

Concise Review: Human-Animal Neurological Chimeras: Humanized Animals or Human Cells in an Animal?

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ABSTRACT

Blastocyst complementation is an emerging methodology in which human stem cells are transferred into genetically engineered preimplantation animal embryos eventually giving rise to fully developed human tissues and organs within the animal host for use in regenerative medicine. The ethical issues surrounding this method have caused the National Institutes of Health to issue a moratorium on funding for blastocyst complementation citing the potential for human cells to substantially contribute to the brain of the chimeric animal. To address this concern, we performed an in-depth review of the neural transplantation literature to determine how the integration of human cells into the nonhuman neural circuitry has altered the behavior of the host. Despite reports of widespread integration of human cell transplants, our review of 150 transplantation studies found no evidence suggestive of humanization of the animal host, and we thus conclude that, at present, concerns over humanization should not prevent research on blastocyst complementation to continue. We suggest proceeding in a controlled and transparent manner, however, and include recommendations for future research with careful consideration for how human cells may contribute to the animal host nervous system. STEM CELLS 2019;00:1–9

SIGNIFICANCE STATEMENT

Due to a severe shortage of human organs and tissues, thousands of patients die each year due to an inability to procure organs for transplantation. Blastocyst complementation is a methodology that has the potential to generate large quantities of functioning human organs and tissues but is hindered by a National Institutes of Health moratorium on funding, citing concern over substantial human cell contribution to the brain of the animal. This review summarizes published, peer-reviewed studies on human–animal neural transplantation and suggests that this concern over neurological chimerism should not prevent research to continue in a controlled and transparent manner.

INTRODUCTION

In the broadest of definitions, a chimera is “a single biological entity that is composed of a mixing of materials from 2 or more different organisms” [1]. In Greek mythology, the chimera was a composite organism of different body parts from wildly divergent species. More recent variations of the chimera can be found in North American folklore (jackalope, hodag, Jersey Devil, etc.) as well as in modern literature such as Margaret Atwood’s speculative fiction *Maddaddam* trilogy. Far from the mythical and bizarre, however, chimerism—using the above definition—can commonly be found within the human brain. Microchimerism, the natural transfer of cells from a fetus which can cross the placenta and integrate within the maternal host, has

been observed within the brain of over half of sampled women [2]. Similarly, female recipients of bone marrow transplantation contain neural and non-neural cells derived from the male donor marrow [3]. Human–human neurological chimeras have also existed as part of clinical trials investigating the efficacy of cell-mediated therapies for devastating neurological disorders such as Parkinson’s disease (PD), Huntington’s disease (HD), and spinal cord injury (SCI).

Blastocyst Complementation

Advances in mammalian gene editing, pluripotent stem cell culture, and embryo micromanipulation technology have culminated in attempts to grow authentic interspecies organs through blastocyst complementation (for a comprehensive review, see [4]). This emerging methodology

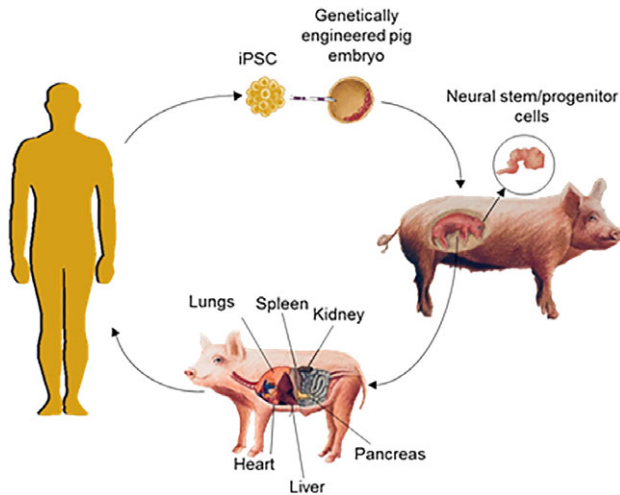


Figure 1. Cartoon schematic of blastocyst complementation. Human pluripotent stem cells grown in vitro are microinjected into genetically engineered porcine blastocysts which are then transferred to surrogate sows. The chimeric blastocysts develop to a fetal stage in which neural stem/progenitor cells can be harvested from the brain or to live-born animals where adult organs are processed for transplantation into patients.

has the potential to generate whole organs and tissues comprised entirely of cells from a single human donor (Fig. 1). To accomplish this, embryos from one organism are genetically engineered so that they lack functional gene(s) necessary for the development of the tissue of interest. The organogenesis-disabled embryos are then microinjected with healthy pluripotent stem cells (PSCs) from a second organism and are then transferred into a maternal surrogate. Through normal mammalian development, the microinjected PSCs occupy the niche left by the gene knockout and develop into a functional organ. This technique has successfully generated functioning allogeneic or xenogeneic pancreata in mice, rats, and pigs [5–8]. Microinjection of human cells into the wild-type porcine embryo has also led to human–animal chimerism across multiple organ systems, including neural cells [7].

