Consensus statement on heart xenotransplantation in children: Toward clinical translation

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Gene-editing has made an enormous impact on our ability to create a bioengineered pig heart for clinical transplantation (Figure 1). Survival of up to 9 months in experimental life-supporting pig heart xenotransplantation has been achieved, 2,3 and, following decades of collaborative research, 4 the first transplantation of a genome-edited pig heart into a human became a reality. 5,6 This offered some promise and sparked renewed interest for clinical translation of cardiac xenotransplantation. 9,10

This progress has largely been achieved by the combination of 2 approaches—(1) the transplantation of organs from pigs genetically engineered to protect their organs from the primate innate immune response 11 and (2) the administration of novel immunosuppressive agents that prevent the adaptive immune response by blockade of the CD40/CD154 T cell costimulation pathway.12 Gene editing has included deletion of expression of 3 major carbohydrate xenoantigens and growth hormone receptor as well as addition of transgenic expression of human “protective” proteins, such as complement- and coagulation-regulatory proteins.11

Although the most suitable patient population for pig heart xenotransplantation is yet to be determined, 6,13,14 neonates and infants with complex life-threatening congenital heart disease may benefit most. 15,17 Herein, we review the need and applicability of heart xenotransplantation in this vulnerable population of young children and discuss the prospects for clinical translation. This review is not aimed at discussing the ethical and regulatory issues but is rather focused on clinical aspects and feasibility.

HEART FAILURE MANAGEMENT IN NEONATES AND INFANTS WITH COMPLEX CONGENITAL HEART DISEASE

Despite significant progress in surgical and intensive care management, outcomes in neonates and infants with complex congenital heart disease who develop heart failure are poor, particularly in those with univentricular circulation. The Single Ventricle Reconstruction (SVR) trial demonstrated that 15% of patients who survived to hospital discharge after a Norwood procedure develop heart failure by 6 years of age. 18 Mortality in children awaiting heart
transplantation remains high.\textsuperscript{19} Newborns and infants have the greatest waiting-list mortality.\textsuperscript{20} In particular, 1-year survival of infants placed on the cardiac transplantation waitlist was found in some studies to be as low as 55%, with especially high mortality for children weighing <2.5 kg at the time of listing or those on ventilatory or

\begin{figure}
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\includegraphics[width=\textwidth]{producing_genetically_modified_pig}
\caption{Producing a genetically modified pig from somatic cells. Nuclear transfer of the edited genetic code to enucleated mature pig oocytes is performed to produce cloned genetically modified pigs as founder animals of the herds. Subsequent cycles of breeding and genetic modification produce the genetically modified pig suitable for organ donation.}
\end{figure}
mechanical circulatory support. Although successful durable ventricular assist device (VAD) support in these children has been reported,22,23 such support is associated with high mortality and morbidity, which are, in turn, often associated with efforts made to maintain the difficult balance between the systemic and pulmonary circulations. Recent reviews suggested that survival of young children with univentricular circulation on VAD support was poor, with less than 50% survival by the 3-month mark.24,25

Although VAD support in neonates and infants with failing univentricular circulation is feasible,26 bridging this subgroup to heart transplantation remains challenging. Furthermore, those surviving beyond infancy with staged univentricular palliations continue to need lifelong complex medical and surgical management that may eventually lead to heart transplantation.27,28 Thus, should a reliable source of “donor” hearts become available, primary heart transplantation would be an attractive option in neonates and infants with univentricular circulation.

