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Xenotransplantation

ABSTRACTS

Oral/Mini-Oral Abstracts

105.1 | Incidence and Management of Proteinuria in a Living Recipient of a 10 Gene-Edit Xenokidney Transplant

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Introduction: The etiology of high-grade proteinuria reported in nonhuman primate xenotransplantation is poorly understood. Significant proteinuria was not reported in the first two renal xenotransplant recipients performed at MGH and NYULH. Here, we report the incidence and management of proteinuria in the living recipient of the longest-surviving renal xenotransplant.

Methods: A 53-year-old Black woman donated a kidney in 1999. In 2004, she developed pregnancy-induced hypertension, eventually leading to hemodialysis-dependent end-stage kidney disease in 2016. The presumed cause of her kidney disease was APOL1-mediated, as genomic analysis revealed two risk alleles. On November 25, 2024, she underwent a 10-gene-edit xenokidney transplant. We monitored urine protein and albumin excretion post-transplant and performed protocol and for-cause biopsies.

Results: The xenograft functioned immediately. Serum creatinine (SCr) decreased from 7.6 mg/dL to 1.0 mg/dL within 72 h. Electron microscopic (EM) examination of a protocol biopsy on POD 9 demonstrated intact podocyte foot processes with no evidence of glomerular basement membrane (GBM) remodeling or electron-dense immune deposits. Within the first 2 weeks, the mean urine protein/creatinine (u p/cr) was 1.3 ± 0.44 g/g. On POD 17, although SCr remained stable at 1.0 g/dL, the u p/cr acutely increased to 12.7 g/g (Figure 1), prompting a biopsy. EM revealed near total foot process effacement (Figure 2). Since a circulating factor or podocyte-specific xeno-antibody-associated focal and segmental glomerulosclerosis could not be ruled out, five sessions of therapeutic plasma exchange (PLEX) were initiated. This resulted in a decrease in u p/cr to 0.9 g/g within 72 h. The u p/cr averaged 0.98 g/g until POD 33, when it acutely increased to 9.9 g/g. PLEX again improved u p/cr to 1.1 g/g. After this, the patient had variable but significant proteinuria (5.8 ± 5.8 g/g), though her graft function remained excellent with a stable SCr of 1.59 ± 0.32 mg/dL. However, on POD 127, she presented with an acute rise in SCr to 10.3 mg/dL in the setting of decreased immunosuppression due to infection. She was diagnosed with acute rejection resulting in xenograft failure. The increase in u p/cr in the final days noted in Figure 1 likely represents a significant overestimate since the urine samples were obtained during oliguric acute kidney injury.

Conclusion: We report episodic nephrotic-range proteinuria in the longest-functioning pig-to-human kidney transplant. Proteinuria was initially responsive to PLEX and did not impede graft function. Further studies are needed to elucidate the etiology of proteinuria, which may include antibody-mediated rejection, immune complex-mediated glomerulonephritis, or podocyte-specific xeno-antibodies. Determining the mechanism of injury to the filtration barrier holds the key to developing targeted therapies aimed at preventing or reversing proteinuria should it occur.

Funding: This data is supported by funding from United Therapeutics Corporation, PBC. Tal Eitan was supported by the National Cancer Institute, National Institutes of Health, through Grant Award Number T32 CA193111.

105.2 | Study Design of the First-in-Human Clinical Trial of a 10 Gene-Edited Xenokidney

Andrew Cameron[1], Robert A. Montgomery[2], John Gill[3], Kristina DeSmet[4], C. Q. Deng[4], Margaret R. Sketch[4], Marc I. Lorber[4], Jayme E. Locke[4], Victoria Lacasse[4], Jeff Sigman[4], Mary Boshkoff[4], Noah Byrd[4], David Ayares[4], Leigh Peterson[4], Allan Kirk[5]

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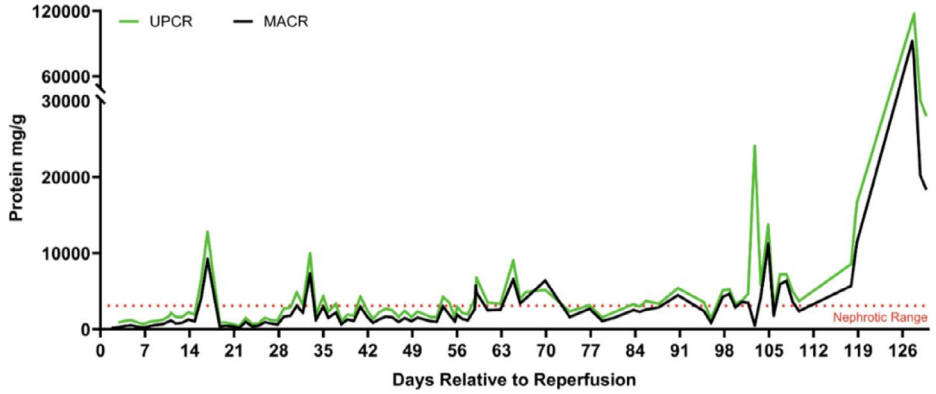


Figure 1: Daily trend of urine protein and albumin excretion and serum creatinine.

FIGURE 1 | 105.1

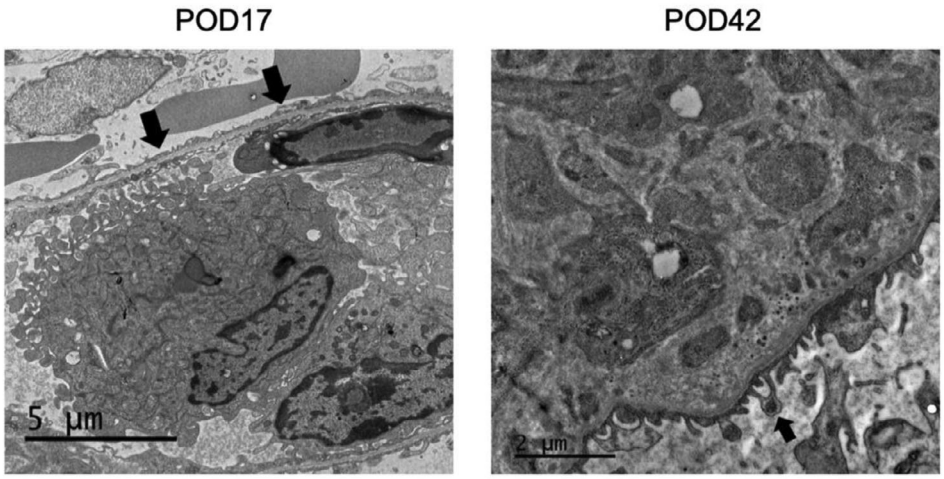


Figure 2: Electron micrographs showed extensive podocyte foot process effacement (black arrows) on POD17 and subsequent improvement on POD42.