A primary goal of blastocyst complementation is to meet the high-demand for human organs by producing fully functional human tissues and organs to be well-matched and ready for transplantation. Aside from the clinical potential of blastocyst complementation, the procurement of healthy human tissue also has the potential to impact the basic- and translational-sciences through disease modeling, drug discovery, and studies of transplantation biology.

Objections to Human–Animal Chimerism

A major concern echoed throughout the public response period to the National Institutes of Health (NIH) proposed changes in the guidelines regarding human–animal chimera research (NOT-OD-16-128) is the “creation of human–animal beings with partly or substantially human brains” and whether such chimeras possess “humanized” characteristics. Given the current NIH moratorium on funding research proposals involving human–animal chimeras at the preimplantation embryo stage, it is difficult to secure funding to answer the question, “Will generation of human neural tissue within animals through blastocyst complementation produce ‘humanized’ animals?” However, we can ask

the following surrogate question: “Has biomedical research involving transplantation of human tissue into the central nervous system (CNS) of animals altered the cytoarchitecture of the host brain resulting in an altered cognitive and behavioral state of the animal which could be considered human-like?”

In this review, we examine the outcomes of 150 transplantation studies in 112 peer-reviewed publications in which human cells have been targeted to the mammalian CNS (Fig. 2). These studies, not under moratorium by NIH, range from basic- to translational-science, and our focus is on the types of cells being transplanted within the nonhuman mammal and the degree to which the transplanted human cells are integrated. Although behavioral tests to identify human-specific attributes have not been performed in any transplant study, to date, we will also examine whether the transplanted human cells have enhanced the cognitive/behavioral abilities of the host to levels above wild-type animals. Because the ethical, legal, and social implications (ELSI) of human–animal chimerism and the potential for humanization of the animal host have been explored elsewhere [9–11], do not discuss the ELSI issues at length. The review aims to provide a necessary empirical foundation for those important ELSI debates.

HUMAN–ANIMAL CHIMERISM

General Neurological Chimerism

In an attempt to provide insight into the early stages of human neural development, several labs have transplanted clonally expanded human neural stem cells into the brains of perinatal mice and rats, a point at which neural development is still occurring, translating to mid-gestation in human prenatal development [12]. In these studies, up to 1 million cells from dissociated neurospheres were transplanted into the ventricles or subcortical regions. In some of these animals, human cells were observed over 1 year following transplantation [13,14] with integration throughout the brain and migration of human cells along the rostral migratory stream to the olfactory bulbs [13–16] and into the proliferative subventricular zone (SVZ) [14]. The phenotype of the transplanted cells ranged from immature neuronal cells weeks following transplantation [15–18] and region-specific mature neuronal phenotypes months following transplantation [13,15–17]. Ourednik and colleagues were interested in identifying the migration and differentiation potential of fetal-derived human neural stem cells following intraventricular transplantation into the brains of fetal Bonnet macaque at 12–13 gestational weeks [19]. This group observed terminal differentiation of human cells into neurons with appropriate cortical laminae that appeared to match the development of the host. These studies have provided evidence that a single cell type is likely to give rise to most cell types within the developing brain.

Experiments in transplantation of neural stem/progenitor cell (NS/PC) derived from fetal tissue [16,20–25], ESCs [24,26–30], or other sources [31–33] have been published that describe survival, neural maturation, and integration of unique cell lines. Survival of transplanted cells was highly variable with instances of complete cell rejection [27,33], fewer than one observed transplanted cell per cubic mm [20,28], or a complete doubling of transplanted cells [21]. The transplanted cells differentiated toward an immature neuronal phenotype early following transplantation

Counts of human neurological chimera studies (by cell line)

