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Chimeric Humanized Vasculature and Blood: The Intersection of Science and Ethics

Daniel J. Garry,[1,2,3,*] Arthur L. Caplan,[4] and Mary G. Garry[1,2,3]
[1]Lillehei Heart Institute, Department of Medicine, University of Minnesota, Minneapolis, MN 55455, USA
[2]Stem Cell Institute, University of Minnesota, Minneapolis, MN 55455, USA
[3]Regenerative Medicine and Sciences Program, Paul and Sheila Wellstone Muscular Dystrophy Center, University of Minnesota, 2231 6th Street SE (CCRB 4-146), Minneapolis, MN 55455, USA
[4]Division of Medical Ethics, New York University Langone Medical Center, New York, NY 10016, USA
*Correspondence: garry@umn.edu
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The only curative therapy for diseases such as organ failure is orthotopic organ transplantation. Organ transplantation has been limited due to the shortage of donor organs. The huge disparity between those who need and those who receive transplantation therapy drives the pursuit of alternative treatments. Therefore, novel therapies are warranted. Recent studies support the feasibility of generating human-porcine chimeras that one day would provide humanized vasculature and blood for transplantation and serve as important research models. The ethical issues they raise require open discussion and dialog lest promising lines of inquiry flounder due to unfounded fears or compromised public trust.

The only curative therapy for many chronic diseases and end-stage organ failure such as advanced heart failure, renal failure, or diabetes is orthotopic organ transplantation (Garry et al., 2005a). Organ transplantation is and has been limited by the shortage of donor organs. It is estimated that no more than 1%–2% of Americans who could benefit from organ transplantation actually receives this life-saving therapy (Giwa et al., 2017). The huge disparity between those who need and those who receive transplantation therapy drives the pursuit of alternative treatments (Garry et al., 2005a).

The use of human cells, tissues, and organs in non-human vertebrates has been extensively examined in biomedical research laboratories (Suchy and Nakauchi, 2018; Wu et al., 2016). Previous studies have examined the impact of delivering human cells for the treatment of cardiovascular disease(s), hematological diseases, hepatic diseases, diabetes, and others in an array of animal hosts including rodents (rat and mice), sheep, pigs, and non-human primates (i.e., macaque monkeys) (Alexander and Bruneau, 2010; Garry et al., 2005b; Garry and Olson, 2006; Jennings et al., 2015; Takebe et al., 2013; Ye and Cheng, 2010). These human/animal chimeric models have importance for safety assessment of new therapies that would benefit patients with chronic diseases. Furthermore, the use of teratoma formation in mice has been extensively utilized and has proved invaluable with regard to the characterization of the pluripotent capacity of human stem cell populations (Masaki et al., 2015; Takahashi et al., 2007; Yu et al., 2007). An example of an important chimeric human/animal model for preclinical assessment of cell therapy is the delivery of human cardiomyocytes into the injured heart of the macaque resulting in remuscularization, decreased scar formation, and restoration of cardiac function (Liu et al., 2018). Furthermore, human hematopoietic stem cells have been shown to successfully engraft and survive in the bone marrow and spleen of the immunodeficient mouse (Audige et al., 2017). Collectively, these and other human/animal chimerism studies have enhanced the understanding of basic human biology and the benefits and adverse effects of established and emerging drug and cell therapies.

Chimerism-related research was further advanced as outlined in the recent publication in *Nature Biotechnology* by Das et al. (2020). Previous studies in mice using gene-disruption strategies demonstrated that *Etv2* null embryos lacked endothelial and blood lineages and were non-viable by embryonic day 9.5 (Ferdous et al., 2009; Lee et al., 2008; Rasmussen et al., 2011, 2013). Additional studies identified the mechanisms that governed Etv2 expression and activation of gene expression (Koyano-Nakagawa et al., 2012, 2015; Rasmussen et al., 2012; Shi et al., 2014) and provided a platform for the study by Das et al. (2020). In this latter study, the role of ETV2 as a master regulator for hematoendothelial lineages in mouse and pig was demonstrated. Using CRISPR/Cas9 gene editing, ETV2 was deleted in pig fibroblasts and then used with somatic cell nuclear technology to produce *ETV2* null pig embryos. Human induced pluripotent stem cells (hiPSCs) and engineered hiPSCs (to overexpress BCL2) were then used as donors and delivered (complemented) into the *ETV2* null porcine host (or recipient) embryo and implanted in surrogate porcine gilts. Human/porcine chimeric embryos were analyzed at less than 30 days' gestation, which is equivalent to the midpoint of the first trimester in the human, using morphological and molecular techniques (note that the normal porcine gestational age ranges from 114 to 116 days). These studies established that the efficiency of the chimerism was quite low, but the engineering of hiPSCs (resulting in BCL2 overexpression) resulted in a marked