FIGURE 2 | 105.1

Purpose: Xenotransplantation is a promising potential solution to address the critical shortage of available organs for transplantation. Here we describe the study design of the first-ever registration-supporting clinical trial of a xeno-organ, the 10GE Xenokidney (UKidney), a xenokidney derived from a 10 gene-edited (10GE) source pig (*GGTA1*KO.*B4GALNT2/B4GALNT2L*KO.*CMAH*KO.*GHR*KO.h*CD46*.h*CD55*.h*THBD*.h*PROCR*.h*CD47*.h*HMOX1*).

Methods: The EXPAND (GPK-KF-101) study (NCT06878560), "A Prospective Study to Evaluate the Safety and Efficacy of the 10GE Xenokidney in Patients with End-stage Renal Disease (ESRD)," is a Phase 1/2/3, multicenter, open-label study initially consisting of a cohort of six participants across two centers, followed by cohort expansion of up to 50 participants. This first-in-human clinical study will use calcineurin-based immunosuppression and aims to assess the safety and efficacy of the 10GE Xenokidney in two groups of participants: ESRD patients who have been assessed and determined to be ineligible for a conventional allogeneic kidney transplant for medical reasons; and ESRD patients who are on a kidney transplant waitlist but are more likely to die or go untransplanted than receive a deceased donor kidney transplant within 5 years. Among other eligibility criteria, participants must be 55 to 70 years of age, have been on hemodialysis for at least 6 months, and be negative xeno-crossmatch by complement-dependent cytotoxicity (CDC). After transplant of the 10GE Xenokidney, participants will be followed for 24 weeks post-transplant, where safety and efficacy endpoints will be evaluated, after which, participants will be followed for their lifetime. Efficacy endpoints include participant survival rate, 10GE Xenokidney survival rate, change in measured glomerular filtration rate, and change in participant quality of life at 24 weeks post-transplant. Overall survival time of participants receiving a 10GE Xenokidney and survival time of the 10GE Xenokidney will also be assessed. Safety endpoints will include incidence of adverse events and serious adverse events, all-cause mortality, and the incidence of proteinuria, zoonotic infection, and opportunistic infection. After the initial cohort of six participants reaches at least 12 weeks post-transplant, safety and efficacy data will be reviewed. If safety and efficacy results are supportive, the study will proceed, and the sample size will be increased to a total of up to 50 participants to enable the study to support registration, with additional transplant centers added to the study.

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Conclusion: The prospectively designed EXPAND clinical trial is a significant step toward supporting xenotransplantation as a therapeutic option for a large population of ESRD patients who are unlikely to receive an allogeneic kidney transplant.

105.3 | Endothelial Injury and Fibrogenesis Drive Post-Transplant Response After Pig-to-Human Heart Xenotransplantation

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Introduction: The recent pig-to-human cardiac xenotransplants (XenoTx) performed at the University of Maryland Medical Center are at the forefront of continued significant advancements in the field of xenotransplantation. The cellular profiles of these XenoTx provide an avenue to better understand molecular perturbations needed to improve post-transplant (post-Tx) xenograft function. Evaluation of the cell-type-specific expression profiles in context of the post-Tx clinical course and strategize ways to overcome the obstacle of antibody-mediated rejection (AMR) needed to advance the field of cardiac xenotransplantation.

Methods: We utilized post-Tx post-mortem wedge biopsies of the heart xenograft explant from each patient (Pt) for single-nuclei transcriptomic (snRNAseq) profiling using the 10X Genomics technology. We assessed quality control and generated raw gene