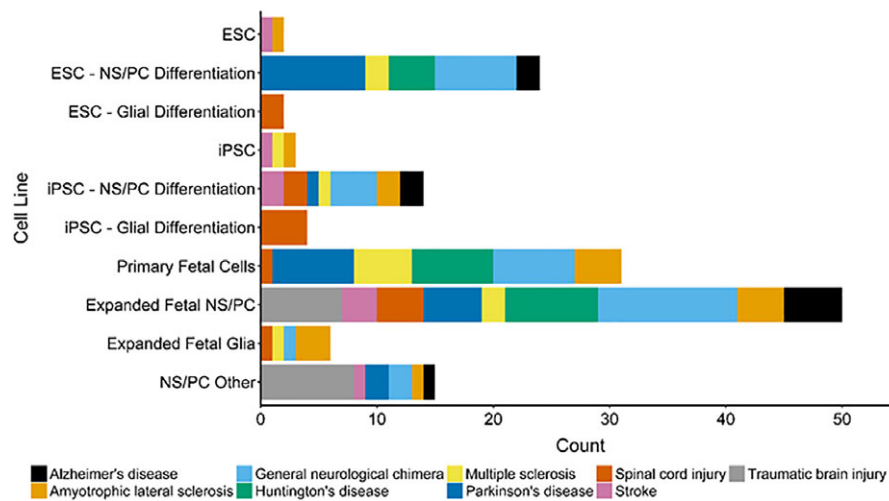


Figure 2. Human cells used for preclinical or biomedical neurological research. For scientific review, we sampled a small fraction of the available peer-reviewed primary research articles in which human cells are transplanted into the CNS of mice, rats, and nonhuman primates. From these studies, a variety of cell lines were used which primarily fall into 3 categories: Embryonic stem cell (ESC) derived, induced pluripotent stem cell (iPSC) derived and fetal-derived. These cell lines can either be expanded then differentiated into neural stem/progenitor cells (NS/PC) or glial progenitors, or transplanted directly with minimal in vitro manipulation (fluorescent- or magnetic-activated cell sorting) and no in vitro expansion (primary fetal cells). Within our review, an additional category of NS/PC cell lines is identified, which includes direct conversion of somatic tissue to NS/PC and the teratocarcinoma-derived Ntera2/D1 cell line.

that appeared more developed starting around 10 weeks post-transplantation [20, 28, 31]. Primary fetal tissue isolated from the developing cortex, thalamus, and striatum were transplanted into adult rats and were observed up to 40 weeks following transplantation and differentiated into immature or mature neurons as well as astrocytes that were spread throughout the SVZ, rostral migratory stream, and in the white matter of the corpus callosum [17, 24, 34, 35].

More recently, the group from the Gage lab developed a system to observe the growth and maturation of human brain-like structures, termed organoids [36]. In this study, human ESC derived organoids were transplanted into a cavity in the retrosplenial cortex of immunocompromised mice. The transplanted organoids were observed to mature and extend axons that integrated with the host neuronal circuitry, as measured by optogenetics and electrophysiology. Furthermore, the organoids were vascularized by the host and infiltrated with microglia. In a test of spatial learning and memory, none of the transplanted mice displayed significant alterations in behavior.

Transplantation of healthy neural cells into a nondiseased brain has improved our knowledge of neural development, cell migration and terminal differentiation. Although most of these studies did not directly measure behavior of the transplanted animals, none of the authors suggested the animals displayed altered characteristics that could be construed as human-like. Furthermore, none of these studies suggested an alteration of the cytoarchitecture of the host brain. To the contrary, multiple studies noted that the host organism dictated migration and transplanted cells followed the differentiation cues from the host [13, 14, 16, 19, 21].

Neurological Chimerism and Behavior

Neurological diseases and injuries can cause profound deficits in behavior and cognition. Current estimates for the prevalence of Alzheimer's disease (AD), provided by the Alzheimer's Association,

suggest 5.5 million Americans are living with AD, a number expected to rise dramatically [37]. Transplantation of human NS/PCs into either transgenic mice or hippocampal lesioned rats ameliorated learning and memory deficits in the Morris water maze (MWM) task [38–41]. As AD is a disorder of global brain degeneration, the targeted site for transplantation is a debated issue. Direct transplantation into the affected hippocampal formation demonstrated a limited degree of migration [38, 39, 42], whereas transplantation into the lateral ventricles migrated out through the SVZ to the hippocampus and other subcortical regions through white matter tracts [40, 43]. Transplantation of human cells has also been used to model AD. In one study, human ESC-derived NS/PCs functionally integrated into the neonatal AD transgenic mouse brain and were present up to 8 months post-transplantation, during which time the human cells were showing signs of degeneration similar to AD patients [44]. Transplanted cells from all of these studies were observed to survive and differentiate into immature neurons as well as astrocytes and glia, but mature neurons expressing choline acetyltransferase were rarely observed [38, 39, 44].

