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Original Article

Gene-edited pig cardiac xenotransplantation as a bridge to allotransplantation in infants: Progress in a pig-to-baboon model

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ABSTRACT

Gene-edited pig hearts may have an application for critically ill infants who are poor candidates for mechanical support. We established a pediatric animal model of gene-edited pig orthotopic cardiac xenotransplantation (OCXT) in baboons to assess its potential as a bridge to allotransplantation. Fifteen OCXTs were performed from genetically-engineered infantile pigs into size-matched baboons. Maintenance immunosuppression was founded on CD40/CD154 costimulation pathway blockade and rapamycin. After being sustained by xenografts for >4 months, 3 xenograft recipients were selected for transition to cardiac allotransplantation. Outcomes were tracked by invasive hemodynamic monitoring, surface echocardiography, and serial blood tests. After OCXT, 8

Abbreviations: AMR, antibody-mediated rejection; CHD, congenital heart disease; CMR, cell-mediated rejection; DSA, donor-specific antibody; Ig, immunoglobulin; IGA, intentional genomic alteration; IL, interleukin; IS, immunosuppression; IVC, inferior vena cava; mAb, monoclonal antibody; OCXT, orthotopic cardiac xenotransplantation; PBMC, peripheral blood mononuclear cell; POD, postoperative day; SD, standard deviation.

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of 15 (53%) baboons achieved survival of >1 month, with 6 surviving for >3 months. Mortality was more common early in the study, followed by longer and more uniform survival later. The longest survivor lived >24 months postxenotransplantation. There has been no evidence of significant xeno- or allo-sensitization during xenograft support. The aims of these studies were (1) to demonstrate that a gene-edited pig heart can confer months-long survival in pediatric-sized recipient baboons, and (2) to determine whether prolonged xenograft exposure does not preclude subsequent allotransplantation. Our data suggest that these aims may be achievable and warrant further study.

1. Introduction

Modern CRISPR-Cas9 gene-editing technology has enabled the production of pig organs whose cells lack the 3 major carbohydrate antigens known to be factors in hyperacute rejection in pig-to-nonhuman primate organ transplantation. In addition, the triple-knockout pig organs express selected human cell surface and intracellular proteins that aid in protecting from inflammation, coagulation, and the adaptive immune response of a primate recipient.[1] These modifications have enabled substantial progress in the longevity of life-supporting cardiac xenografts[2,3] and have prompted the US Food and Drug Administration to approve 2 expanded access applications of gene-edited pig heart transplants in adult humans.[4,5] Both patients succumbed to antibody-mediated rejection (AMR) of their cardiac xenografts, but there remains optimism that this technology may ease the human donor organ shortage.[6]

Medical ingenuity throughout the last 70 years has saved the lives of countless children with congenital heart disease (CHD). Surgical outcomes have plateaued,[7] but we now care for a population of patients who are at ongoing risk for heart failure and the need for cardiac replacement.[8] The pediatric heart transplant list has grown every year since 2010, yet the number of suitable human donor hearts available for transplantation remains inadequate. Children <1 year of age experience the greatest negative impact. They have the lowest rate of cardiac allotransplantation[9] and carry the highest risk of waitlist mortality.[10] Thirty-four percent of children <1 year of age will die while waiting a median time of 110 days for a new heart,[9] with wait times significantly exceeding this in certain geographic locations.[9]

These poor results are explained, in part, because available technology is not capable of safely bridging this fragile population to allotransplantation.[11] We believe that pig cardiac xenotransplantation is poised to disrupt this paradigm, and we have developed a preclinical pediatric model to prove the concept of orthotopic cardiac xenotransplantation (OCXT) as a bridge to allotransplantation. Herein, we present the results of those efforts.

2. Materials and methods

2.1. Donor and recipient selection

2.1.1. Pigs

Gene-edited pig donors were produced according to previously published techniques.[12] Given the preliminary nature of this work, we explored the viability and impact of varying gene edits on the long-term survival of OCXTs. From pilot studies and from work funded by the US National Heart, Lung, and Blood Institute, 4 different genetic constructs were tested in a pig-to-baboon model (Table 1).

Pigs in the pilot cohort were Large Whites (*Sus domesticus*, OCXT #1-4), whereas those studied under the US National Heart, Lung, and Blood Institute grant were Yucatan miniature swine (*Sus scrofa,* OCXT #5-15). The desired expression and functionality of intentional genomic alterations (IGAs) were confirmed using RNA sequencing, flow cytometry, and immunohistochemistry on ear punch samples (predonation) from all pigs selected as sources of organs. Immunohistochemistry on the aorta (postdonation) was also performed for the same purpose. Pigs were of blood type O, either sex, aged 5 to 15 (mean ± standard deviation [SD], 8.9 ± 2.9) weeks, and weighed 4.1 to 13.2 (mean ± SD, 8.3 ± 3.1) kg, and were cytomegalovirus-negative (Table 2).

2.1.2. Baboons

Candidate olive baboons (*Papio anubis*) were selected to mimic the size of our target human population, ie, human infants with complex CHD. They were of either sex, all AB blood types, aged 13 to 30 (mean ± SD, 20.5 ± 6.2) months, weighed 4.5 to 9.5 (mean ± SD, 6.4 ± 1.6) kg, and were cytomegalovirus-negative (Table 2). All baboons came from a pathogen-free colony maintained at the Michale E. Keeling Center.

2.1.3. Pig-baboon matching

Sera from potential baboon recipients underwent screening using flow cytometry to measure preformed levels of anti-pig

Table 1
Variability among swine donors for orthotopic cardiac xenotransplantation based on pig breed and intentional genomic alterations.