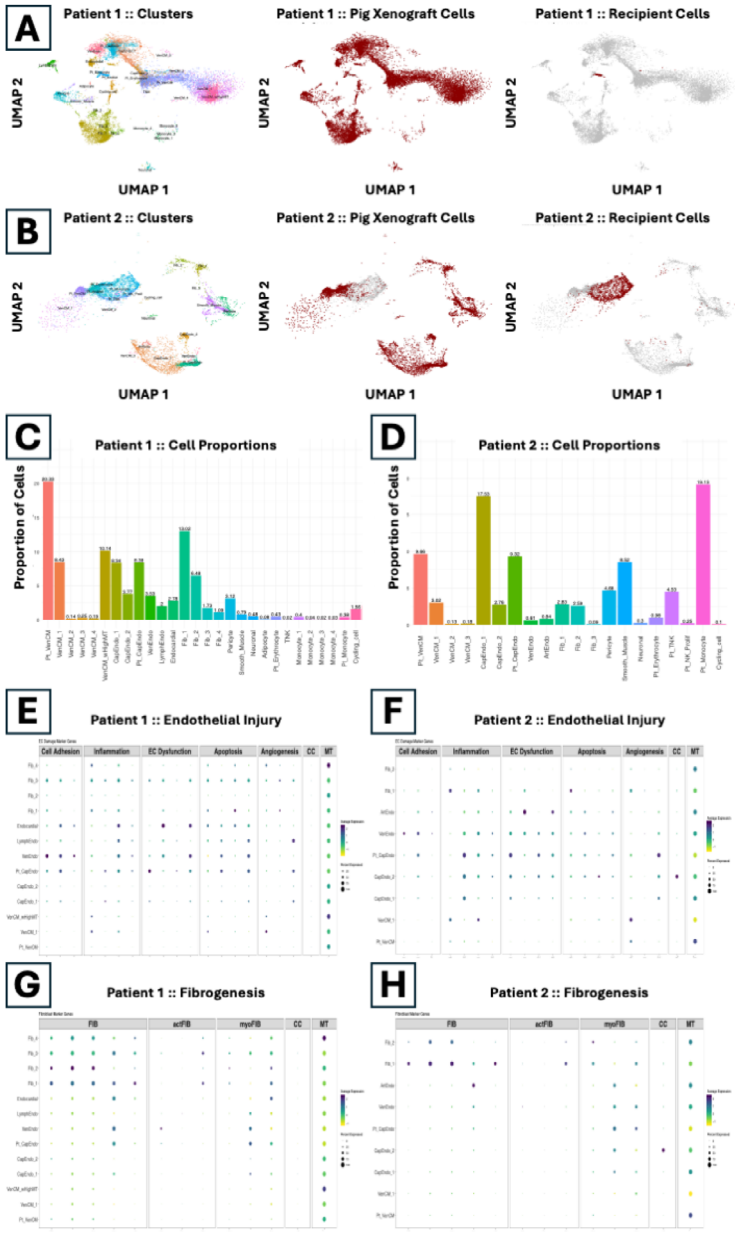


FIGURE 1 | 105.3

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count data using the CellRanger software. We integrated gene expression profiles for each Pt with snRNAseq profiles from native pig heart (nH) and analyzed the data using Seurat. Unsupervised cell clustering was performed using UMAP, and cell-type-specific molecular profiles were computed independently for each Pt.

Results: A total of 58 700 high-quality nuclei (nH: 39 400; Pt 1: 12 600; Pt 2: 6700) were analyzed and categorized into major cardiac cell-types, namely ventricular cardiomyocytes, endothelial, fibroblasts, smooth muscle, and neuronal (Figure 1A,B). We identified less than 1% of both resident pig and human immune cells in Pt 1 (Figure 1C), while Pt 2 showed about 24% of human immune cells (Figure 1D). Endothelial cells in both Pts showed overexpression of marker genes associated with cell adhesion, inflammation, endothelial dysfunction, and apoptosis, indicating increased levels of endothelial injury (Figure 1E,F). Greater than 50% of fibroblasts from both Pts were *PDGFRA*$^{+}$ and *PDGFRB*$^{+}$ compared to fibroblasts from nH that were only *PDGFRA*$^{+}$ (Figure 1G,H). In Pt 2, recipient-derived T and NK cells were enriched for genes associated with TCR signaling, NK-mediated cytotoxicity, and allograft rejection, while recipient-derived monocytes were enriched for genes associated with chemokine signaling, neutrophil degranulation, and allograft rejection.

Conclusions: Overall, we identified heterogeneous cellular compositions in the two XenoTx Pts with increased endothelial injury, increased fibrogenesis, and associated molecular profiles in both Pts. Immune profiles in Pt 2 also provided insights into early AMR and post-Tx immunosurveillance. Overall, our results provide clinicians a better understanding of genomic determinants of post-Tx xenograft function.

105.4 | Complement Monitoring in Cardiac Xenotransplantation: Insights From the Second Porcine-to-Human Live Heart Transplant

Michael Cole[1], Andy Tully[2], Alison Grazioli[2], Javier Galindo[2], Avneesh K. Singh[2], Idris Boudhabhay[3], Erwan Morgand[4], Fariza Mezine[4], Lubka Roumenina[3], Alexandre Loupy[4], Robert Brodsky[1], David Ayares[5], Bartley P. Griffith[2], Muhammad M. Mohiuddin[2]

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Background: Activation of complement is a major barrier in xenotransplantation. We report a detailed monitoring strategy in a 58-year-old man who received a 10-gene-edited porcine heart. Despite expression of human complement regulators CD46 (membrane cofactor protein) and CD55 (decay-accelerating factor) in the xenoheart, early biopsies revealed significant complement deposition, prompting changes in therapeutic approach.

Methods: The initial strategy for the inhibition of complement used the C1 esterase inhibitor (C1INH) to target classical/lectin pathways. After the first endocardial biopsy performed on Day 13, the endomyocardial biopsy (EMB) showed diffuse capillary C3d and C4d deposition, and therapy was switched to a C5 inhibitor, eculizumab. We monitored complement activity via immunohistochemical staining (C3c, C3d, C4d, C5b-9) on biopsies, serum soluble C5b-9 (sMAC) levels via ELISA, and a bioluminescent modified Ham test (bmHam) using HEK293 cells lacking complement regulators (CD46, CD55, CD59). The bmHam assay measured serum-mediated cell lysis and included titrated mixes with normal human serum to gauge complement inhibition functional strength.

Results: EMB (POD13) revealed widespread C3c, C3d, and C4d capillary deposition and endothelial activation, suggesting early innate complement-mediated injury. After initiating eculizumab, upstream complement fragments (C3c, C3d, C4d) remained detectable in biopsies (as expected, given known tissue persistence), but capillary C5b-9 deposition was only focally evident on later biopsy (POD30) (Figure 1). Ex vivo assays indicated that switching from C1INH to eculizumab improved complement control: serum sC5b-9 levels dropped to low levels, and bmHam showed reduced complement-mediated lysis consistent with effective terminal pathway blockade (Figure 2A). In dilutional bmHam tests, patient serum obtained on eculizumab required significantly higher proportions of normal serum to reach 50% cytotoxicity, indicating stronger complement blockade compared to serum on C1INH. Notably, plasma exchange (TPE) and massive transfusion events correlated with transient rises in sC5b-9 and bmHam cytotoxicity, suggesting reduced drug levels and complement "breakthrough" that were promptly identified by these assays (Figure 2B).

Conclusions: Activation of complement contributed to early porcine-to-human cardiac xenograft injury despite transgenic human complement-regulatory transgene expression. A shift from upstream (C1) to terminal (C5) inhibition aligned with a marked reduction in circulating and tissue terminal complement activity. Immunohistochemical detection of C3d/C4d provided evidence of complement activation but could not discriminate ongoing injury due expected persistence of tissue complement deposits. By contrast, real-time assays (sC5b-9 and bmHam) proved valuable for monitoring functional complement activity and guiding therapeutic adjustments.

Funding: This work was supported in part by the National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health (NIH), which helped generate preliminary data for FDA submission (5U19AI090959).

