Review Article

The potential of cardiac xenotransplantation for management of infants with complex congenital heart disease

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Abstract: Gene editing of the porcine genome has enabled the production of pigs that do not express the three known carbohydrate antigens that are associated with hyperacute rejection of a pig organ xenotransplant. In addition, it is now possible to insert a variety of human transgenes to protect against the human immune response, e.g., to protect from complement and coagulation activation. As a result, cardiac xenotransplantation of the gene-edited porcine heart is progressing towards clinical application. Many hope that it will definitively address the disparity between organ supply and demand. The role of cardiac xenotransplantation in pediatric care remains controversial but we believe there is an infant patient population with complex congenital heart disease (CHD) (not optimally managed by conventional surgical approaches) that is ideally suited to initial clinical application of this new technology. The most efficacious start would be to initiate clinical use as a short-term bridge to allotransplantation, particularly in infants with single ventricle pathology and significant risk factors for first stage Norwood palliation. Infants with end-stage heart failure after first stage palliation would represent a second target population. Infants experience unacceptably high mortality and morbidity when placed on mechanical circulatory support as a bridge to allotransplant. Effectively bridging these vulnerable populations could promote acceptance of cardiac xenotransplantation, allowing indications and use to expand, e.g., by (I) bridging patients with failed second and third stage single ventricle disease, or (II) with complex biventricular CHD, or (III) those with a restrictive or dilated cardiomyopathy. Finally, there is a reasonable expectation that the immunologic privilege of infants will allow porcine heart xenotransplantation to be destination therapy for some patients. In summary, heart allotransplantation in infants offers superior outcomes when compared to three-stage single ventricle palliation, but there is a continual shortage of deceased human donor organs. We should pursue research towards the application of xenotransplantation in patients with single ventricle pathology, in whom the results of staged palliation are likely to be suboptimal. There are many remaining issues to be resolved before cardiac xenotransplantation enters regular pediatric clinical use, but experience in this field is progressing rapidly.

Keywords: Gene-editing; heart; infants; neonates; xenotransplantation

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Introduction

Current status of pediatric cardiac transplantation

Background

Cardiac transplantation is the most effective therapy for treating end-stage heart failure in children. Short and long-term outcomes have reached historical highs. A total of 30 pediatric heart transplants took place in 1984 with a median survival of 3.5 years (1). Today, over 600 children worldwide undergo cardiac transplantation each year with an expected overall median survival of 18 years. Results are age-dependent and superior in patients who undergo transplant at an earlier age. Median survival is 24.5 years in infants compared to 14.3 years for those transplanted between the ages of 11–17. Greater than 60% of infants who survive the first post-transplant year are still alive 25 years later (2).

Despite these excellent results, the annual number of transplants has remained stagnant over the last 10 years. Twenty to thirty percent of children listed for transplant will die on the waitlist each year before a suitable organ can be found (3). The primary reason for this is simple—the need for transplant exceeds the available supply of deceased human donor hearts.

Combating waitlist mortality

Physicians caring for children with heart failure are combating waitlist mortality through three strategic efforts: (I) reducing demand for transplantation; (II) increasing the available supply of donor hearts; and (III) seeking alternatives to safely extend waitlist times. The first of these efforts centers on judicious listing and appropriate prioritization of children waiting for transplant. This is reflected by modifications made in 2016 to the allocation system for pediatric heart transplantation by the Organ Procurement and Transplant Network (4). In addition, stakeholders are advocating to implement risk models to determine transplant candidacy. They argue that limited resources should be protected, and the medical community must reduce the use of cardiac transplant for patients at the highest risk of death within 1 year of transplant (5).

The second effort has providers working to increase the supply of donor hearts either by improving utilization of currently offered organs or seeking alternative methods to increase human donation. Reports indicate that 18–57% of offered hearts are not presently transplanted (6). The International Society for Heart and Lung Transplantation (ISHLT) has spearheaded efforts in conjunction with other groups to define the impact of donor characteristics on transplant outcomes (7-10). They sought to dispel perceived limitations on organ acceptance based on donor characteristics so that fewer hearts offered in donation go un-transplanted.

The community continues to push the boundaries of ABO-incompatible heart transplantation in children. This initiative expands the pool of donors from which any given recipient can receive an organ. Depending on the country, children as old as four are candidates to safely undergo transplantation of a heart from a donor of an incompatible ABO blood group, provided that isohemagglutin titers are below 1:16 (11). Additionally, certain pediatric groups have followed the lead of colleagues working with adults and have renewed their focus on procuring hearts following circulatory death. Limited pediatric data suggest acceptable outcomes, albeit inferior to hearts obtained following brain death (12).