**IMPACT OF THE IMMUNE SYSTEM ON HEART TRANSPLANTATION IN NEONATES AND INFANTS**

An International Society for Heart and Lung Transplantation report demonstrated that median survival for infants after heart transplantation was 22.3 years, which is substantially greater than in any other age group.29 In contrast, the SVR heart transplantation was 22.3 years, which is substantially higher than that after the Norwood operation.31,32 Most importantly, long-term survival after neonatal heart transplantation for hypoplastic left heart syndrome (HLHS) was only 65%. Thus, survival in patients with HLHS after primary heart transplantation in the neonatal period appears to be greater than that after the Norwood operation.31,32 Most importantly, long-term survival after neonatal heart transplantation for HLHS was reported to be 73.9%, 64.4%, and 55.8% at 10, 20, and 25 years, respectively.33

It appears that the immature immune system of neonates and infants may bring important advantages.17,34,35 Following the initial description of ABO-incompatible heart transplantation in infants,36 several studies demonstrated that ABO-incompatible allotransplantation in young children (with immature immune systems) is not only possible but also provides good long-term results.37,39 It appears that ABO-incompatible heart transplantation also results in persistent alteration of the immune response and tolerance to the donor blood group antigens.30 Interestingly, these changes in immune maturation are also associated with reduced response toward human leukocyte antigen epitopes and lower risk of posttransplantation infection.41

**THYMIC MANIPULATION**

Thymectomy in neonates and infants alters T-cell responses.44 Although the effects of thymectomy in young children undergoing heart transplantation are not yet fully understood,42 thymectomy may result in altered development of the T-cell profile, especially when combined with T-cell depletion treatments,43-45 and play an important role in mitigating the immune response to an allograft (or xenograft) and in the induction of immunological tolerance.33,46 However, this appears to be associated with atopic disorders after heart transplantation.77 In contrast to patients with DiGeorge syndrome, who lack normal thymic function, there has been no reported increase in infection in children after neonatal thymectomy, which may be related to the fact that some T cells are produced and matured during fetal and early neonatal life.38 Even after visually complete thymectomy, T cells are observed in peripheral blood.39 After thymectomy in the neonatal period, the cotransplantation of pig thymic tissue could possibly contribute to induction of immunological tolerance to a bioengineered pig heart.50,51 The Markert technique, by which cultured thymic tissue is implanted into the quadriceps muscle, may induce tolerance52,53 and has recently been applied in clinical heart transplantation.54

**RAPID GROWTH OF BIOENGINEERED DONOR PIG HEARTS**

Rapid growth of porcine xenografts after transplantation in nonhuman primates was reported as long ago as 200055 and has been confirmed by several groups.56-58 It appears that the growth of the transplanted heart is multifactorial, and recipient’s blood pressure may play an important role.59 Rapid growth of a bioengineered pig heart after transplantation in neonates and infants may be problematic if heart growth is disproportionate to the recipient’s somatic growth. Growth of pig organs can be reduced by deletion of the gene for the growth hormone receptors.59-61 An alternative would be to use a miniature pig as the source of the xenograft heart, eg, Auckland Island pigs or Yucatan pigs. Furthermore, there is evidence that inclusion of rapamycin in the immunosuppressive regimen appears to be associated with less rapid growth of the pig organ xenograft.56,57

**BRIDGING TO HUMAN OR SECOND BIOENGINEERED HEART TRANSPLANTATION**

Pigs, with their average life-expectancy of 12 to 18 years, do not live as long as humans. Thus, even if perfect immunological tolerance developed, it is possible that pig heart xenograft longevity might be limited (although a second size-matched porcine heart would be readily available). Thus, as an initial step in clinical xenotransplantation, the implantation of a pig heart as a bridge to human heart transplantation is compelling.16,17 Xenotransplantation could be performed as a primary operation or after failed initial palliation. In children with previous cardiac surgery, previous testing for antibody binding to pig cells will be required to exclude the possibility of sensitization.62 In contrast, sensitization to porcine xenoantigen, if it occurs, does not appear thus far to be detrimental to subsequent allotransplantation.63-65 As an alternative, if tolerance to a pig heart develops, it might
be possible to retransplant with a second size-matched pig heart, if retransplantation is required due to lack of growth of the organ. It is also tempting to speculate that eventually an autologous human bioengineered heart might be “manufactured” using blastocyst complementation or some other method.66 Thus, in summary, if xenotransplantation is performed in neonates and infants, it could potentially be carried out as a bridge to human heart transplantation (either an autotransplantation or, if an autologous heart could be produced in the future using the patient’s own tissues, autotransplantation) or to a second xenotransplantation using a size-matched pig heart.