OCXT #	1-2	3-4	5, 7-10	6, 11-15
Swine breed	*Sus domesticus*	*Sus domesticus*	*Sus scrofa (ESUS 1417)*	*Sus scrofa (ESUS 1784)*
Intentional genomic alterations	GGTA1-KO, hCD55	GGTA1-KO, hCD46, hTBM	3KO hCD46	3KO hCD46
			hCD55	hCD55
			hTM	hTM
			hEPCR	hEPCR
			hCD47	hCD47
			hTFPI	hHO-1
			hHLA-E	hA20
			hB2M	Inactivated PERV
			Inactivated PERV	

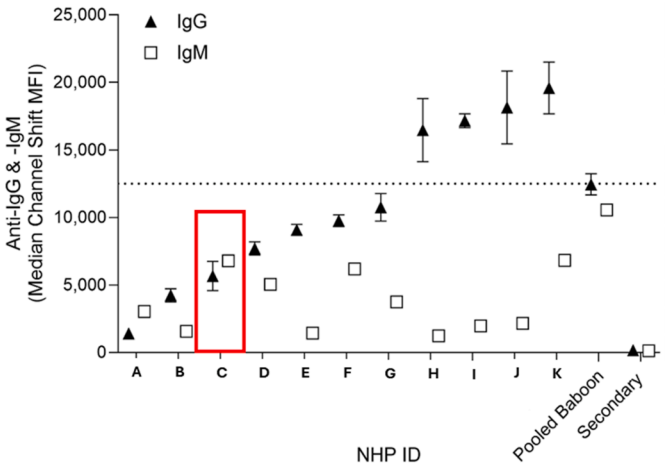
GGTA1-KO, α-1,3-galactosyltransferase knock out; hA20, tumor necrosis factor alpha-induced protein 3; hB2M, β-2 microglobulin; hCD55, complement decay-accelerating factor; hCD47, integrin-associated protein; hCD46, membrane cofactor protein; hEPCR, activated protein C receptor; hHLA-E, human leukocyte antigen-E; hHO-1, heme oxygenase-1; hTFPI, tissue factor pathway inhibitor; hTM, human thrombomodulin; OCXT, orthotopic cardiac xenotransplantation; PERV, porcine endogenous retrovirus; 3KO, triple knockout.

Table 2
Baboon and pig demographics for all orthotopic cardiac xenotransplantation.

OCXT	Donor age (wk)	Donor weight (kg)	Donor heart weight (g)	Recipient age (mo)	Recipient weight (kg)	Recipient heart weight (g)	Donor/recipient weight ratio	Donor/recipient heart weight ratio
#1	15	13.2	n/m	28	9.0	n/m	1.5	n/a
#2	14	12.1	n/m	30	9.5	n/m	1.3	n/a
#3	11	9.9	n/m	24	8.0	n/m	1.2	n/a
#4	10	6.5	n/m	17	6.0	n/m	1.1	n/a
#5	7	8.5	60	14	5.0	20	1.7	3.0
#6	6	5	n/m	13	4.5	n/m	1.1	n/a
#7	6	6.5	63	14	5.0	28	1.3	2.3
#8	10	12	70	28	8.5	44	1.4	1.6
#9	6	5.5	35	16	5.6	28	1.0	1.3
#10	11	14	68	29	7.0	32.2	2.0	2.1
#11	5	4.1	26	15	4.8	19.2	0.9	1.4
#12	10	8.2	54	20	5.3	25.6	1.6	2.1
#13	7	6.5	45	25	5.9	27.3	1.1	1.6
#14	8	6	40	13	4.7	20.9	1.3	1.9
#15	7	6.2	48	21	7.5	34	0.8	1.4
Mean ± SD	8.9 ± 2.9	8.3 ± 3.1	51 ± 14	20.5 ± 6.2	6.4 ± 1.6	28 ± 7	1.3 ± 0.3	1.9 ± 0.5

n/a, not applicable; n/m, not measured; OCXT, orthotopic cardiac xenotransplantation; SD, standard deviation.

immunoglobulin (Ig)G and IgM. Baboons with serum levels less than the mean level in a pooled sample of 20 naïve baboon sera were considered candidates for cardiac xenotransplantation (Fig. 1). Within this acceptable group, a specific recipient was selected based on a good size match with the porcine donor (based on standardized growth curves for the breed of pig being used). Our goal was to either match donor and recipient weights exactly or oversize up to a ratio of 1.5:1.0 between donor and



Preference of NHP ID (Lowest IgG first)	Animal ID
1	A
2	B
3	C
4	D
5	E
6	F
7	G
8	H
9	I
10	J
11	K

Figure 1. Example of preoperative baboon screening for preoperative levels of anti-pig immunoglobulin (Ig)G and IgM. The dashed line represents the upper limit of acceptable levels of preformed anti-pig antibody. Animal C was selected for orthotopic cardiac xenotransplantation #13 due to sizing needs and low levels of preformed anti-pig IgG and IgM compared with a pooled baboon sample. AMFI, mean fluorescence intensity; NHP ID, non-human primate identification.

recipient weight. Baboons that lived >4 months after OCXT were considered candidates for xenograft explant and cardiac allotransplantation from a prospectively size-matched baboon donor.

2.2. Operative procedures

Donor pig cardiac procurement was coordinated in the same operating room with the baboon recipient operation to minimize cardiac xenograft ischemic times (Table 3). Baboons underwent OCXT with bicaval anastomosis.[13] Cardiac xenograft replacement with a baboon allograft was carried out in a similar fashion.

2.3. Immunosuppressive regimen

Baboons were jacket and tether trained beginning 2 weeks before OCXT. Baboons who did not acclimate to wearing a jacket were not enrolled in the study. Induction immunosuppression (IS) was started on day minus 3 (ie, 3 days before OCXT) via a tunneled internal jugular central venous catheter. Details of the IS regimen and variations throughout our experiments have been previously described.[14] Although there was some variation between experiments (Table 4), induction of IS included antithymocyte globulin, an anti-CD20 monoclonal antibody (mAb; rituximab), and C1-esterase inhibitor. Maintenance IS therapy consisted of CD40/CD154 costimulation pathway blockade (primarily Tegoprubart, OCXT #5-15, a mAb directed against CD154), rapamycin, low-dose corticosteroids, and, in some cases, tocilizumab, an interleukin (IL)-6 receptor blocker (Table 4).

There were some inconsistencies in our IS regimen throughout the study. Anti-CD40 was used in the first 4 experiments, as there was no available anti-CD40L medication that was not associated with diffuse platelet activation and thrombosis. Once Tegoprubart was available, we utilized it exclusively (OCXT 5 onwards). Etanercept for tumor necrosis factor-α blockade was utilized for the first 4 experiments but had no impact on cytokine profiles or outcomes. Tocilizumab was included in the regimen starting with OCXT 10 onwards, given reproducible spikes in measured levels of IL-6 during prior experiments.[14]

In the first baboon that underwent secondary cardiac allotransplantation, no further induction therapy was administered. Maintenance IS was transitioned from costimulation blockade and rapamycin to conventional IS therapy (tacrolimus and mycophenolate mofetil). The associated experimental outcome dictated a need for induction IS therapy for the second and third attempts to transition back to a cardiac allograft.