increase in the efficiency of human/porcine chimera formation. This study also established that a majority (>85%) of the hiPSCs populated the hematoendothelial lineages with a small minority of the hiPSCs either arresting in their development or contributing to other lineages.

These studies also have limitations that will need to be examined in the future, one of which is the relatively early stages that were examined in the chimeras, which were purposeful and followed the guidelines established by institutional Stem Cell Research Oversight Committee. Examination of later-stage chimeras will address questions of vascular and hematopoietic maturation, the contribution to other later-developing lineages, and immunological responses of the recipient or host (pig) to the donor (hiPSCs) cell populations or tissues. A second limitation of this technology is the inefficiency of hiPSCs to form human/animal chimeras. In the study by Das et al. (2020), hiPSCs were engineered to overexpress BCL2 (B cell lymphoma 2) as a proof-of-concept strategy, but ultimately the engineering of humanized vasculature and blood will require hiPSCs that do not have sustained or persistent overexpression of factors such as BCL2 that can cause cancer.

Overall, these studies provide a platform for the engineering of gene-edited pigs that harbor human vasculature and human blood products, which will serve as important research models for the testing of devices, medications, and/or toxicology of future therapies. It is possible that one day these technologies may provide organs that have human vasculature or will be utilized for transfusion for the more than 5 million Americans who do not have access annually to blood transfusion therapies.

As with every scientific advance, there are ethical issues that warrant discussion (Caplan, 2014; Häyry, 2018; Lake, 2019). These studies utilized human stem cells that generate little ethical concern regarding their provenance (unlike human embryonic stem cells) as they were derived from non-embryonic, adult sources (i.e., somatic cells) and reprogrammed (to hiPSCs) using the Yamanaka factors (Takahashi et al., 2007). However, ethical issues surrounding the engineering of human/animal chimeras are real. For example, it will be essential to prevent chimeric animals from reproducing. Furthermore, it will be essential to monitor and limit the contribution of human stem cells to off-target lineages (i.e., the contribution to the neuronal and germ cell lineages). Third, how does the generation of chimeric animals affect the moral status of the animal? Fourth, are the issues related to the degree of chimerism that is ethically acceptable—a percentage of a whole organ, a whole organ, or multiple organs? As a consequence of these and other issues, it will be important to have a phased approach to examine and characterize the human stem cell contributions to the animal host. Such studies require transparency and dialog. Ongoing chimera studies in all countries will require real-time dialog and discussion with results, data, and methods published in peer-reviewed scientific journals.

Issues that accompany ongoing human/animal chimerism studies (such as rodent models with a human liver, human teratoma studies, or human bone marrow in rodent models) (Audige et al., 2017; Grompe and Strom, 2013) require a dialog about how much chimerism can be engineered before an organism is considered more human than not. Moreover, are there research fields that should not be examined using this technology? For example, would a chimera with human skin or a chimera with human hair be unacceptable—would these tissues have too many visible human features? Similarly, are there research areas that should be encouraged (e.g., heart, kidney, lung) where issues related to chronic disease, organ shortage, and quality of life suggest that new therapies should vigorously be pursued?

This is an emerging field that holds tremendous promise. The opportunity to work together and proceed in a measured fashion with transparency will allow us to address these and other issues as they arise to promote this and future technologies.

Chronic and terminal diseases shorten life and affect the quality of life of many. Novel therapies are warranted. Recent studies support the feasibility of generating human/porcine chimeras that one day would provide humanized vasculature and blood for transplantation and would serve as important research models. The ethical issues they raise require open discussion and dialog lest these promising lines of inquiry flounder due to unfounded fears or compromised public trust.

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