SOURCE OF THE BIOENGINEERED HEART AND TRANSLATIONAL ISSUES

In the United States, organ-source pigs would need to be bred and housed in a designated pathogen-free (DPF) facility approved by the US Food and Drug Administration to prevent transmission of zoonotic microorganisms.67,68 The bioengineered heart from the pig that was transplanted into the patient at the University of Maryland at Baltimore carried latent porcine cytomegalovirus (pCMV)7 that escaped the currently validated detection assay that was available and was later detected by using very sensitive nested polymerase chain reaction in donor spleen and transplanted heart. There are studies in progress to evaluate whether this latent virus replicated and caused damage to the transplanted heart. There is no definitive evidence so far that this virus caused any pathology in the patient. It should be noted that the pig donor used for transplantation was not raised in a DPF facility, although it was a clean facility at which the pigs are individually housed. Monitoring for potentially pathogenic microorganisms in the organ-source pig therefore requires meticulous attention.66 It would be crucial to have sensitive screening for pCMV69-72 while creating genetically modified pigs for xenotransplantation.72 Early weaning of piglets from the sow decreases the risk of pCMV infection.

Although there is no evidence that they would be pathogenic in humans, transmission of porcine endogenous retroviruses (PERVs), which are present in the genome of all porcine cells, has been of some concern.73 However, if believed to be necessary, this potential risk can be prevented or negated by PERV gene deletion74 or downregulation75,76 or using PERV-C–free donors.77 It may be unnecessary to delete all PERV, as the risk of PERV can be minimized by deleting just PERV-C by selective breeding or broader genetic engineering techniques. Only PERV-C has been shown capable of infecting human cells in vitro.

PRACTICAL ASPECTS OF CLINICAL PIG HEART TRANSPLANTATION

Ischemic Myocardial Injury of Pig Hearts

Porcine hearts appear to be more vulnerable to ischemia than human hearts, particularly if the period of cold ischemia is prolonged,2,78,79 although successful myocardial preservation has been reported using cold static storage.17 Thus, nonischemic preservation may be advantageous to prevent perioperative cardiac xenograft dysfunction.2,78,80 Continuous perfusion with the XVIVO perfusion system (XVIVO)78,79 has been achieved in preclinical studies, and this approach was used in the first pig-to-human heart transplantation.7 Since 2015 the XVIVO perfusion system has been used in baboons weighing 15 to 20 kg.80 However, this system is currently available for children only at the Royal Children’s Hospital in Melbourne. Further, it is not yet validated for children less than 30 kg and must therefore be modified for use in neonates and infants.

An alternative approach to continuous perfusion would be to transport the donor pig to the hospital in which the transplantation is to be performed, thus minimizing the ischemic time by synchronizing the donor and recipient operative procedures (Figure 2). It should be kept in mind that it would not be possible to maintain a pig in DPF conditions during transport to the hospital and appropriate methods of transportation must be determined. Thus, transporting the pig to the hospital may not be a viable option. The unavoidable ischemic time during transplantation can be further minimized by completion of left atrial and aortic anastomoses first and subsequent completion of the pulmonary artery and caval anastomoses with the heart beating (Figure 2). Thus, it would be crucial to establish a strategy for myocardial protection during organ procurement specifically designed for pig hearts.