2.4. Monitoring of recipient baboons

Standard laboratory tests, including complete blood count, serum chemistry, and troponin level (initially analyte I, but subsequently switched to troponin T), were routinely checked, as were levels of rapamycin and the costimulation blockade agent. Baboons were sedated weekly for surface echocardiography to measure graft function and ventricular septal thickness, as well as a general health assessment by the veterinary team. Immunologic response following induction IS was tracked using flow cytometry to examine trends in absolute values of lymphocyte subpopulations every other week. Donor-specific serum reactivity was tracked by incubating recipient serum with donor-derived pig cardiac endothelial cells. Flow cytometry on these samples identified measured levels of IgG and IgM that bound to donor cardiac endothelial cells. The significance of post-xenotransplant levels was determined by comparison to pre-transplant levels. Pretransplant and posttransplant samples were run simultaneously to improve precision and comparability.

Serum from candidates for cardiac xenograft explant and allotransplantation was prospectively cross-matched against cells from potential baboon cardiac donors of acceptable size and ABO blood type. Sera from baboons with an active OCXT were incubated in separate aliquots with candidate baboon donor peripheral blood mononuclear cells (PBMCs). Flow cytometry identified measured levels of bound anti-baboon IgG and IgM. Serum reactivity to all PBMCs and specific reactivity to donor $CD3^+$ and $CD20^+$ cells were documented. In view of the

Table 3
Perioperative and outcomes data.

OCXT	Total graft ischemic time (min)	CPB time (min)	Survival time	Outcome
#1	136	122	<24 h	PCXD
#2	77	119	90 d	AMR
#3	71	81	241 d	AMR
#4	68	85	<24 h	SIXR
#5	63	80	<24 h	Technical error
#6	60	85	9 d	AMR, CMR
#7	59	90	2 d	SIXR
#8	43	85	734 d	Healthy at euthanasia
#9	31	79	3 d	SIXR
#10	43	70	238 d (133 d Xenotransplant + 105 d Allotransplant)	AMR, CMR
#11	40	68	147 d	Technical error during allotransplant
#12	44	58	242 d	AMR
#13	29	64	41 d	AMR and CMR
#14	38	68	52 d	AMR
#15	46	77	<24 h	Technical error
Mean ± SD or median (IQR)	56.5 ±25.6	82.1 ± 17.4	41 (1.5, 193)	

AMR, antibody-mediated rejection; CMR, cell-mediated rejection; CPB, cardiopulmonary bypass; IQR, interquartile range; OCXT, orthotopic cardiac xenotransplantation; PCXD, primary cardiac xenograft dysfunction; SD, standard deviation; SIXR, systemic inflammatory xenograft response.

Table 4
Immunosuppression regimen and changes according to experiment number over time for all orthotopic cardiac xenotransplantation.

Baboon OCXT #	1-4	5-9	10-15
Induction			
ATG 5 mg/kg IV (PODs –3 and –1)	+	+	+
Rituximab 10 mg/kg IV (POD –2)	+	+	+
Methylprednisolone 10 mg/kg IV (POD 0)	+	+	+
C1-esterase inhibitor 20 units/kg IV (PODs 0, 4, and 8)	+	+	+
Tocilizumab 10 mg/kg (PODs –1 and 7)	–	–	+
Etanercept 1 mg/kg IV (POD 0), 0.5 mg/kg IV (PODs 3, 7, and 10)	+	–	–
Maintenance			
Anti-CD40 mAb 50 mg/kg IV (PODs 0, 4, 7, 10, and 14, weekly)	+	–	–
Anti-CD154 mAb 20 mg/kg IV (PODs 0 [×2], 2, 7, 10, and 14, weekly)	–	+	+
Rapamycin 1 mg/kg orally ×2/d (from POD-14) (trough 8-12 ng/mL)	+	+	+
Methylprednisolone 5 mg/kg tapering to 0.125 mg/kg IM (daily)	+	+	+

ATG, antithymocyte globulin; IM, intramuscularly; IV, intravenous; mAb, monoclonal antibody; OCXT, orthotopic cardiac xenotransplantation; POD, postoperative day.

lack of availability of granular characterization and reagents for the entire complement of baboon major histocompatibility complexes, these nonspecific tests served as a surrogate for traditional major histocompatibility complex reactivity testing. Preallotransplantation levels were normalized and analyzed for significance by comparing them to prexenograft exposure levels run simultaneously.

At the experiment's end, for each animal, a complete necropsy with histopathology was conducted. Tissues were fixed in 10% neutral buffered formalin, processed for standard histopathology, and slides were stained with hematoxylin and eosin. Additionally, immunohistochemistry was performed on cardiac samples to identify C4d deposition as well as lymphocyte subtypes infiltrating the myocardium (if present).

2.5. Data analysis

Data were tabulated and analyzed using SAS 9.4. Standard descriptive statistics were utilized. Normality of continuous data was determined with the Shapiro-Wilk test. Continuous data with normal distribution are presented as median with SD, whereas those with non-normal distribution are presented as median with quartiles.

3. Results

3.1. Primary orthotopic cardiac xenotransplantation

3.1.1. Xenograft survival and recipient survival

A total of 15 OCXTs was performed. Mean cardiopulmonary bypass and graft ischemic times were 82.1 and 56.5 minutes, respectively (Table 3). Fourteen of 15 baboons were successfully weaned from cardiopulmonary bypass, although 3 died within 24 hours. Eight of 15 baboons survived >1 month with a functioning xenograft. Median survival for the entire series was 41 days. Median survival for baboons that lived >30 days post-OCXT (n = 8) was 193 days. The longest survivor lived just beyond 24 months post-OCXT, at which time she was electively euthanized with no significant clinical abnormalities.

Of the 6 baboons that died within the first week post-OCXT (Table 3), causes included: (1) primary graft dysfunction related to inadequate myocardial preservation[15] (University of Wisconsin solution with cold ischemia of 60 minutes) (n = 1); in all subsequent experiments del Nido solution was used and the ischemic time was minimized[12]; (2) management complications leading to hypovolemic shock in baboons with excellent graft function (n = 2), and (3) respiratory failure from pulmonary edema and pleural effusions in the presence of excellent cardiac function (n = 3), possibly associated with a systemic inflammatory response[16,17]; protocol changes including administration of tocilizumab and a method for active pleural space drainage for 10 days resulted in no further deaths from respiratory failure. Histologic evaluation of cardiac grafts from all animals that died <7 days following OCXT showed no features of rejection (Fig. 2A). Most of these animals demonstrated significant pulmonary edema and associated inflammation (Fig. 2B).