Neurological injuries occurring as a result of ischemic stroke or traumatic brain injury (TBI) can also have a profound impact on cognitive and behavioral function. Reported statistics within the United States place the yearly incidence of stroke at nearly 800,000 individuals [45] and roughly 2,800,000 emergency department visits related to TBI [46]. Long-term disability is common in individuals surviving these injuries, often requiring years of physical-, speech-, and occupational-therapy. Although these disabilities are difficult to measure experimentally in animals, the MWM task can assess spatial learning and memory that are significantly impaired in stroke and TBI animals. Transplantation of NS/PCs derived from fetal brain as well as the NT2N cell line were able to attenuate MWM deficits back toward baseline levels as early as 2 weeks post-transplantation and lasting up to 12 weeks post-transplantation [47–51]. Hippocampal

transplantation of fetal-derived NS/PCs 48-hours following middle cerebral artery occlusion reduced early sensorimotor deficits in the sticky-dot task in mice [49]. Many studies observed that transplanted cells remained in an immature neuronal phenotype up to 13 weeks post-transplantation [52–58]. Transplanted cells were also observed to differentiate toward a glial phenotype [47, 59, 60].

Cell-based regenerative therapies for AD, stroke and TBI are at a nascent stage in development, with a variety of hurdles to overcome before it is seen as a viable therapeutic option. Preclinical transplantation of human cells into rodent models were able to ameliorate some of the cognitive and behavioral deficits albeit rarely returning to, or rising above, healthy levels. This benefit may be due, in part, to trophic effects of the transplants, rather than integration of the graft as few studies noted alterations in synapse formation of host cells [38, 44, 49] and no study noted an alteration in the cytoarchitecture of the host brain.

Neurological Chimerism and Motor Function

Transplantation of neural progenitor cells as a therapy for PD has arguably advanced the furthest of any neurological cellular therapy, to date. Although PD affects multiple systems throughout the body, the clinical diagnosis of PD occurs as a result of the loss of dopaminergic neurons within the substantia nigra (SN) projecting to the striatum (STR). It is estimated that the prevalence of PD is near 2 individuals per every 1,000 in the population, with increased risk associated with age [61]. Preclinical success of cellular transplantation for PD is due to the identification of a population of neural progenitors that can innervate the STR and release dopamine to near physiological levels, which can be achieved using a variety of cell sources [62, 63]. Fetal ventral mesencephalic tissue is a dopamine-rich source of cells that are capable of integrating with the denervated STR and has resulted in the rescuing functional deficits in the rat and nonhuman primate following transplantation [34, 64–69]. Within these grafted animals, a strong outgrowth of tyrosine hydroxylase (TH) fibers from the graft core into the host STR has been observed, suggesting that the grafts are integrating with the host neurons.

Due to the limited availability of human fetal tissue and the variability of cell populations in transplants derived from fetuses of different gestational ages, several labs have focused on identifying alternative sources of cells for transplantation into PD. Differentiation of PSCs, *in vitro*, toward a dopaminergic progenitor has advanced largely due to the dual SMAD inhibition and floor-plate specification protocols [70, 71]. Using variations of this protocol, multiple groups have focused on differentiation of ESCs, which have observed strong integration of graft-derived TH⁺ fibers in the host STR of mice, rats, and nonhuman primates [72–79]. Transplantation of iPSC derived dopaminergic progenitors have similarly displayed robust engraftment and functional benefits in unilaterally lesioned rats [80].

From analysis of post-mortem tissue correlated with behavioral outcomes in human fetal tissue clinical trials, Hagell and Brundin suggest, at a minimum, 100,000 surviving TH⁺ neurons need to be present for a sustained therapeutic benefit [81]. Within the rat, numerous studies report amelioration of amphetamine-induced rotational deficits with as little as 1,000 human TH⁺ neurons. In some cases, up to 20,000 human TH⁺ neurons were observed in the rodent striatum [71, 76] more than doubling the estimated number of dopaminergic neurons in the healthy rat SN pars compacta [82].