The third effort, i.e., to safely extend waitlist times, centers on the application of technology to improve cardiac output until a suitable organ can be found. Pediatric mechanical circulatory support (MCS) utilization is on the rise worldwide. Thirty-seven percent of children listed for transplant are now bridged to transplant with a ventricular assist device (VAD) and outcomes have improved with each passing year. The most recent Pediatric Interagency Registry for Mechanical Circulatory Support report indicated a positive outcome (alive on device or successfully bridged to transplant/recovery) for 82% of VAD recipients 6 months following implant (13).

However, there remains a disparity in the use of this technology among children with congenital heart disease (CHD) versus those with dilated cardiomyopathy (2). VAD outcomes are inferior for patients with CHD, particularly for those patients with first stage palliated single ventricle disease (13). Despite these efforts, waitlist mortality among neonates and infants remains disproportionately high, warranting further exploration into alternatives.

Cardiac xenotransplantation

Background

Medical professionals have long dreamt that xenotransplantation would be the definitive solution for patients with end-stage heart failure. Transplant pioneer Norman Shumway has often been quoted, “Xenotransplantation is the future of transplantation, and always will be.” This pessimistic sentiment is

Table 1 Carbohydrate xenoantigens that have been deleted in gene-edited pigs

Carbohydrate (abbreviation)	Responsible enzyme	Gene-knockout pig
1. Galactose-α1,3-galactose (Gal)	α1,3-galactosyltransferase	GTKO
2. N-glycolylneuraminic acid (Neu5Gc)	Cytidine monophosphate-N-acetylneuraminic acid hydroxylase (CMAH)	CMAH-KO
3. Sda	β-1,4N-acetylgalactosaminyltransferase	β4GalNT2-KO

understandable considering highly publicized failures such as those of Dr. James Hardy and Dr. Leonard Bailey who transplanted nonhuman primate (NHP) hearts into humans who succumbed fairly rapidly (14,15). Advances in molecular biology and genome editing are beginning to shift attitudes once again and encourage physicians to hope that xenotransplantation will soon have clinical utility.

Since the 1990s, scientists have been experimenting with genome editing in pigs for the purpose of producing organs compatible with the human immune system (16). Early molecular biology techniques in addition to lack of identified xenoantigen targets precluded rapid and widespread clinical translation. The development of CRISPR-Cas9 technology (clustered randomly interspaced short palindromic repeats and the associated protein 9) made genome editing easier, quicker, and less expensive (17,18).

In addition, scientists identified three major carbohydrate antigens expressed on pig vascular endothelium against which humans have natural antibodies (*Table 1*). Genetically engineered "triple knockout" (TKO) pigs that do not express any of these antigens are now available (19). Human infant serum antibody binding to TKO pig cells is non-existent or minimal (*Figure 1*). Researchers have introduced other genetic modifications, e.g., protective human transgenes, which add further protection against the human immune response (21). This progress has prompted a recent push for clinical application, including one clinical heart transplant in an adult on 'compassionate' grounds (22) followed recently by a second.

There continues to be concern for the potential of cross-species transmission of potentially infectious microorganisms, but expert opinion is that most post-transplant infections are likely to be the same nosocomial infections as those seen after allotransplantation (23). The presence of porcine endogenous retroviruses (PERV) in pig cells was a concern, but these can now be inactivated in the pig (24).

Community attitudes

The history, ethical considerations, and psychosocial impact surrounding xenotransplantation make it important to delineate community attitudes before clinical implementation. Early surveys distributed to pediatric medical providers and families of children with CHD indicate potentially high levels of acceptance (25-28). If the functional performance of the xenograft is proven inferior to that of an allograft, acceptance rates among stakeholders were lower (particularly for the use of a xenograft as a bridge to transplant). Unfortunately, these early surveys were limited by their broad scope and the limited background information provided to participants. A more focused follow-up study helped identify specific concerns (religious beliefs, animal ethics, stigma about how pigs are viewed, organ allocation logistics, and impact on quality of life) to target for improved acceptance by better education (29). Further studies are warranted to explore attitudes across providers and families from a broad scope of backgrounds to ensure widespread applicability of these findings.