There was 1 death at 9 days from AMR and cell-mediated rejection (CMR) resulting from inadequate intake and levels of IS. Deaths after 30 days were primarily attributable to rejection (n = 6) (Fig. 3 and Table 3). Baboons OCTX #13 and OCTX #14 died with AMR on postoperative days (PODs) 41 and 52, respectively.

3.1.2. Immunologic recovery

Following induction, IS lymphocyte numbers were suppressed by 80% to 100%. T cell recovery began as early as POD 14 and generally reached 50% to 60% recovery in the following weeks in most baboons. Unlike $CD4^+$ T lymphocytes, which followed the recovery pattern of $CD3^+$ T cells, the recovery of $CD8^+$ T cells was slower and fluctuated between 30% and 40% of the pretreatment number.

The recovery of B cell numbers was also generally slower. Although in some baboons, recovery to 40% to 50% pretransplant numbers occurred within the first 2 months, in others it was slower and never reached >50% to 70%. However, baboon OCXT #13 died from rejection before recovery of B cells.

The absolute number of monocytes fluctuated but remained largely unchanged or slightly higher than pre-OCXT (Supplementary Fig. S1).

Anti-pig IgG and IgM remained near pretransplant levels throughout the post-OCXT course (Fig. 4). Despite prolonged xenograft exposure, there were no increases >2-fold above

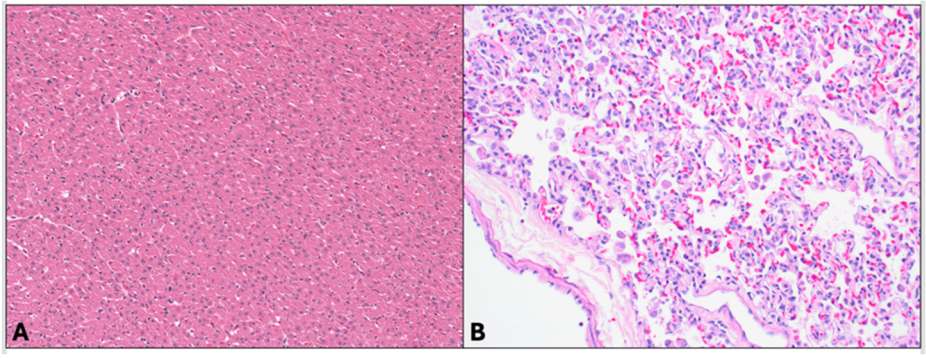


Figure 2. Representative histologic images from OCXT 9, who died at postoperative day (POD) 3 from respiratory failure. (A) Hematoxylin and eosin (H&E), 200× magnification, The myocardium was overall unremarkable, with a few areas of acute hemorrhage and individual myocyte necrosis consistent with agonal changes related to hypoxia but no significant inflammatory cell infiltrates or other evidence of rejection; (B) H&E, 200× magnification, In the lungs, the interstitium was expanded by increased clear space, and alveoli contained markedly increased numbers of foamy macrophages and eosinophilic material, consistent with pulmonary edema. OCXT, orthotopic cardiac xenotransplantation.

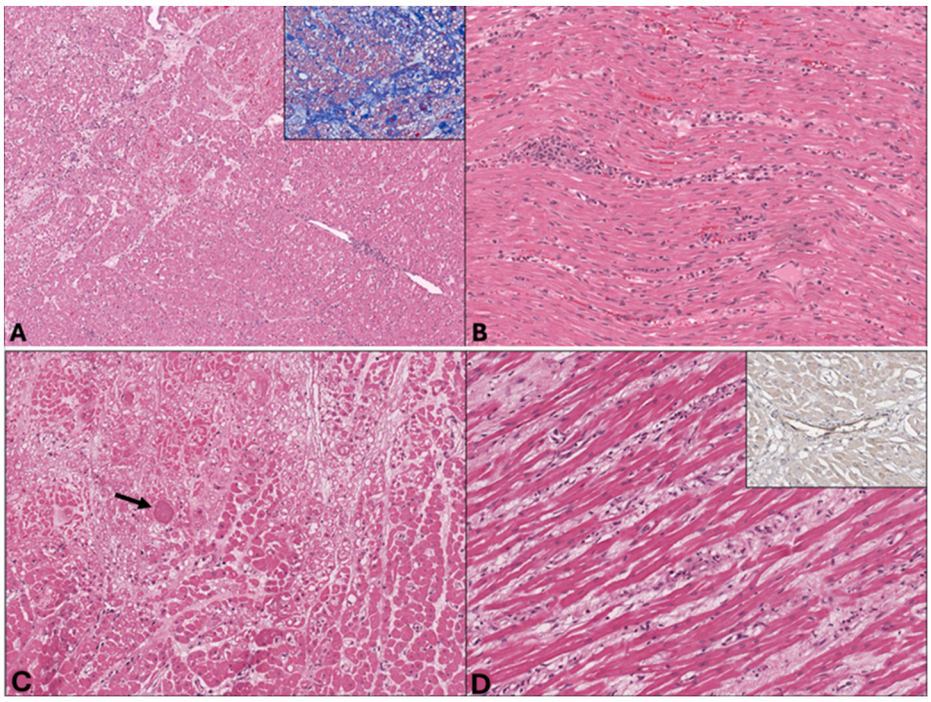


Figure 3. Representative histologic images of xenografts from orthotopic cardiac xenotransplantation (OCXT) 11 (A, B) and OCXT 12 (C, D) demonstrating varying degrees of rejection. (A) Hematoxylin and eosin (H&E), 100× magnification; areas of the xenograft demonstrated chronic myocardial degeneration and fibrosis. Inset: trichrome stain demonstrating collagen fibers of fibrosis in blue. (B) H&E, 200× magnification; additionally, there were mild multifocal areas of myocardial degeneration and necrosis with infiltration of low numbers of inflammatory cells, predominantly lymphocytes and macrophages. (C) H&E, 200× magnification; fibrin thrombi (arrow) were identified within small vessels throughout the myocardium, with ischemic necrosis of surrounding areas. (D) H&E, 200× magnification; diffusely, cardiac myofibers were separated by edema with low numbers of inflammatory cell infiltrates, predominantly lymphocytes and macrophages. Inset: C4d immunohistochemistry demonstrating positive reactivity of the lining of vessels, evidence of antibody-mediated rejection.

pretransplant levels, and prospective cross-matching before planned allograft transition was negative. Anti-baboon IgG and IgM levels measured on PODs 71, 115, and 223 in OCXT #10, 11, and 12, respectively, were similar to levels measured before OCXT (Fig. 5). Reactivity was also unchanged to prospective donor baboon $CD3^{+}$T and $CD20^{+}$B cells (Supplementary Figs. S2 and S3).