Similar to PD, HD is a movement related disorder that is a result of an autosomal dominant mutation of the *Huntingtin* gene leading to the death of medium spiny neurons within the STR and glutamatergic neurons of the cortex [83]. Transplantation of fetal tissue into HD patients has been seen as a relative success in clinical trials [84–86]. Preclinical data suggested that tissue derived from the human fetal ganglionic eminences could engraft into the STR in the quinolinic acid lesion model of HD, survive up to 9 months post-transplantation, and ameliorate apomorphine-induced rotational deficits [34, 87–90]. Primary fetal grafts from 6 to 11 postgestational week ganglionic eminences, still containing dividing cells, resulted in differentiation toward immature neurons and few region-specific mature neurons [91, 92].

Similar to the issues associated with fetal transplants in PD, the research field in HD moved preclinical transplantation toward finding new sources of tissue, such as *in vitro* expanded or *Myc* immortalized fetal-derived NS/PCs. Studies in which fetal NS/PCs were transplanted into the rodent striatum demonstrated poor outcomes with little or no improvement in behavioral measures, and poor survival or integration into the host circuitry [92–98]. However, differentiation of PSCs toward a neural stem cell fate or toward a medium spiny neuronal fate show promise in reducing deficits associated with the quinolinic acid model [99–102]. Transplants of PSC-derived NS/PCs survived up to 4 months post-transplantation, differentiated toward medium spiny neurons and also expressed synaptic marker PSD95, suggestive of integration with the host [102–104].

Transplantation of human tissue into animal models of PD or HD has proven invaluable as new regenerative therapies are advancing toward the clinic, providing benefit for the individuals diagnosed with these devastating neurological disorders. Early preclinical studies transplanting fetal-derived tissue into the animal host has laid the groundwork for PSC derived progenitor cells. In all of these studies, no group has reported findings in which the grafted cells altered the behavioral state of the animals above baseline levels or altered the cytoarchitecture of the host brain.

Neurological Chimerism in the Spinal Cord

SCI affects more than 250,000 people in the United States, with vehicular and fall-related injuries being the most common causes [105]. Injury of the spinal cord results in partial or complete loss of limb sensation and function depending on the severity of the injury. Rodent models of SCI attempt to reproduce injury from either a temporary compression or contusion of the spinal cord or a complete transection. In these rodent models, human ESCs or iPSCs differentiated toward NS/PCs or glial cells are most commonly transplanted into the lesion epicenter or surrounding vertebrae. Two- to six-months following transplantation, counts of surviving cells ranged from less than 1% to as high as 23.9% [106–109]. Another study observed a plateau of approximately 400,000 surviving human cells within the spinal cord, regardless of initial dose [110]. Although the vast majority of transplanted cells remain within the transplantation site, there have been reports of migration more than 7 mm distal to the site of transplantation, corresponding to approximately 3 vertebrae [108, 110, 111]. Multiple studies have observed that transplanted cells differentiate into oligodendrocytes, suggesting that the human cells may be supporting endogenous surviving neurons [106, 109–111]. Similarly, terminal differentiation of transplanted human NS/PCs into astrocytes also provides a therapeutic benefit,

likely due to increased trophic support [107, 112]. Transplantation human cells into SCI rats demonstrate marginal behavioral improvements in open-field, flat beam, and rotarod tests, relative to SCI control animals [108–110, 113, 114].

The most common motor neuron disorder in adults is amyotrophic lateral sclerosis (ALS), which is characterized by the loss of both upper and lower motor neurons resulting in muscle atrophy and ultimately death most commonly due to the inability to contract the diaphragm. Over 90% of ALS cases are considered idiopathic, and death usually occurs 3–5 years after the onset of symptoms [115]. Variants of the superoxide dismutase 1 (*SOD1*) gene have been linked to familial ALS and is present in 5%–10% of all human ALS cases. Both ESCs and iPSCs differentiated toward NS/PCs or glial cells have been transplanted directly into the spinal cord of rodent models of ALS. Human cells have been observed within transplanted animals up to 9 months post-transplantation and have been shown to form neurites, axons, and even functional neuromuscular junctions [116–118]. However, the terminal differentiation of these cells is variable. One study demonstrated that human cells are still in an immature state at 9 months post-transplant, suggesting the human cells may not be at a phenotypic state to completely integrate into the rodent CNS [119]. Two separate groups observed that up to 1,600,000 human cells integrated into the spinal cord of mutant *SOD1* transgenic rodents up to 9 months post-transplant, with the majority of transplanted human cells differentiating toward astrocytes [117, 120]. Although some studies have shown grafted human neural stem cells to extend axons and innervate muscle, it is likely that the benefits seen are at least in part due to the neuroprotective mechanisms exerted by the engrafted human cells [116, 119, 121–125].