Immune privilege of children

Pediatric application of xenotransplantation may offer immunologic advantages over the adult population. The case for this argument is made strong through understanding the outcomes of neonatal and infant cardiac allotransplantation in addition to the outcomes of a few key *in vitro* studies. Current ISHLT data indicate that neonates and infants with heart allografts experience less rejection, allograft vasculopathy, post-transplant lymphoproliferative disease, and need for re-transplant, but greater survival compared with all other age groups that receive a cardiac transplant. In fact, the half-life of a heart transplanted prior to one year of age has yet to be determined (2,30). Many argue that this superiority is driven by an immunologic advantage inherent to children less than one-year-old, i.e., inducibility. Research dating back to 1945 suggests that immune responses can be modified in young patients with early antigen exposure (30,31). However, such immunologic advantage remains ill-defined and difficult to target with therapies intended to augment it.

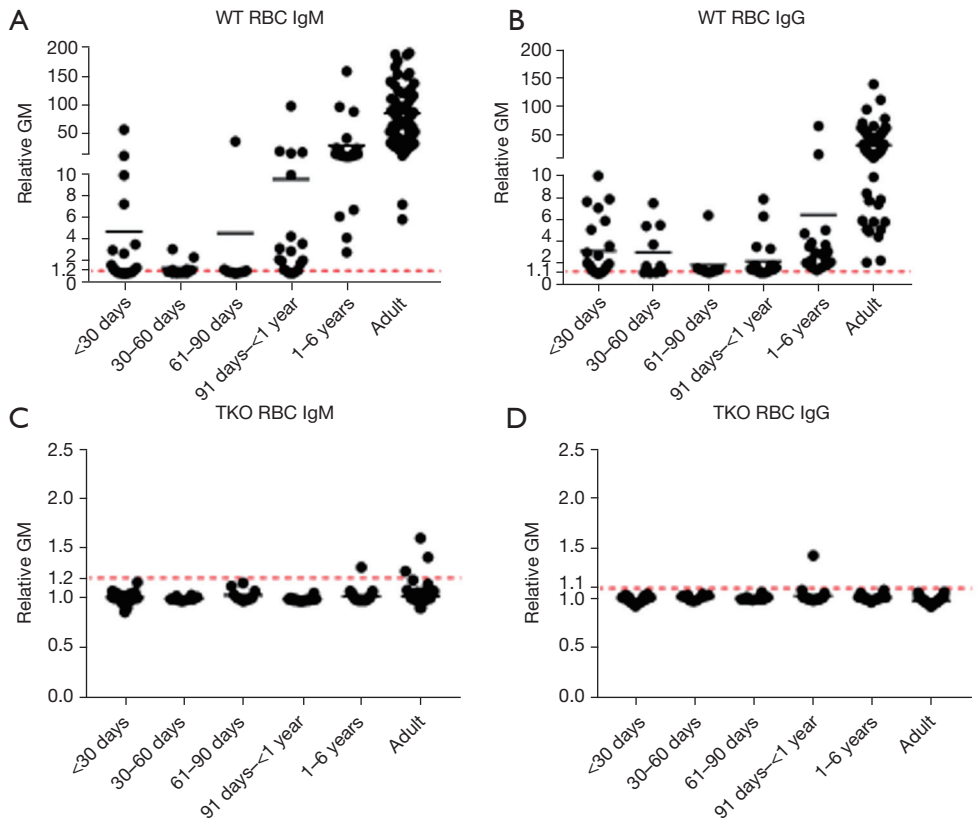

Figure 1 Correlation between human serum antibody binding to pRBCs (by rGM) and age. Human serum IgM and IgG antibody binding to WT (i.e., genetically unmodified) pRBCs (top) and to TKO pRBCs (bottom). The dotted lines indicate no IgM or IgG binding (Note the great difference in the scale on the Y axis between WT and TKO). Adapted from original data set from Li *et al.* (20). RBC, red blood cell; pRBC, pig RBC; rGM, relative geometric mean; WT, wild-type; TKO, triple knockout.

A second factor that must be taken into consideration is the fact that most neonates and infants undergo total thymectomy at the time of heart transplantation. Although this is not associated with many infectious complications, it certainly reduces the patient's T cell response and may well contribute to the good results achieved after heart transplantation in this age group (31).

Cardiac allotransplantation across the ABO blood group barrier remains an excellent example of tolerance by the infant immune system. In 2000, West and colleagues theorized that, because human infants lack strong humoral responsiveness to stimulation by carbohydrate antigens and have low levels of antibodies to non-self A and B blood group antigens, they would accept grafts from ABO-incompatible donors (32). Not only were the transplants uniformly successful, with excellent survival and low rates of rejection, but follow-up studies indicated that patients who received an ABO-incompatible organ failed to develop specific antibodies to the donor blood group (33). They named this phenomenon "donor-specific B-cell tolerance". We predict that infant humans will demonstrate a similarly blunted and accommodating humoral response to a xenograft (34).

