight committees authorized to decide the need for real-time changes in protocols and, if necessary, the withdrawal of animal subjects. Additional recommendations for stem cell-based animal studies of the CNS are as follows. These track closely to the ISSCR Ethics and Public Policy Committee white paper on this topic when the ISSCR first released stem cell research guidelines in 2006 (Hyun et al., 2007):

1. Additional data collection and monitoring by animal research committees should be commensurate with the anticipated characteristics of the modified animal in the context of the proposed research. Issues regarding the possible change in or enhancement of an animal's behavior or operationally assessed cognition should be addressed through diligent application of accepted principles for the humane treatment and protection of animals in research; these should proceed through regular animal research oversight mechanisms.
2. Monitoring and data collection should be based upon a sound assessment of the developmental trajectories of the animal host that may be further affected by taking into account the environmental and epigenetic context in which the donor genes or cells are going to be deployed. It should be grounded in existing knowledge of such trajectories, with reasonable scientific inferences as to their phenotypic and fate potential, with thorough reference to the physiological and behavioral tests and assessments currently available by which to assess the host species.
3. Research involving the modification of the CNS, as established with the introduction of human stem cells or their neural and/or glial derivatives in a way that they contribute to the brains or spinal cords of animal hosts, may attempt to model or directly mimic aspects of human neurological and neuropsychiatric function. As such, this research may demand specialized cognitive and behavioral assessments of the sort conducted in neuroscientific research. There may be an irreducible degree of uncertainty about the internal cognitive processes of any new animal model, in particular how it would manifest distress, anxiety, or other aspects of animal welfare. In such cases, as with transgenic animals, researchers and institutions should familiarize themselves with available options for behavioral response assessment. A baseline of normal behavioral data for the test species and strain should be available before experimentation is permitted, so as to enable clear and rapid identification of behavioral differences or abnormalities associated with treatment and/or human cell transfer. Investigators and institutions should also consider requiring limited pilot studies to produce initial data on the effects of experimental interventions on modified animals, monitoring all deviations from normal behaviors, with prescribed discussion with pertinent animal welfare committees before proceeding to definitive experiments.
4. Investigators and institutions should also make appropriate adjustments to research protocols to take into account new data or unanticipated responses from animal subjects that may inform or alter the continued permissibility of the animal's participation in the study. These include identifying any novel signals suggesting a material change in an animal's condition, comfort, or behavioral state or repertoire, whether by way of deterioration or enhancement. Regular reassessment of animal welfare during the course of experimentation is essential.
5. Research with a known, intended, or well-grounded significant potential to create some aspect suggestive of human cognition, self-awareness, behavior or behavioral pathology, while not prohibited, should be subject to close scrutiny, taking care to ensure the humane protection of animal subjects. Such studies require a clear and compelling justification, grounded in the potential for significant scientific breakthrough, clinical advance, or both.
6. Through retained advisors or committee diversity, animal research review committees should ensure that they have sufficient scientific and clinical expertise to make appropriate judgments concerning the matters discussed in these recommendations.

In addition to endorsing and updating these key recommendations from the 2007 ISSCR Ethics and Public Policy Committee white paper, our subcommittee also drafted additional recommendations to help stem cell investigators and oversight committee members who may be working with large animal studies for the first time. In these cases, investigators using large and often complex animal models, such as NHPs and livestock, should follow international standards for NHP and livestock animal research, which call for frequent monitoring of animals whenever



there is the potential for unexpected outcomes and unanticipated phenotypes.

With regard to stem cell studies involving NHP host species (excluding, as mentioned above, the great and lesser apes), we strongly recommend that stem cell investigators familiarize themselves with the unique challenges posed by working with NHPs. First, we cover some common practical issues, followed by issues that are pertinent for stem cell studies of the CNS in postnatal NHPs.

Keeping NHPs in the laboratory creates a number of problems that are not shared with other commonly used laboratory mammals. Unlike domesticated species, NHPs are potentially aggressive wild animals and are highly reactive to any unfamiliar stimuli. In addition to posing a bite and scratch hazard, NHPs can be challenging and difficult to handle safely because they possess great strength, dexterity, and intelligence.

Because of the many physical and behavioral characteristics of NHP species and the many factors to consider when using these animals in a biomedical research setting, personnel competent in the behavior of each species of NHP should be available for advice, and species-specific plans for housing and management should be developed. For animal care staff and scientists working with NHPs, training should include species-specific information such as unique biological and behavioral requirements, environmental enrichment, methods used for the introduction and removal of animals, and social dynamics.