3.2. Bridging to allotransplantation

Three baboons were enrolled for xenograft explant in exchange for a prospectively cross-matched allograft (Table 3).

1. OCXT #10 transitioned 133 days after xenotransplantation. Histology of the explanted xenograft demonstrated healthy

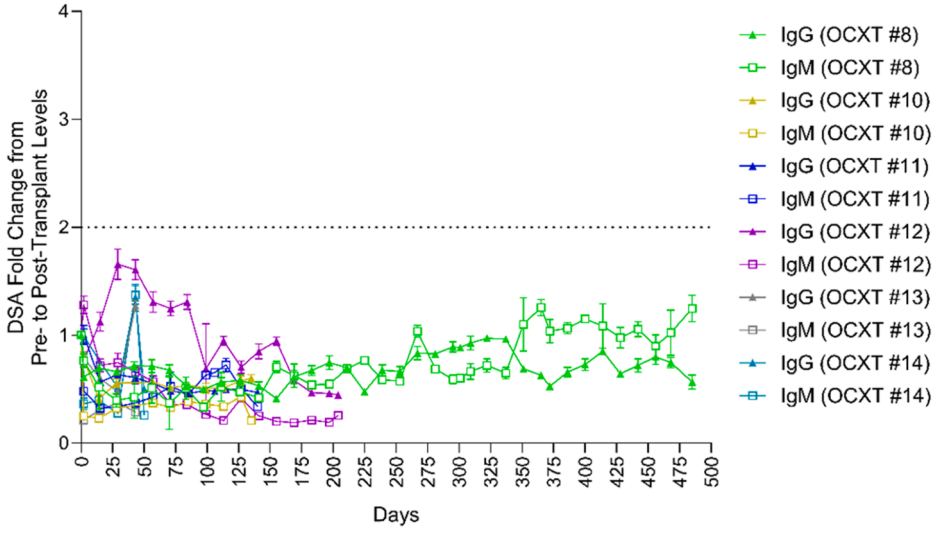


Figure 4. Measured anti-pig immunoglobulin (Ig)G and IgM levels for NHPs who survived for greater than 30 days following orthotopic cardiac xenotransplantation (OCXT), referenced as a fold change compared with pre-OCXT values. Samples from OCXT #2 and 3 were not obtained for measurement. DSA, donor-specific antibody.

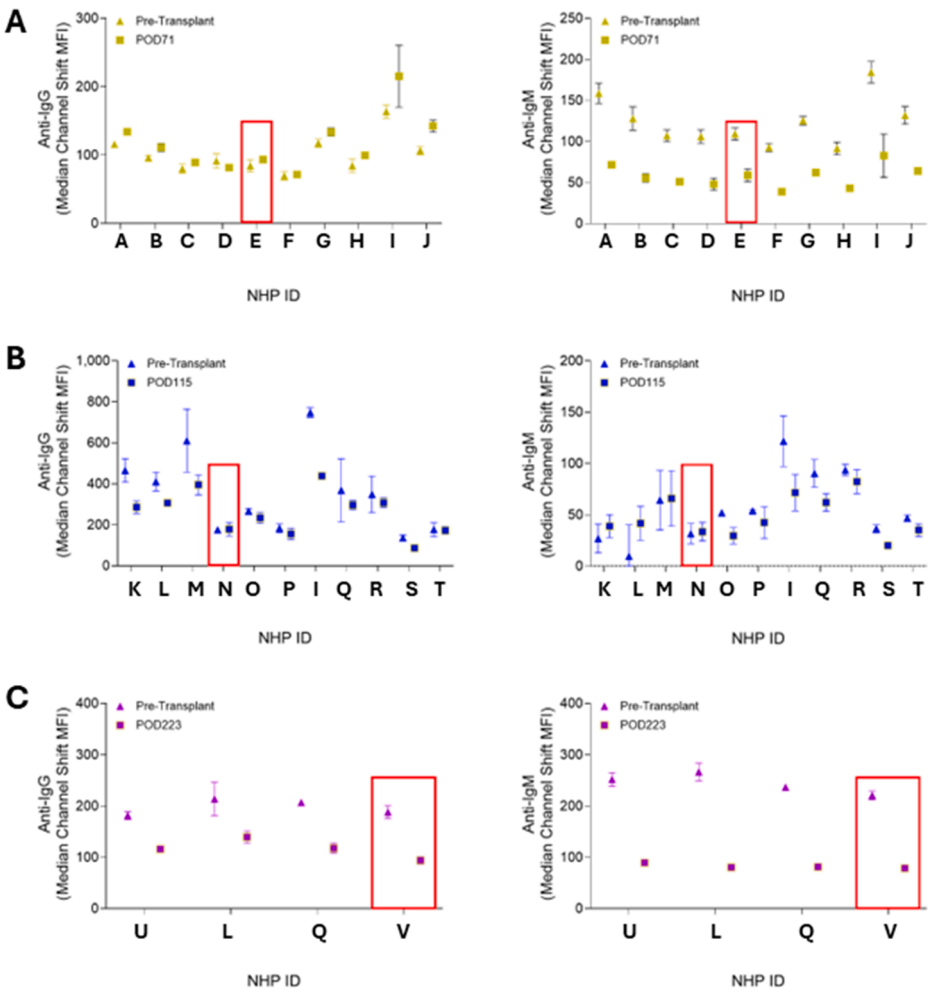


Figure 5. Prospective crossmatches to candidate baboons for all 3 planned transitions to cardiac allotransplantation. (A) OCXT 10; (B) OCXT 11; and (C) OCXT 12. Red boxes highlight selected baboons for allograft donation. Ig, immunoglobulin; MFI, mean fluorescence intensity; NHP ID, non-human primate identification; OCXT, orthotopic cardiac xenotransplantation; POD, postoperative day.

myocardium with scattered areas containing mild inflammatory cell infiltrates and minimal myocardial degeneration (Fig. 6). To facilitate the surgical procedure, a small piece of pig inferior vena cava (IVC), comprising 50% of the posterior wall of the vessel, was left in place. At the time of allotransplantation, (1) no induction therapy was administered, and (2) the maintenance IS regimen was changed to conventional tacrolimus and mycophenolate mofetil. The baboon survived for 105 days after allotransplantation.