To investigate this, our group performed early *in vitro* assessments of neonates and infants both pre- and post-cardiac surgery to determine their native reactivity to porcine antigens. No patients less than one-year-old had performed IgM or IgG antibodies to TKO pig red blood cells, regardless of whether they had undergone prior cardiac surgery (20). This was not the case in older children and adults, several of whom registered significant levels of antibodies to TKO pig antigens. Most intriguing, newborns who underwent a Norwood procedure, which requires implantation of an allograft patch for arch reconstruction and generous blood transfusion, did not express levels of anti-pig IgM or IgG that would result in humoral rejection (20). Further studies

are necessary to delineate infant human cellular porcine immunity along with their humoral reactivity to nucleated cells from pigs.

Bridge to allotransplantation

New technology is most ideally applied in clinical settings where it addresses a large gap within current care paradigms. Xenotransplantation has the potential to target the problems associated with contemporary pediatric VAD support which weigh heavily on patients and the healthcare system. Mortality associated with pediatric VADs has improved but remains at 30% for all children placed on support as a bridge. Morbidity is also high, mostly centered on thrombosis and the complications of therapies intended to prevent it. Independent of underlying diagnosis, children on a VAD demonstrate a 30% stroke rate and 25% risk of life-threatening bleed during their time on support. Furthermore, 30% of children will develop a major infection during VAD support, in part because all devices have externalized components, but primarily due to ongoing nosocomial exposure (13). Patients with small body surface areas on MCS cannot be discharged from hospital. Patients successfully bridged require prolonged inpatient hospital stays, resulting in numerous infections and ultimately a heavy financial burden on the healthcare system.

Pretransplant MCS significantly increases hospital costs compared to those of recipients not on MCS (35), MCS adding almost $69,000 to the adjusted excess cost per case basis in post-operative CHD surgery (36). While the additional costs associated with infant cardiac xenotransplantation are hitherto unknown, the potential for early home discharge with outpatient follow-up until allotransplantation would almost certainly lower the costs compared to continued hospitalization of an infant supported by a currently available MCS (in addition, home care would be greatly beneficial from a psychological perspective for the patient and the family).

A fully implantable, biologic pump capable of delivering adequate biventricular cardiac output would revolutionize the current pediatric bridge-to-transplant paradigm. A xenograft's natural endothelial lining would preclude the need for intensive anticoagulation. Our current standard of care in animal experiments of xenotransplantation is simply to give recipients only 40 mg of aspirin on alternate days. The xenograft's lack of externalized hardware would eliminate the risk of infection inherent to exposed drivelines and cannulas. This may be a neutral benefit when accounting for the risks of required immunosuppression.

However, the graft's ability to adjust to physiologic demands could allow for safe discharge to home during the bridge period. This would help reduce the length and cost of prolonged inpatient hospitalization along with the added benefit of diminishing exposure to nosocomial infections. As experience with the technology improves, the transplant community will likely support efforts to discharge xenotransplant recipients to home while retaining a stable United Network for Organ Sharing listing status.

There are, however, remaining barriers to the clinical application of cardiac xenotransplantation in infants. To date, no research group has demonstrated consistent six-month survival of an orthotopically-placed cardiac xenograft in an infant NHP. Nevertheless, all of the evidence to date indicates that the development of an immune response to a TKO pig heart will not result in the production of antibodies that cross-react with human leukocyte antigens (HLA) and so will not prevent successful subsequent allotransplantation (37).

As a research group, we are actively evaluating orthotopic cardiac transplants from TKO pigs into juvenile baboons (4–6 kg in weight). Our goal is to demonstrate consistent 4–6 months survival of life-supporting cardiac xenografts with no evidence of cross-reactivity of anti-pig antibodies with human antigens that would preclude subsequent allotransplantation. To ensure clinical feasibility, at 4–6 months we will excise the pig heart and replace it with a baboon allograft, which we would monitor for a further 2–4 months. If these milestones are achieved, we believe a clinical trial in infants failing traditional single ventricle palliation is warranted.

There is clearly a risk in this approach because, in the event of graft failure, there is likely to be no alternative therapy available. However, without a xenograft, the risk of death on MCS is also high. We would not proceed to a clinical trial unless our laboratory studies in the pig-to-baboon model indicated that a clinical trial would have a realistic chance of success. As NHPs are more likely to have preformed antibodies to TKO pig cells whereas our *in vitro* data indicate that no human infants have anti-pig antibodies (*Figure 1*) (and our current immunosuppressive regimen successfully prevents the production of *de novo* antibodies) we believe that rejection is unlikely to be problematic.