Acknowledgments: We thank L. Faucette and his family for the opportunity to participate in his care, learn alongside his journey, and be inspired by his persistence and sense of humor. We thank Machaon Diagnostics (Mike Ero, Brad Lewis, and Jamey Kain) and their laboratory staff for assistance on the measurement of sC5b-9. We thank the laboratory and clinical staff for their contribution to this case, referred to here as the University of Maryland Cardiac Xenotransplantation Working Group.

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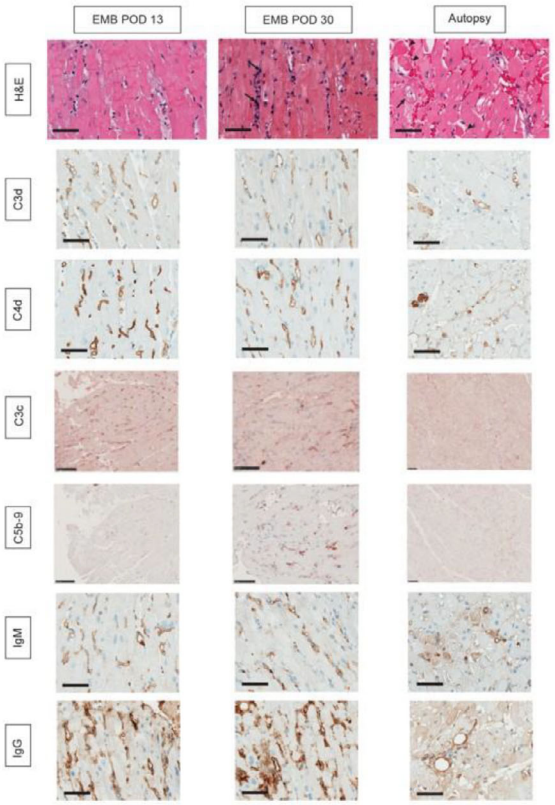


Figure 1. Histopathological examination of xenograft samples obtained from endomyocardial biopsies at post-operative days (POD) 13 and 30, plus autopsy specimens from POD 40. H&E-stained sections from the right ventricle at POD 13 and 30 show endothelial cell apoptosis (marked by arrows), while the left ventricular autopsy specimen (POD 40) exhibits fibrin deposition within necrotic vessels (arrow) and myocyte ischemia (arrowheads). These findings demonstrate worsening microvascular damage and subsequent ischemic changes in cardiomyocytes. POD13 Immunostaining for complement components (C3d, C3c, C4d, C5b-9) and immunoglobulins (IgM, IgG) reveals diffuse, intense linear staining for C3d, C3c and C4d, while C5b-9 was negative at this timepoint. By POD30, C3d, C4d, and C3c staining became heterogeneous and segmental, while C5b-9 staining was intense and linear. At autopsy, all staining (C3d, C3c, C4d, C5b-9) were negative, corresponding with electron microscopy findings of endothelial denuding (data not shown). Light microscopy images taken at 40× magnification (scale bars: 60 μm). H&E, C3d, C4d, IgG, and IgM staining previously presented and adapted from Griffith et al (2024).

FIGURE 1 | 105.4

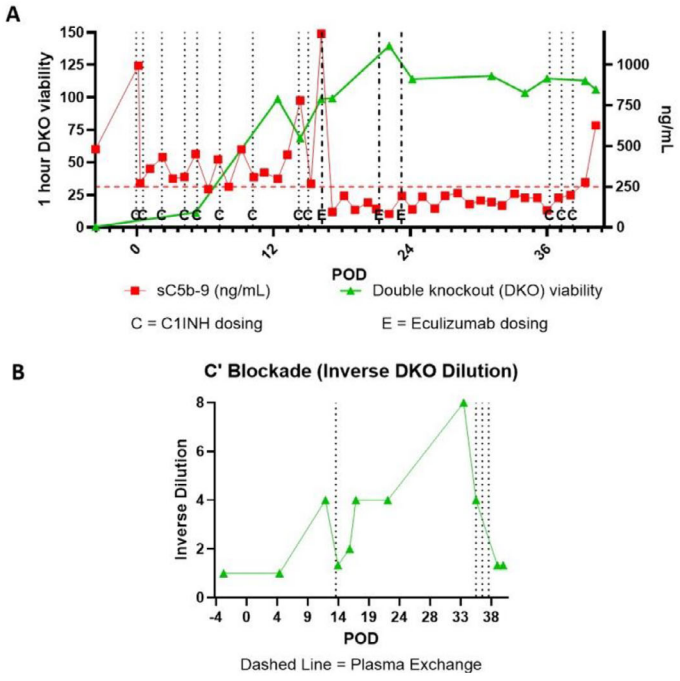


Figure 2. Ex vivo complement activity assays before and after complement inhibition switch. (A) Time-course of soluble C5b-9 levels (sMAC, red line) and bioluminescent mHam assay results (green line) in the patient's serum. The left y-axis shows sC5b-9 in ng/mL, and the right y-axis shows cell viability in the bmHam on DKO cel lline (percentage luminescence relative to heat-inactivated control). While on C1INH, sC5b-9 levels were elevated (300–500 ng/mL) and bmHam viability was low, denoting active complement causing cell lysis. After switching to eculizumab, sC5b-9 promptly fell to near baseline and mHam viability rose to ~90–100%, consistent with effective complement blockade. Transient drops in mHam viability (with corresponding rises in sC5b-9) are seen around POD14 and POD 36/37/38 corresponding to plasma exchange or heavy transfusion events. **(B)** Complement inhibitory titer determination using the bmHam dilution assay. The graph shows inverse of dilution of patient serum with NHS to obtain >50% cell death in the bmHam. Higher inverse dilution corresponds to greater protective effect of the complement inhibitor. Vertical dashed lines indicated plasma exchange.