It is crucially important to take seriously these and other factors that could have an impact on the well-being and behavior of NHPs used in neurological stem cell studies. Failure to do so not only could lead to the unnecessary and wrongful suffering of NHPs, but also could confuse monitors' assessments of whether an investigational stem cell-based intervention is itself causing observable effects on the animals' behavior that should inform future research. This point is overlooked in the stem cell ethics literature. As a case in point, the ethical discourse around stem cell-based neurological chimerism thus far has not taken into account the potential impact of practical issues such as animal housing.

Housing NHPs in social groups best replicates the social interactions they experience in the wild and thereby promotes species-typical behaviors and psychological well-being (Tardif et al., 2013). For some NHP species, temporary removal of an individual from its social group may cause it acute stress, and permanent removal may cause distress (the inability to cope with stress). Because of this variability, investigators and veterinarian staff must be aware of normal behaviors of individual NHPs and must know how to identify potential signs of stress and distress. Any singly housed modified NHPs should be kept so for the minimum length of time required. The need for single

housing should be reviewed by animal research committee members and veterinary staff. Because NHPs are social animals, single housing can produce a reduced range of species-typical behavior, increased environmental stressors, and self-inflicted wounding or withdrawn behavior. Not only could these outcomes affect the welfare of modified NHPs, but they might also confound investigators' and regulators' judgments about any potential behavioral changes caused by the transplantation per se of human stem cells or their direct derivatives.

Within the next 5–10 years, some investigations from stem cell scientists may be directed to generate transplantable human organs in livestock animals. To help prepare investigators and their regulators for this possibility well in advance, our subcommittee recommends familiarity with the following issues and best practices so that stem cell protocols can be designed appropriately.

First, the use of agricultural animals in research is subject to the same ethical considerations as for other animals in research. Regardless of the category of research (agricultural or biomedical), institutions are expected to provide oversight of all research animals and ensure that pain and distress are minimized.

Second, the study parameter, rather than the category of research, should determine the setting (farm or laboratory). Management systems for all farm animals should accommodate their natural behaviors, such as the need to graze, forage, and exercise. For animals maintained in a farm setting, the *Guide for the Care and Use of Agricultural Animals in Research and Teaching* is a useful resource (Federation of Animal Science Societies, 2010).

Third, most agricultural animals are social species, and attention to conditions and space needs that allows appropriate social interaction to occur is imperative (Edwards et al., 2018).

Fourth, personnel (animal care, veterinary, and researchers) should have experience working with livestock. The use of positive reinforcement techniques for acclimatization of these larger species to handling and research-related procedures contributes to the safety of personnel and of the animal subjects.

Finally, veterinarians should be knowledgeable about the health status of the species on study. Unlike most traditional laboratory animal species, biosecurity is not consistent among sources for livestock animals. Commercial suppliers of laboratory animals and land grant institutions generally maintain herds with known disease status. Conversely, disease status and health records may not be readily available for animals obtained from smaller farms or producers. Prepurchase review of animal health records and appropriate quarantine procedures can assist in preventing the introduction of species-specific and zoonotic pathogens.



Conclusion

Stem cell-based animal research continues to be an active area of stem cell and translational science. The recommendations in the 2021 ISSCR guidelines discussed in this report aim to promote the responsible advance of these activities. Much of what is in this report is neither strikingly new nor flashy from the standpoint of research ethics, despite the fact that animals with some human composition or elements have long been in the public imagination. This last point reinforces the need for stem cell researchers to avoid communicating about their chimera research projects with the public in misleading or inaccurate ways. While we are aware that some individuals would prefer to prohibit this research outright, we proceed from the position that under the correct conditions and with appropriate oversight, this research can provide valuable knowledge and so can ethically be undertaken. Thus, our recommendations are intended to help our audience—researchers and regulators—navigate and move forward with designing, conducting, and overseeing stem cell research protocols involving the use of animal hosts. These coordinated efforts should be seen as part of a much broader constellation of animal and human embryo research that has driven broad advances in biomedicine of the past several decades. We see no need to reinvent the wheel of research ethics when considering the transfer of human stem cells and their derivatives into animal models—rather, the addition of a few stem cell-specific spokes as needed should be sufficient.

AUTHOR CONTRIBUTIONS

All authors contributed to the ISSCR subcommittee deliberations, which led to this summary paper. And all authors edited and approved the final content of this work.

CONFLICTS OF INTEREST

S.A.G. is a part-time employee and stockholder of Sana Biotechnology, a cell therapy company, and his lab receives sponsored research support from Sana. He is also on the editorial board of *Stem Cell Reports*. R.A.P. is an advisor and holds stock options in BIT BIO.

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