Transplantation studies of human cells into the diseased and injured spinal cord of rodents has demonstrated a therapeutic benefit with encouraging cell survival and integration of the human graft with animal host tissue. Preclinical transplantation of human NS/PC or glial progenitors into the spinal cord of rodent models of SCI and ALS have provided new therapeutic options for future clinical trials. In these 2 conditions, transplantation of human cells has not altered the behavioral state of the host above baseline levels, nor altered the cytoarchitecture of the host CNS.

Neurological Chimerism Using Human Glial Progenitors

The demyelinating disease multiple sclerosis (MS) is characterized by the progressive loss of myelinating oligodendrocytes in the CNS, resulting in numbness, tingling, tremors, and loss of mobility. Human cell transplantation studies in adult rodent models of MS have shown variable results. Transplants of fetal glia progenitors into the adult brain as well as ESC- or iPSC-derived NS/PCs into the adult spinal cord have shown limited benefit, beyond an altered immunomodulatory effect [126–128]. Transplants of human fetal-derived NS/PCs were found to survive up to 9 weeks in the demyelinated primate brain while remaining in a progenitor state [129] and 25 weeks in the brain of the shiverer transgenic mouse focally myelinating cells around the site of transplantation [130].

The greatest degree of neurological chimerism in any of the studies and disease states mentioned thus far has been observed in multiple studies by Goldman and colleagues at the University of Rochester. In these studies, human glial precursors isolated from fetal NS/PCs are transplanted into the corpus callosum of neonatal shiverer transgenic mice, resulting in the ultimate replacement of the endogenous mouse glia with human glia [131]. This replacement

has developed an entire human glial network within the mouse brain, where 300,000 initial human glial precursor cells proliferated into an estimated 12 million human glial cells throughout the entire shiverer mouse CNS [131–134]. Adult shiverer glial chimeric mice showed reduced seizure activity and extended overall survival as well as myelination patterns and glial networks similar to what is observed in wild-type mice. Human glial progenitors derived from human iPSCs have demonstrated similar effectiveness in myelinating the shiverer mouse CNS [135].

One study that deserves special attention transplanted fetal-derived glial precursors into neonatal immunodeficient mice and found human glia throughout the entire brain within 12–20 months and was organized in a laminar structure, a phenotype previously thought to be present only in humans and nonhuman primates [136]. The human astrocytes maintained a human astrocyte morphology (i.e., larger nuclei, long projections) within the mouse brain. Functionally, human astrocytes propagated calcium waves significantly faster than mouse astrocytes resulting in enhanced rates of field excitatory postsynaptic potentials and long-term potentiation. Behaviorally, chimeric mice displayed an improvement in the speed of acquisition of an auditory fear conditioning response, reduced latency to escape the Barnes maze, and an increase in the ability of mice to remember the locations of objects in the object-location memory task, relative to wild-type mice. It is likely that the enhanced ability to learn in the human/mouse astrocyte chimeras is a result of subtle differences between the functions of human and mouse astrocytes rather than human astrocytes forming novel neuronal pathways. However, the enhanced ability of these human/mouse chimeras to learn, regardless of whether the neuronal architecture is altered, is an important observation that necessitates further research.

DISCUSSION

The purpose of this review was to summarize the literature in which human tissue has been transplanted into the CNS of mice, rats, and nonhuman primates, in order to answer the question: “Will generation of human neural tissue within animals through blastocyst complementation produce humanized animals?” Although the ultimate answer to this question requires additional research, our review of the scientific literature finds that human/animal chimerism has not yet generated animals that possess an altered cognitive or behavioral state which trends toward “human-like.” To the contrary, few studies transplanting human cells into a diseased or injured animal restored cognitive or motor function to levels of healthy animals.