FIGURE 2 | 105.4

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105.5 | Clinical Correlations With Longitudinal Echocardiography and Left Ventricular Global Longitudinal Strain in the First and Second Genetically Modified Porcine to Human Cardiac Xenotransplantations

Sarah Leventhal[1], Muhammad Mohiuddin[3], Javier Galindo[3], Andrew Tully[3], Sarah Cipriano[1], Marco Oldsman[1], Alison Grazioli[2], Timm Dickfeld[1], Allison Lankford[4], Akash Shah[3], Brian Barr[1], Sadegh Asadi[1], Albert J. Hicks[1], Anuj Gupta[1], Avneesh Singh[3], Erika Feller[5], David Ayares[6], Bartley P. Griffith[3], Susie N. Hong-Zohlman[1], Manjula Ananthram[1]

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Introduction: After heart transplant, left ventricular global longitudinal strain (LV-GLS) is often used with invasive testing such as right heart catheterization to better understand graft health. Cardiac power output-to-mass (CPOM) is a valuable noninvasive prognosticator in patients with LV dysfunction, with higher CPOM correlating with more mechanically efficient ventricles. Our institution performed the first two genetically modified porcine cardiac xenotransplantations in humans. Serial longitudinal transthoracic echocardiograms (TTE) with LV-GLS were performed for both patients to identify evidence of xenograft dysfunction. For both recipients, marked myocardial hypertrophy with worsening LV-GLS was noted at the time of graft failure. It was hypothesized that a ratio of posterior papillary muscle thickness (PPMT) to end diastolic diameter (EDD) would correlate with LV-GLS in this specific clinical situation. It was also hypothesized that cardiac power output (CPO) and CPOM would correlate with LV-GLS, and therefore predict xenotransplant graft failure and mortality.

Methods: Serial TTE measurements obtained included LV-GLS, PPMT, EDD, and parameters for calculation of LV mass by Devereux formula and CPOM (LVOT VTI, LVOT diameter, LV internal diameter end diastole [LVIDd], interventricular septum thickness [IVSd], and left ventricular posterior wall thickness [PWd]). Peason's correlation coefficients between LV-GLS and echocardiographically-derived CPO and CPOM (CO = LVOT area × LVOT VTI × HR; CPO = CO × MAP/451; LVM = 0.8 (1.04 ((LVIDd + IVSd +PWd)^3 − LVIDd^3)) + 0.6; CPOM = (CPO × 100)/LVM) were calculated. Serial echocardiographic measurements and ratios were subsequently compared to endomyocardial biopsies and clinical correlation, including graft failure and death.

Results: For both xenografts, the LV-GLS worsened by an average of 42.3% (CI 42.2%−42.4%) when the PPMT/EDD ratio reached between 0.5 and 0.6, demonstrating a strong positive linear correlation ($r = 0.81$, $p < 0.001$), at which point the xenografts terminally failed due to diastolic and/or systolic dysfunction. For the second xenograft, there was a strong negative correlation between CPO and LV-GLS, both with contrast ($r = -0.80$, $p < 0.001$) and without contrast ($r = -0.96$, $p < 0.01$). There was also a strong negative correlation for the second xenograft between LV-GLS without contrast and CPOM ($r = -0.96$, $p = 0.01$).

Conclusion: Increased ratio of PPMT to EDD, decreased CPO, and decreased CPOM were associated with worsening LV-GLS, cardiac xenotransplant graft failure, and recipient mortality. TTE with LV-GLS provided real-time clinical and noninvasive histological correlation in the first and second cardiac xenotransplants. Such serial noninvasive measurements identifying early graft failure will be crucial for future monitoring and management after xenotransplantation.

Funding: This work was supported by Revivicor, a United Therapeutics Company.

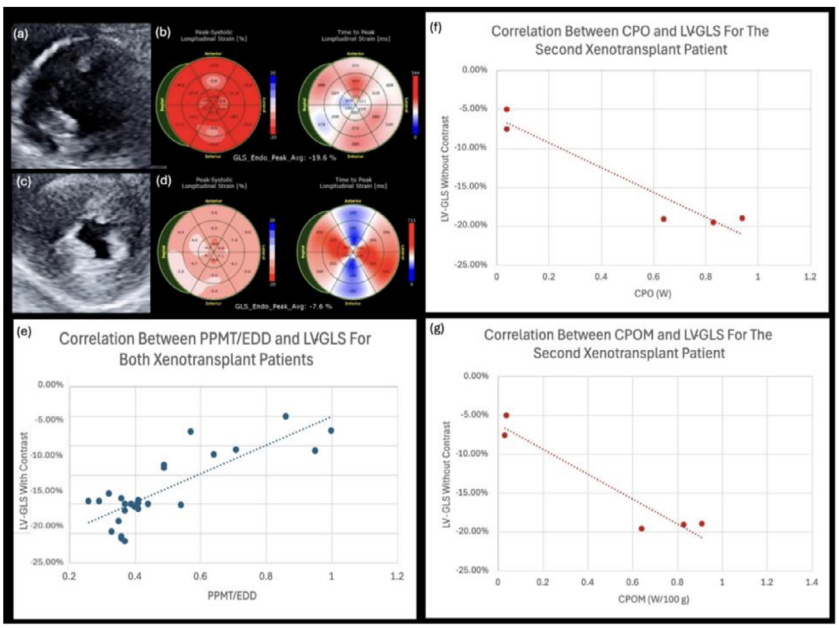


FIGURE 1 | 105.5

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106.1 | The Expression of Human Erythropoietin in Pig Kidneys Can Mitigate the Problem of Anemia After Kidney Transplantation

Yong Wang[1], Jiaxiang Du[1], Hao Feng[2], Man Zhang[2], Gang Chen[2], Shaoping Deng[1], Dengke Pan[1]

[1]Genetic Engineering Department, Chengdu Clonorgan Biotechnology Co., Ltd., Chengdu, People's Republic of China; [2]Institute of Organ Transplantation, Tongji Hospital, Wuhan, People's Republic of China

Introduction: Post-transplant anemia (PTA) is a frequent complication observed in 40% of allogeneic kidney transplant recipients. The erythropoietin (EPO) produced by the kidney is a glycoprotein that stimulates the production of red blood cells; the molecular incompatibilities between pig and human EPO may lead to the aggravation of anemia after porcine to human transplantation. Genetic modification of donor kidneys to overexpress human EPO may offer a novel therapeutic strategy for ameliorating PTA in transplant recipients.