On POD 71 after allotransplantation, increases in anti-pig and anti-baboon IgG and IgM were detected (Supplementary Figs. S4 and S5). This was also associated with a rise in serum troponin levels. The measured level of tacrolimus was within the desired therapeutic range (10-15 ng/mL). Carrying out a myocardial biopsy was not feasible at the time, and so based on available data, we correctly presumed that rejection was occurring and administered bolus steroids over 3 days together with a single dose of anti-CD20 mAb and an additional dose of Tegoprubart. Without access to an assay that measures serum mycophenolate mofetil levels, we were unable to confirm adequate drug consumption and absorption. We elected to replace it with intramuscular rapamycin. Levels of donor-specific antibodies (DSAs) decreased (Supplementary Fig. S4). The response of anti-baboon DSA could not be monitored beyond postallograft day 71 because of a lack of available donor-specific PBMCs for testing. Despite a decrease in troponin level and improved cardiac systolic function on echocardiography, we elected to euthanize the baboon on POD 105 due to deteriorated clinical condition.

At necropsy, the animal had systemic edema, pleural effusions, and abdominal ascites. The allograft demonstrated multiple foci of moderate cellular infiltrates along with edema, myocyte injury, hemorrhage, fibrosis, and coronary endarteritis. A diagnosis of moderate-to-severe AMR/CMR was made based on positive staining for CD3, CD20, and CD4d on histopathology (Fig. 7), with >50% stenosis of the IVC orifice with fibrosis and severe eosinophilic and granulomatous inflammation.

2. Baboon OCXT #11 underwent allograft transition on POD 147. Histopathologic evaluation of the xenograft showed mild edema and infiltration by low numbers of inflammatory cells, predominantly lymphocytes ($CD3^+$ and $CD20^+$) and macrophages, with

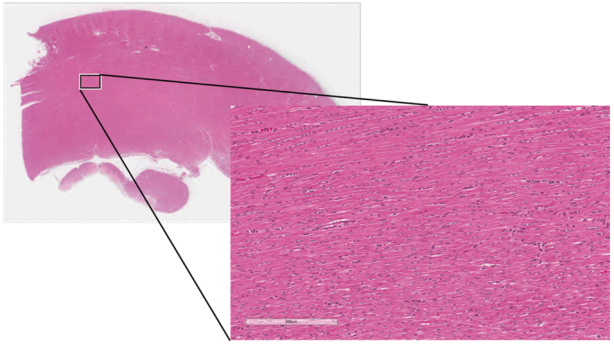


Figure 6. Histologic images from the cardiac xenograft electively explanted from OCXT 10 at POD 133 for planned allograft exchange. The explanted xenograft had overall unremarkable myocardium in most areas (inset). There were mild multifocal infiltrates of inflammatory cells comprised primarily of lymphocytes with fewer macrophages in some scattered areas. OCXT, orthotopic cardiac xenotransplantation; POD, postoperative day.

areas of chronic myocardial degeneration and fibrosis. There were areas of C4d reactivity, possibly representing an early/mild stage of AMR and CMR (Fig. 3A, B). To remove all swine tissue during the explant, the pulmonary artery branches were made discontinuous; reconstruction of the pulmonary artery was unsatisfactory, and the baboon was euthanized on the operating table.

3. In baboon OCXT #12, troponin levels began to increase on POD 200 after cardiac xenotransplantation, reaching a peak of 67 ng/mL. This persisted along with other clinical signs of rejection, including increasing myocardial thickness and echogenicity on echocardiography (similar to the clinical experience of the Maryland group).[6] The baboon was treated with a single, extra dose of anti-CD154 mAb (Tegoprubart) on POD 210 and then, with a view to replacing the xenograft with an allograft, underwent a full course of induction therapy beginning on POD 239. Following a second antithymocyte globulin infusion, the baboon succumbed to acute respiratory failure and was euthanized on POD 242. We hypothesized that, although systolic function was normal on echocardiogram, diastolic function was impaired and that an acute volume overload of induction medications resulted in acute pulmonary edema.

Necropsy demonstrated signs of heart failure, including bilateral thoracic effusions, pulmonary edema, and hepatic congestion, as well as infarction of the right kidney. Histopathology of the xenograft revealed diffuse myocardial edema, vasculitis, and multifocal fibrin thrombi, with multiple areas of