Several key variables need to be considered when interpreting these findings. First, most studies implanted human cells into mice (30%) or rats (64%). The mouse brain contains an estimated 70 million neurons and 23 million glia, the rat brain contains an estimated 200 million neurons, and the human brain contains an estimated 86,000 million neurons and 85,000 million glia [137–140]. It would be unlikely that even a majority of human neurons within the rodent brain would substantially alter the cognitive abilities of the chimeric animals, due to the small size, limited cytoarchitecture and connectome of the rodent brain. Relevant to blastocyst complementation, the domestic pig has a gyrencephalic brain with roughly 2.5% of the total number of neurons relative to humans, similar to the total number of neurons in the rhesus macaque [141, 142]. Of the 6% of studies in this review in which human cells were transplanted into the nonhuman primate brain, no team identified an observational alteration in the behavior of the nonhuman primate.

The second variable that needs to be considered is the age of the host at the time of transplantation. The majority of studies transplanted human neural tissue into adult animals (84%) compared with prenatal or neonatal animals (16%). It is likely that the age of the transplant recipient will impact the degree of chimerism as the developing brain is more plastic to the integration of transplanted cells. One study observed that the neonatal mouse brain promoted the survival and migration of transplanted human cells when compared with adult mice [143]. Multiple studies transplanting human fetal glial progenitor cells into demyelinated neonatal mice show the human cells integrate and out-compete endogenous mouse glia, effectively creating a largely human glial network within the demyelinated mouse brain [131–136], whereas transplantation of other cell types into adult demyelinated animals results in limited engraftment [130, 144]. Although it is likely the case that transplant recipient age affects the outcome of transplantation, more work needs to be done to determine the extent of these effects.

Ethical concerns have been raised about the possibility that the introduction of human stem cells into a nonhuman blastocyst could potentially alter the brain connectome of the host to the degree to which we would observe evidence of neural connections of the type and magnitude that would be required to produce human-like thought and behavior [9]. Although more research is required to address the concern more completely, work by the Nakauchi group demonstrated that through generating a rat pancreas within the mouse, using blastocyst complementation, the size of the pancreas was similar to that of a normal mouse and not the rat. This suggests that in the context of interspecies complementation, the development of the host species likely dictates the ultimate size of the complemented organ [5]. Likewise, we speculate that generating human neural cells in the nonhuman host would produce a connectome that would be dictated by the host species. Thus the elaboration of human behavior through a human connectome would be unlikely.

A final consideration is that preclinical and clinical studies using human fetal brain tissue for transplantation demonstrate that neural precursors derived from the fetal brain are the most suitable for transplantation. Therefore the need to allow chimeras to come to term for the harvesting of neural cells for transplantation is not required and addresses the concerns of chimeras being born that express human behaviors.

RECOMMENDATIONS FOR FUTURE COMPLEMENTATION RESEARCH

The 150 transplantation studies reviewed here suggest that complementation of nonhuman mammalian embryos with human stem cells is not likely to substantially alter the behavior of the chimera in a manner which can be construed as humanized. However, research on the potential humanization of chimera animals remains limited, and further investigation is required to more fully

explore the plausible risks. Research in blastocyst complementation should be allowed to continue, carefully and with transparent milestones, in order to better evaluate these unknown risks. As the scientific community begins to discuss these milestones, we recommend the following be included:

- Complementation of human cells in the livestock embryo should not surpass mid-gestation without determining the extent of chimerism in all tissues, including neurogenic regions, thus providing an early time point to alter or abandon the experimental protocol.
- Blastocyst complementation for the purpose of non-neurological organs should not be allowed to come to term until a thorough and reproducible analysis of neurological structures can determine the extent of chimerism in pre-term fetuses, with the goal of limiting neurologic chimerism.
- Blastocyst complementation for the purpose of treating neurological disorders should harvest tissue at a progenitor stage, and therefore chimeric animals will not be allowed to come to term. However, neurological chimeras should be analyzed for the purity of human cells within the target structure as well as outside of target structure.
- Blastocyst complementation for the purpose of neurological disease modeling should be allowed to come to term after a thorough analysis of the preterm brain has been established and if on-target chimerism is limited to motor regions of the brain, allaying concerns over human chimerism to prefrontal cortex and hippocampal regions.
- Chimeric animals should be separated at weaning and not allowed to breed, in case human gametes are found in chimeric livestock.

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AUTHOR CONTRIBUTIONS

A.T.C.: conception and design, manuscript writing, final approval of manuscript; J.P.V.: manuscript writing; F.X.S.: manuscript writing, final approval of manuscript; W.C.L.: manuscript writing, conception and design, final approval of manuscript.

DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

W.L. declared leadership position with Regenevida. The other authors indicated no potential conflicts of interest.

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