Posttransplant Management

The detailed immunosuppression regimen used in the first pig-to-human heart transplantation has been described.7 Similar to the regimens described in animal trials, relatively intensive induction immunosuppression was administered, and peripheral B cells (CD20+) were depleted, and T cells were reduced with polyclonal antithymocyte globulin. In addition, complement activation was inhibited using a C1-esterase inhibitor. A humanized monoclonal antibody (mAb) (KPL-404; Kiniksa Pharmaceuticals) was used for maintenance immunosuppression to block CD40 costimulation.7 Apart from the difference in maintenance immunosuppressive therapy necessitated by the administration of an anti-CD154 mAb or anti-CD40 mAb, postoperative management would be similar to that of patients at greater immunological risk (human leukocyte antigen sensitization) undergoing cardiac allotransplantation today. It should also be kept in mind, however, that in young children with a more forgiving immune system and greater risk of adverse effects of immunosuppression, such a regimen would far exceed routine immunosuppressive strategies.81 Many pediatric centers tend to avoid polyclonal T-cell depletion in young children due to the increased risk of...
posttransplantation lymphoproliferative disorders and greater frequency of atopic disorders. At present, there is no clinical experience with costimulation blockade or C1-esterase inhibition in pediatric patients. The interplay between B cells and the complement system appears to play a key role in the development of tolerance toward incompatible blood group antigen in ABO-incompatible heart transplantation in infants, who show a natural deficiency in T-cell–independent B-cell activation. Accordingly, targeting the complement system may enhance the development of tolerance in early childhood.

Monitoring for rejection of a pig heart in a nonhuman primate recipient is today similar to that of a human cardiac allograft. Xenotransplantation would require frequent monitoring of antipig antibodies, which is different from allotransplantation. It appears that costimulation blockade is critical for xenograft survival. Although therapy with an anti-CD40 mAb or an anti-CD154 mAb has usually been combined with another immunosuppressive agent, eg, mycophenolate mofetil or rapamycin, and a corticosteroid, it may eventuate that costimulation blockade alone may be sufficient as maintenance therapy.

**COMMENT**

The ethical issues associated with xenotransplantation are several and have been discussed previously, as have the attitudes of health care professionals, patients, and their families to this form of therapy. These important topics have therefore not been reviewed here. Needless to say, all aspects of research, clinical-scale production of organs, and clinical translation require an utmost ethical scrutiny (Figure 3).

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**FIGURE 2.** Transporting the pig or pig heart for clinical heart xenotransplantation. (1) The pig can be transported to the hospital under high-efficiency particulate air–filtered biosecurity conditions so that ischemia of the heart is eliminated almost entirely. In this case, the ischemic time is basically reduced to that required for the surgical procedure of pig heart removal and implantation. (2) The heart can be removed from the pig at the animal production facility and transported to the recipient hospital using cold nonischemic heart preservation. Regardless of the mode of donor heart delivery, the intraoperative ischemic time could be further reduced by anastomosis of the 2 left atria and 2 aortas first to allow myocardial perfusion to be initiated before the caval and pulmonary artery anastomoses are completed.

**FIGURE 3.** Heart xenotransplantation: key points pertinent to research, industry, and clinical translation. Successful clinical translation of heart xenotransplantation is dependent on several key points; each key point, in turn, requires utmost ethical scrutiny.
Pig heart xenotransplantation in neonates and infants with complex congenital heart disease, particularly univentricular circulation, offers a potential therapeutic option that could save the lives of children who would not survive otherwise. Initially, bridging with a pig heart until a human heart became available would allow experience to be gained without committing the patient to a lifetime supported by a xenograft. However, there are many issues to be resolved, and a significant body of work needs to be carried out to achieve consistent, safe, and reproducible translation to clinical practice.

Conflict of Interest Statement
Dr Cooper is a consultant to eGenesis, but the opinions expressed in the manuscript are his own and not necessarily those of eGenesis. Dr Rothblatt is Chairperson and Chief Executive Officer of United Therapeutics Corporation. Revivicor, Inc, a subsidiary of United Therapeutics Corporation, owns a variety of issued patents and pending patent applications broadly relevant to xenotransplantation. All other authors reported no conflicts of interest.

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