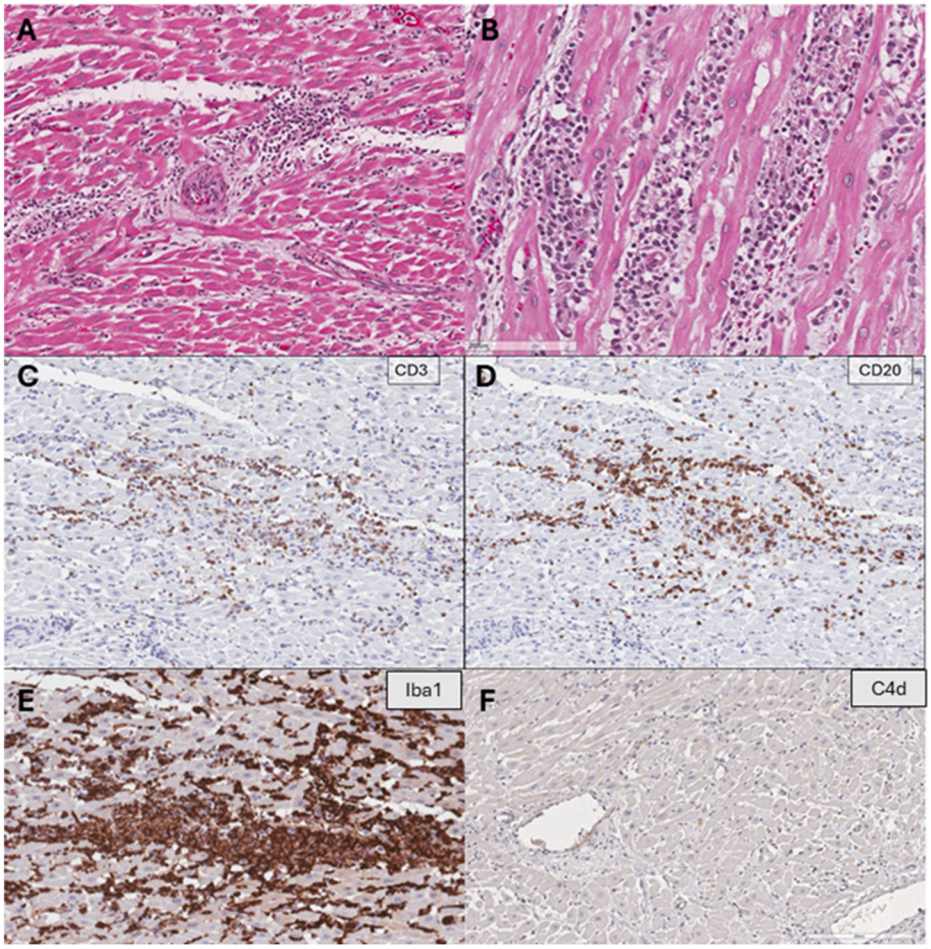


Figure 7. Histologic analysis of OCXT 10's allograft following necropsy. There are multiple foci of moderate cellular infiltrates along with edema, myocyte injury, hemorrhage, and mild fibrosis (A) and (B). Positive inflammatory cell infiltrate staining for (C) $CD3^+$ T cells, (D) $CD20^+$ B cells, (E) $Iba1^+$ macrophages, and (F) positive C4d staining of capillaries indicated a mixed cellular and antibody-mediated rejection. OCXT, orthotopic cardiac xenotransplantation.

ischemic necrosis, likely related to AMR as indicated by positive C4d staining (Fig. 3C, D).

4. Discussion

We set out to explore the potential of cardiac xenotransplantation as a bridge to allotransplantation in children with complex CHD for which no other therapy is available. We have grown weary of watching them linger and die in the intensive care unit, succumbing to prolonged heart transplant waiting times and the associated waitlist mortality. In our opinion, currently available genetically modified pig hearts may represent a novel solution to this problem. Although utilizing such hearts as destination therapy for these fragile children would be ideal, ethical concerns and public perception in the setting of a pediatric human application, as well as lack of xenograft longevity data, make a short-term bridge option with eventual transition back to a human heart attractive. The results of our work in an animal model indicate a viable pathway forward, but with each subsequent success or failure, new questions are introduced.

The deficits of this current study are many and worthwhile addressing. Exploratory in nature, we introduced a wide swath of variables between each experiment. Myocardial preservation changed once, aspects of the IS regimen were modified 3 times, postoperative care paradigms were changed several times, and perhaps most crucially, we utilized 2 different donor pig breeds with 4 distinct genetic patterns. The introduction of multiple variables can be considered a deficit to the science and reproducibility of this work. However, we believe each adaptation marked a pivotal point that has allowed us to progress. Fifteen OCXTs later, the end goal is in sight with what we believe to be a viable protocol requiring validation.

Several notable discoveries have been made during this body of work.

1. Respiratory failure associated with pulmonary edema and pleural effusions caused the demise of 3 baboons early in our experience. A baboon-proof system was established to actively drain the pleural space while preventing the animal from accessing it, thus eliminating this problem as a cause of death. The addition of IL-6 receptor blockade to the IS regimen did not appear to impact the pleural fluid output or the measured IL-6 levels.[14]
2. Despite the profound immunogenicity of a pig xenograft, an effective IS regimen largely (but not always) prevented the development of AMR and/or CMR, enabling several recipients to survive for a sufficiently long period of time, ie, >4 months, necessary for most human infants to bridge successfully to a time when a suitable allograft would become available. We personally believe that both rapamycin and Tegoprubart (anti-CD154) are key contributors to this outcome. Costimulation blockade and rapamycin have tolerogenic properties individually and in combination[18] that may lead to some degree of graft accommodation, particularly in young animals with immune plasticity. In support of this, donor-specific antibody production in our baboons with an OCXT appeared to be negligible in the testing we performed. However, the observed deposition of C4d in OCXT #2, 6, and 12 to 14 indicates that AMR may still have contributed to the mortality in recipients that survived for >30 days. An improved understanding of the mechanism of rejection and potential sequestration of anti-pig DSA in the graft is required.
3. There is substantial recovery of native immunologic function in the setting of our maintenance IS regimen for cardiac xenografts. This was most clearly manifested in the immunologic failure following transition to allograft for OCXT #10. Our group elected not to give repeat induction therapy before allograft transition because we inappropriately assumed a persistent state of adequate immunologic suppression following prior induction and ongoing suppression with steroids, rapamycin, and costimulation blockade. On post hoc analysis of flow cytometry profiles of lymphocyte recovery following OCXT, we recognized the error of this reasoning. Despite ongoing suppression of B cell and monocyte populations, T cells had recovered to 50% of pre-OCXT levels (Supplementary Fig. S1). A second, limited induction regimen focused on T cell suppression may have aided subsequent reactivity to the newly implanted allograft. We also strongly believe that no amount of induction therapy would have precluded eventual rejection of residual pig IVC tissue in the setting of isolated tacrolimus/mycophenolate mofetil IS and that this reactivity would induce a systemic inflammatory response that also initiated allograft rejection.

We began our animal model studies with hearts from Large White pigs (final adult weight >200 kg) containing limited gene edits. However, our goal of bridging human infants to allotransplantation necessitates hearts that start small and grow slowly. We therefore transitioned to using hearts from Yucatan miniature swine (final adult weight 60-70 kg) with more sophisticated and comprehensive IGAs. Of the 11 OCXTs with hearts from Yucatan miniature swine, 5 were from ESUS 1417 donors, and 6 were from ESUS 1784 donors (Table 1). It remains unclear whether either one has a major advantage. However, we have witnessed increased rates of rejection in recipients of ESUS 1784 vs ESUS 1417 hearts (4/6 vs 0/5). This comparison is extremely limited, and early noncardiac mortality in 3 of 5 recipients of hearts from ESUS 1417 donors precludes any definitive conclusions. Further experiments in the setting of a refined protocol that minimizes early mortality may help to elucidate any impact the different IGAs may have on long-term outcome.

The published literature suggests that prolonged exposure to porcine antigens does not increase sensitization to alloantigens in animal models.[19] We confirmed this in 3 baboons but failed to translate this serologic finding to prolonged allograft survival after xenograft transition. Lessons learned from this experience included (1) the need to plan for the technical success of a future allotransplant at the time of the initial xenotransplant, (2) the importance of removing all pig tissue at the time of xenograft explant, (3) the likely need for at least some induction IS therapy at the time of transition, and (4) the potential advantage of maintaining costimulation pathway blockade for several weeks before switching to a conventional IS regimen.