Methods: Based on GTKO/CD55 pigs, the CRISPR/Cas9 system was utilized to insert an expression vector into the COL1A1 locus via homologous recombination, generating transgenic pigs with endothelial cell-specific expression of human EPO. Serum from the pigs was isolated and analyzed by ELISA to quantify human-derived EPO levels. Primary endothelial cells were also isolated from the pigs for expression validation, with detecting human EPO level in the cell culture supernatant. Subsequently, pig kidneys were transplanted into rhesus monkeys to monitor post-transplant hemoglobin levels and comparative assessment of functional maintenance between organ-intrinsic EPO expression and exogenous rhEPO administration.

Results: By genetic editing of GTKO/CD55 pig fibroblasts, six positive cell clones were obtained. Through somatic cell nuclear transfer (SCNT), five piglets expressing human EPO were ultimately generated. Human EPO was not detected in wild pig serum, compared to human serum (6.90 mIU/mL), elevated levels of human-derived EPO were detected in the serum of EPO transgenic pig serum (78.23 mIU/mL). Porcine endothelial cells were isolated, and human EPO could also be detected in the culture supernatant (20 mIU/mL). In pig-to-monkey kidney transplantation experiments, two recipient monkeys transplanted with EPO transgenic pig kidneys could maintain relatively stable hemoglobin content within 3 weeks without the exogenous human EPO injection. However, the hemoglobin of those transplanted with GTKO/βGalKO/CD55/TM kidneys gradually decreased 1 week after transplantation, and exogenous rhEPO treatment was necessary. Furthermore, we observed that EPO-transgenic pigs exhibited significantly higher red blood cell counts and hemoglobin levels compared to normal piglets, demonstrating that human-derived EPO can promote erythropoiesis in pigs.

Conclusion: Donor kidneys with human EPO transgene can produce high-level human-derived EPO in pig serum and have demonstrated the potential in alleviating post-transplant anemia, but data were constrained by the limited groups and short observing time, necessitating further investigation in the future.

106.2 | Genetically Modified Auckland Island Pigs as Organ Source for Xenotransplantation

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Introduction: Successful pig-to-human organ xenotransplantation requires careful matching of donor and recipient organ sizes, elimination of potential porcine pathogen transmission, and appropriate genetic modifications in donor pigs to address cross-species molecular incompatibilities. Although various pig breeds and genetic strategies have been explored, challenges remain. Large breeds risk post-transplant organ overgrowth, while smaller breeds like Yucatan Minipigs show a higher incidence of cardiac abnormalities. Additionally, achieving stable, long-term expression of human transgenes in genetically modified (GM) pigs has proven difficult. Here, we introduce Auckland Island (AUCK) pigs as a naturally superior donor breed for xenotransplantation, alongside a gene-editing strategy designed to ensure optimal transgene expression.

Methods: Male and female AUCK pig kidney cells were imported from New Zealand, and a colony was established at the Center for Innovative Medical Models (CiMM), LMU Munich, via somatic cell nuclear transfer. We evaluated their body size, organ dimensions, growth patterns, cardiac function, and genetic diversity. Building on our consistent success with pig-to-baboon orthotopic heart transplantation using 3GM pigs, our genetic modification strategy for AUCK pigs focused on (a) introducing a minimal essential set of modifications (*GGTA1*-KO/*hCD46*-tg/*hTHBD*-tg) and (b) propagating source pigs through breeding rather than cloning.

Results: Cells used to generate the founder AUCK pigs at CiMM were selected to be free of porcine endogenous retrovirus C (PERV-C), ensuring that the Munich AUCK herd meets FDA regulatory requirements. Morphologically, AUCK pigs have a smaller body stature compared to many domestic breeds, making their organ dimensions well-suited for human transplantation. Echocardiographic assessments confirmed normal cardiac structure and function across various age groups. Single nucleotide polymorphism (SNP) analysis revealed that AUCK pigs exhibit higher runs of homozygosity (ROH) than other domestic breeds, including complete homozygosity across the swine leukocyte antigen (SLA) locus, simplifying potential strategies for addressing SLA-mediated xenograft rejection. To generate GM AUCK pigs, CRISPR-Cas9-based homology-directed repair (HDR) was used for targeted transgene insertion at the *GGTA1* locus, employing a one-step knockin-knockout approach. Two GM AUCK lines were produced: 2GM males carrying *GGTA1*-KO and *hCD46*-tg, and 2GM females carrying *GGTA1*-KO and *hTHBD*-tg. Tissue

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expression analysis confirmed robust CD46 and THBD expression in GM pigs, particularly in endothelial cells. Breeding between these 2GM lines will generate 3GM pigs for preclinical trials in baboons.

Conclusion: Given their favorable physiological features, PERV-C-free status, and robust expression of human transgenes, Munich AUCK pigs might be superior donor animals for preclinical and future clinical studies.

Funding: This work was supported by the Deutsche Forschungsgemeinschaft (CRC-TR 127), the Swiss National Science Foundation (Sinergia grant CRSII5_198577/1), and the Leducq Foundation (23CVD01 "Xenogenic and exogenic pig organs for transplantation into humans").

106.3 | Exploring Primate Immune Responses to SLA-Deficient Pig Xenografts in Humanized Mice and Rhesus Monkeys

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Background: MHCs play essential roles in allogeneic and xenogeneic rejection. In this study, we generated SLA-deficient pigs to mitigate primate anti-pig immune responses.

Methods: SLA-deficient pigs (GBC-4F) were produced by deleting three pig genes (GGTA1, B2M, and CIITA) and expressing four human genes (hCD55, hCD46, hEPCR, and hTFPI). Gene editions were confirmed by flow cytometry and confocal microscopy. Pig artery tissue (PAT) and kidney were transplanted into human immune system (HIS) mice and rhesus monkeys (conditioned with or without anti-CD154 antibody), respectively, to evaluate anti-pig xenogeneic immune responses.