Selection of patients

If preclinical animal studies confirm an acceptable safety

profile for orthotopic cardiac xenotransplantation, the question remains: "What clinical setting is the most appropriate for initial application?" Seventy percent of pre- or post-first stage single ventricle patients who need a VAD die before a suitable human heart can be found. The children within this group who receive heart allotransplants undergo MCS for a median of 64 days (13,38). Most successful experiences with bridging first stage single ventricle patients to transplant with a VAD occur in a few specialized centers around the country. Other single center experiences report mortality rates as high as 100% (39).

If this low success rate is exceeded clinically, populations with lower risk VAD profiles could be offered xenotransplantation as an alternative bridge in a hazard-adjusted fashion: (I) failed second stage single ventricles; (II) failed third stage single ventricles; (III) complex failed biventricular CHD; (IV) dilated/restrictive cardiomyopathies. Contrary to adult applications, porcine hearts used in these settings would eventually be removed in favor of a human allograft, which may also promote community acceptance and clinical expansion.

We believe there are significant advantages of our proposal to employ a pig xenograft as a bridge to allotransplantation. No NHP has yet survived beyond 9 months after orthotopic transplantation of a pig heart, and therefore the prospects for destination therapy are presently limited. Bridging of infants for 4–6 months would be much more feasible. Bridging does not commit an infant to a lifetime dependent on a pig heart which, in view of our limited knowledge of the field at present, must be an advantage.

Destination therapy

Looking further into the future, successfully bridging pediatric patients to transplant across all diagnoses could open the opportunity for destination therapy. This has precedent in adult VADs, which began initially as a therapy offered only as a bridge but eventually became destination therapy once results were proven superior to medical therapy alone (40). Large gaps in the current care paradigm of CHD leave room for xenotransplantation to play a role, particularly in the care of single ventricle patients.

The current limits of three-stage palliation

The evolution and refinement of three-stage single ventricle palliation has saved the lives of many children. As those children progressed into adulthood, the medical community discovered that Fontan physiology is not a lifelong solution. Instead, it represents a stable state while patients await inevitable cardiac transplantation to prolong their lives past the third and fourth decades (41-44). Furthermore, we are learning more about the long-term consequences of elevated central venous pressure on end organ function, including but not limited to hepatic, renal, gastrointestinal, pulmonary, neurologic, and lymphatic systems (45-49). Because of this end organ dysfunction as well as the added technical complexity and immunologic impact of multiple prior cardiac operations, adult Fontan patients demonstrate greater post-transplant mortality compared to their biventricular counterparts (50,51). Such profound limitations warrant reconsideration of a well proven fact—single ventricle palliation is offered because of necessity, not superiority among available therapies.

Although bridging by a xenograft will necessitate a second operation to replace the pig graft with an allograft, this is arguably preferable to the multiple palliation procedures and eventual cardiac allotransplant required by many patients.

Cardiac transplantation is superior to palliation

In the 1980s, a few centers in the United States pursued primary cardiac transplantation as the treatment modality for hypoplastic left heart syndrome (HLHS). Simultaneously, the remaining centers in the United States offered three-stage single ventricle palliation. The long-term survival advantage favoring human primary transplant is clear for newborns with HLHS. At 15 years from index operation, 65% of patients with primary transplants are alive versus 40% of three-stage palliation patients (52,53). In addition to better survival, the negative sequelae of prolonged elevation in central venous pressures were avoided in the transplant population. If enough donor hearts were available, cardiac replacement would be the treatment of choice for HLHS. Cardiac xenografts have the potential to fill this need assuming they demonstrate acceptable longevity, associated morbidity, and rates of rejection.

Prenatal diagnosis

Over the past two decades, there have been significant improvements in fetal diagnosis. In the present era, there is an expectation of accurate and detailed diagnosis of complex CHD in fetuses referred to pediatric cardiologists.

Diagnosis before birth would allow sufficient time for counseling of the parents about the potential availability of xenotransplantation among other treatment options. If they wished to explore xenotransplantation, the time before birth would allow prenatal clinical preparation.

The ability to accurately diagnose CHD in the fetus also provides the possibility of modifying the neonatal immune response to xenoantigens and thereby improving the outcome of cardiac xenotransplantation. In fact, this ability could potentially result in cardiac xenotransplantation utilized as destination therapy for a group of neonates that have been documented to have poor prognosis.

Conclusions

Xenotransplantation with gene-edited porcine hearts offers great promise. There is a specific population of vulnerable neonates and infants with CHD who could immediately benefit from its introduction. Our community, comprised of physicians, nurses, families, and patients, must investigate and overcome its current limitations. Once these are resolved, we must boldly push towards clinical application.

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Footnote

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