With respect to future studies, we must seek to utilize the optimal protocol as defined by the work outlined in this manuscript. The animal that attained the longest post-OCXT survival (OCXT #8) may represent the ideal combination of porcine genetic edits, myocardial preservation, surgical technique, and immunosuppressive regimen. This animal survived beyond 2 years before undergoing elective euthanasia and cardiac xenograft explant. The porcine heart contained edits that

included 3 carbohydrate antigen knockouts, 3 human complement modulators, 2 human antithrombotic proteins, 1 human anti-inflammatory protein, 2 human leukocyte antigen protein components, and inactivation of all porcine endogenous retroviruses (ESUS 1417). The heart was preserved with modified del Nido cardioplegia[13] at the time of procurement and implanted via bicaval implant. Maintenance IS was the same as the last 11 of 15 experiments: twice daily oral rapamycin, once weekly intravenous anti-CD154 (CD40 ligand), and daily low-dose prednisone. With this combination of key components to the cardiac xenotransplantation process, we hope to produce the necessary data to safely press forward into human infant application.

5. Conclusions

Prolonged survival with an OCXT beyond the median waitlist time for children with complex CHD is feasible. Exposure to pig xenoantigens in the setting of CD40/CD154 costimulation pathway blockade does not appear to preclude subsequent allotransplantation. More research is necessary to clarify the optimal protocol for human use and perfect the transition from xenograft to allograft. Current progress and results suggest a pathway forward and a reason to hope.

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Declaration of competing interest

The authors of this manuscript have conflicts of interest to disclose as described by *American Journal of Transplantation*. D.K.C. Cooper is a consultant to eGenesis Inc, and K. Getchell, I. Moreno, V. Yeung, and S. Low are employees of eGenesis Inc. S. Perrin and E. Katz are employees of Eledon Pharmaceuticals. However, the opinions expressed in this paper are those of the authors and do not necessarily represent those of the company. The other authors of this manuscript have no conflicts of interest to disclose as described by *American Journal of Transplantation*.

Data availability

Any further data that support the findings of this study are available on request from the corresponding author.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ajt.2025.12.017.

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References

1. Reichart B, Cooper DKC, Längin M, Tönjes RR, Pierson RN, Wolf E. Cardiac xenotransplantation: from concept to clinic. *Cardiovasc Res*. 2023;118(18):3499–3516. https://doi.org/10.1093/cvr/cvac180.
2. Längin M, Mayr T, Reichart B, et al. Consistent success in life-supporting porcine cardiac xenotransplantation. *Nature*. 2018; 564(7736):430–433. https://doi.org/10.1038/s41586-018-0765-z.
3. Mohiuddin MM, Goerlich CE, Singh AK, et al. Progressive genetic modifications of porcine cardiac xenografts extend survival to 9 months. *Xenotransplantation*. 2022;29(3):e12744. https://doi.org/10.1111/xen.12744.
4. Griffith BP, Goerlich CE, Singh AK, et al. Genetically modified porcine-to-human cardiac xenotransplantation. *N Engl J Med*. 2022;387(1): 35–44. https://doi.org/10.1056/NEJMoa2201422.
5. Griffith BP, Grazioli A, Singh AK, et al. Transplantation of a genetically modified porcine heart into a live human. *Nat Med*. 2025;31(2): 589–598. https://doi.org/10.1038/s41591-024-03429-1.
6. Mohiuddin MM, Singh AK, Scobie L, et al. Graft dysfunction in compassionate use of genetically engineered pig-to-human cardiac xenotransplantation: a case report. *Lancet*. 2023;402(10399):397–410. https://doi.org/10.1016/S0140-6736(23)00775-4.
7. Holcomb RM, Ündar A. Are outcomes in congenital cardiac surgery better than ever? *J Card Surg*. 2022;37(3):656–663. https://doi.org/10.1111/jocs.16225.
8. Morales-Demori R, Montañes E, Erkonen G, Chance M, Anders M, Denfield S. Epidemiology of pediatric heart failure in the USA-a 15-year multi-institutional study. *Pediatr Cardiol*. 2021;42(6):1297–1307. https://doi.org/10.1007/s00246-021-02611-3.
9. Colvin MM, Smith JM, Ahn YS, et al. OPTN/SRTR 2022 annual data report: heart. *Am J Transplant*. 2024;24(2S1):S305–S393. https://doi.org/10.1016/j.ajt.2024.01.016.
10. Dipchand AI. Current state of pediatric cardiac transplantation. *Ann Cardiothorac Surg*. 2018;7(1):31–55. https://doi.org/10.21037/acs.2018.01.07.

11. Peng DM, Davies RR, Simpson KE, et al. Seventh Annual Society of Thoracic Surgeons Pedimacs Report. *Ann Thorac Surg*. 2024;117(4):690–703. https://doi.org/10.1016/j.athoracsur.2023.11.035.
12. Anand RP, Layer JV, Heja D, et al. Design and testing of a humanized porcine donor for xenotransplantation. *Nature*. 2023;622(7982):393–401. https://doi.org/10.1038/s41586-023-06594-4.
13. Mitchell CB, Simmons J, Vo C, et al. Preservation of cardiac xenografts in a model of infant human cardiac transplantation. *Xenotransplantation*. 2025;32(1):e70009. https://doi.org/10.1111/xen.70009.
14. Mitchell CB, Simmons J, Hodo CL, et al. Early results of an infant model of orthotopic cardiac xenotransplantation. *J Heart Lung Transplant*. 2025;44(4):503–510. https://doi.org/10.1016/j.healun.2024.12.011.
15. Byrne GW, McGregor CG. Cardiac xenotransplantation: progress and challenges. *Curr Opin Organ Transplant*. 2012;17(2):148–154. https://doi.org/10.1097/MOT.0b013e3283509120.
16. Ezzelarab MB, Ekser B, Azimzadeh A, et al. Systemic inflammation in xenograft recipients precedes activation of coagulation. *Xenotransplantation*. 2015;22(1):32–47. https://doi.org/10.1111/xen.12133.
17. Iwase H, Liu H, Li T, et al. Therapeutic regulation of systemic inflammation in xenograft recipients. *Xenotransplantation*. 2017;24(2). https://doi.org/10.1111/xen.12296, 10.1111/xen.12296.
18. Wolner L, William-Olsson J, Podesser BK, Zuckermann A, Pilat N. Tolerogenic therapies in cardiac transplantation. *Int J Mol Sci*. 2025;26(9):3968. https://doi.org/10.3390/ijms26093968.
19. Cooper DKC, Habibabady Z, Kinoshita K, Hara H, Pierson RN. The respective relevance of sensitization to alloantigens and xenoantigens in pig organ xenotransplantation. *Hum Immunol*. 2023;84(1):18–26. https://doi.org/10.1016/j.humimm.2022.06.003.