Results: The GBC-4F pigs exhibited deficiency of α-Gal, SLA-I, SLA-II, along with widespread expression of hCD55 and hCD46 and low expression of hEPCR and hTFPI. In the PAT transplantation model, GBC-4F PAT grafts were clearly detectable 5 weeks post-transplantation, whereas the wild-type grafts were rejected by 3 weeks. Human T cells from HIS mice grafted with GBC-4F PAT showed significantly reduced anti-pig responses compared to those from wild-type PAT-grafted HIS mice. In the kidney transplantation model, two monkeys without anti-CD154 antibody survived for 17 and 19 days, showing clear evidence of immune rejection. However, two monkeys treated with anti-CD154 antibody survived 51 and 70 days, with deaths likely due to complications such as pneumonia, renal hydronephrosis, and ureteral obstruction.

Conclusion: These findings suggest that SLA deficiency significantly reduces human anti-pig immune rejection in vivo, and anti-CD154 antibody treatment enhances SLA deficient pig kidney survival in monkeys, highlighting the importance of postoperative care for the long-term survival of xenografts.

106.4 | Study of the Suppression on Innate Immune Induced Rejection by Homo HO-1 and *Sus scrofa* HO-1

Shuji Miyagawa, Fitri Wargadipura, Izzati Rafidah, Naoki Kan, Daiki Yasufuku, Akira Maeda, Akihiro Ike, Kengo Nakahata, Takehisa Ueno, Soichi Matsumura, Yoichi Kakuta, Yusuke Yanagino, Shigeru Miyagawa, Shunsuke Saito.

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Introduction: There are three types of HO: HO-1, -2, and -3, and HO-3 is presumed to be a pseudogene. HO-2 is constitutively expressed at low concentrations, whereas HO-1 is induced by various stresses, including oxidative stress. HO-1 breaks down heme into bilirubin, CO, and Fe2+. CO has anti-inflammatory effects. Therefore, human HO-1 (hHO-1) is thought to be effective for maintaining xenografts and has already been introduced into many gene-edited pigs, but most of the reports so far have been fragmentary analyses. In this study, to compare the functions of hHO-1 and sus scrofa HO-1 (sHO-1) in pig cells, we introduced hHO-1 or sHO-1 genes into swine endothelial cells (SECs) and compared their anti-apoptotic functions against attacks by the human innate immune system.

Methods: First, the sequence differences between the two HO-1 clones were examined. Next, cDNAs of the sas and homozygous HO-1 genes were prepared, linked to the green fluorescent protein (GFP) gene using the 2A system, and cloned into the expression vector pCXN2 (chicken β-actin promoter). These genes were introduced into SECs to establish each clone. The results were also confirmed by flow cytometry. First, the changes in α-Gal expression and overall antigenic changes of each HO-1 clone were examined using GSI-B4 and NHS, respectively. Next, normal human pooled serum (NHS), THP-1 (monocyte/macrophage line), peripheral blood neutrophils, and peripheral blood mononuclear cells (PBMCs) were prepared, and the inhibitory function in the cytotoxic activity of the established HO-1 clones was evaluated in comparison with naive SECs. NHS was used for complement-dependent cell lysis. NK cytotoxicity assays were performed using PBMCs cocultured for 4 h.

Results: The difference in the amino acid sequence between the two was 17.36%, showing high homology. As for clones, sus and homo HO-1 clones were established. In both HO-1 clones, almost no change was observed in α-Gal expression and antigenicity. First, neither HO-1 clone showed a protective effect against human serum, i.e., complement attack. On the other hand, both sHO-1 and hHO-1 showed significantly reduced THP-1-mediated cytotoxicity compared to naive SEC (naive: 67.0 ± 4.8, sus: 53.7 ± 2.2, homo: 51.7 ± 6.1). Next, in the neutrophil-mediated cytotoxicity, both clones were significantly less cytotoxic than naive SECs in the 4-h PMA-treated (naive: 26.5 ± 2.5, sus: 18.0 ± 1.8, homo: 16.7 ± 3.3) and 24-h PMA-free (naive: 42.4 ± 4.5, sus: 26.7 ± 3.8, homo: 19.2 ± 2.5) experiments, but no significant difference was observed between the two. In the NK cell-mediated cytotoxicity, both HO-1 cell lines also suppressed cell death compared to naive SECs.

Conclusions: These results suggest that although neither sHO-1 nor hHO-1 was resistant to complement attack, they were significantly resistant to cytotoxicity by human innate immune cells. We are now investigating the changes in endogenous sHO-1 and both introduced HO-1 mRNA in these assays.

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106.5 | Targeted Insertions of Human Transgenes Combining CRISPR/Cas9 and Cre/lox-RMCE for Generation of Genetic Engineered-Pigs for Xenotransplantation

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Xenotransplantation is a solution to alleviate the shortage of human donors for patients on the waiting list for organ transplantation. Genetic pig engineering is required to generate suitable transgenic pigs as organ donors, together with the development of new clinical immunosuppression protocols. In line with the current state of the art, we modified available αGAL-Neu5Gc-SDA KO pig lines targeting six human transgenes into the porcine GGTA1 gene, combining the CRISPR/Cas9 and Cre/lox-RMCE. To achieve this result, 3 different RMCE plasmids were created for targeting the GGTA1 gene by sequential insertion of: (a) GGTA1-landing pad (lox2272-5171); (b) 1st RMCE cassette: hCD55/CD46/CD59/p-hTM (lox2272-P-5171); (c) 2nd RMCE cassette: hCD47/HMOX1 (lox2272-P). In a single experiment, primary fibroblasts (male; GGTA1/CMAH/B4GalNT2-KO) were transfected (Nucleofector-Lonza; W024) at the same time with the three different RMCE plasmids in combination with: Cas9 protein, 1 sgRNA (GGTA1), and Cre recombinase mRNA. Five days post-transfection (dpt), cells were sorted using magnetic beads (CELLection, PanMouse IgG) and plated ($\vec{\varnothing}$ = 150 mm dish; 150 cells/dish) for clonal selection. Growing colonies ($\vec{\varnothing}\cong$5 mm; 13 dpt) were picked up, split, and expanded for cryopreservation and molecular analysis. Lysed colonies were used for sequential PCR-screenings to confirm: (a) the integration of p-hTM, hCD55/CD46/CD59, hCD47/HMOX1 (6TG); (b) the GGTA1 targeting events (left and right side); (c) 2nd RMCE event; (d) the absence of Prokaryotic backbone integration. Only transgenic colonies positive for the 2nd RMCE event were selected for the phenotypic characterization (ICC and WB) to identify ones to use as nuclear donor for the SCNT experiments. Starting from 165 selected colonies, 135 were lysed, and 45 were transgenic for all the 6 transgenes. GGTA1-targeting events were detected in 10 transgenic colonies; among these, 7 were positive for the Cre-mediated insertion of the 2nd RMCE cassette, and 3 were negative for the prokaryotic backbone integration. Their phenotypes were characterized by WB and ICC. Final WB and ICC analyses confirmed the ubiquitous expression of hCD55/CD46/CD59/CD47/HMOX1(5TGexpr) in 6 colonies, except for p-hTM that is driven by a tissue specific promoter. Based on the molecular and phenotypical findings, two colonies (6TG; GGTA1-targeted; 2nd RMCE positive; 5TGexpr) were selected for a SCNT experiment. The resulting embryos were transferred in a synchronized recipient sow, which was pregnant at 30 days. We demonstrated that transfecting a modular Cre/lox-RMCE system combined with CRISPR/Cas9 it is possible to obtain a targeted integration of six human transgenes in a single experiment; however, random integrations of the transfected cassettes needs to be addressed. This modular platform will permit us to perform new RMCE experiments to quickly integrate additional transgenes in the same locus.

107.1 | The Longest-Living Human With a Xenograft: A Qualitative Narrative Inquiry

Macey L. Levan[1,2], Towana Looney[2], Rhiannon D. Reed[1,2], Carolyn Sidoti[1,2], Brendan Parent[1,2], Ian Jaffe[1,2], Tal Eitan[1,2], Aprajita Mattoo[1,2], Karen Khalil[1,2], Elaina Weldon[1,2], Imad Aljabban[1,2], Jeffrey Stern[1,2], Vasishta Tatapudi[1,2], Adam Griesemer[1,2], Dorry L. Segev[1,2], Robert Montgomery[1,2], Jayme Locke[1,2,3]

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Introduction: Creating an unlimited supply of organs through xenotransplantation (XTx) with gene-edited pig kidneys could obviate the waitlist, and the experience of the first humans is vital to developing comprehensive informed consent and patient-centered outcome metrics. The objective of this study is to document the narrative experience of a prior living kidney donor and the longest-living human to receive a pig kidney transplant.

Methods: This study employed a qualitative single-case narrative inquiry to explore lived experience with a pig kidney transplant through in-depth, semi-structured interviews. Data collection consisted of three interviews with the same recipient conducted in-person, each lasting approximately 1 h in duration. The interview guide included open-ended questions designed to elicit the participant's lived experiences, perceptions, and insights regarding the experience of XTx, including perceived quality of communication, care, and quality of life. Interviews were audio-recorded and transcribed verbatim. Approval was obtained from the IRB, and the patient provided informed consent before participation.

Results: Overall, the patient perceived that living with the XTx for 130 days was successful, even after graft rejection and explant. At 14 days post-surgery, the patient stated, "The xenotransplant has saved my life, has given me a second chance at life. The experience has been remarkable." Three days post-removal of the xenograft, the patient continued to express her gratitude for the transplant and the opportunity for the field to learn from the experience, sharing, "I feel like the education shouldn't stop just because we had a let-down, but I don't want to say failure. I wanna say let-down because you know, we can go back and learn from the failure and replace the let-down." The participant noted that their care team clearly explained the risks, benefits, and alternatives to receiving a xenotransplant prior to surgery and maintained a similar level of communication while they monitored her for evidence of rejection: "When I know you're gonna know and we'll know together, we'll learn together." When discussing quality of life, the patient noted improvements in their psychological, social, and functional well-being post-transplant, including a level of energy she had not previously experienced. Unfortunately, that surge of energy ceased with the explant, but the patient remained positive about her own future and other patients considering XTx, stating, "I see it as the future. It's the only future to me. For people like me."

Conclusion: The unique experience of being the longest living person with a pig kidney can be used to support the development of comprehensive informed consent and patient-oriented metrics for quality of life as we move into clinical trials for XTx.

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107.2 | Toward a Patient-Centered Definition of Success in Kidney Xenotransplantation: A Qualitative Study of Two Long-Term Recipients

Macey Levan[1,2], Rhiannon D. Reed[1,2], Jayme Locke[1,3], Carolyn N. Sidoti[1], Towana Looney[2], Timothy Andrews[4], Dorry L. Segev[1,2], Elaina Weldon[1,2], Brendan Parent[1,2], Jeffery Stern[1,2], Karen Kahili[1,2], Adam Griesemer[1,2], Aprajita Mattoo[1,2], Allan Massie[1,2], Vasishta Tapapudi[1,2], Tatsu Kawai[4,5], Winfred W. Williams[4,5], Harsimar Kaur Ahuja [5], Robert Montgomery[1,2], Leonardo Riella [4,5]

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Background: With Food and Drug Administration (FDA) approval of clinical trials of kidney xenotransplantation (XTx) in living humans, understanding the patient experience is critical for defining success beyond graft and patient survival. We explored patient perspectives by interviewing the two longest-living kidney xenograft recipients.

Methods: We conducted semi-structured interviews on patient-defined success, quality of life (QOL), fears about XTx, and healthcare team communication and support. Interviews were recorded, transcribed, and analyzed by two qualitative researchers using an inductive thematic approach.

Results: Both recipients (53-year-old female; 66-year-old male) described a restoration of hope and return to normal life, contrasting with desperation pre-transplant due to sensitization and waning participation in daily life activities. Both recipients found purpose in supporting the broader kidney disease and transplant community and took comfort in their faith. They emphasized that access to transplantation and graft survival requires mutual confidence and commitment between recipients and healthcare teams, as well as trust despite fear and family concerns. To contextualize findings, we mapped themes onto the Wilson and Cleary model for health-related QOL, a validated framework integrating biological, social, and psychological determinants of patient-reported outcomes.

Conclusions: Reassuringly, these XTx recipients define success and QOL similarly to allotransplant recipients. Their insights provide a framework for guiding patient expectations and informing trial design. Ongoing engagement with current and future recipients will further reinforce the role of XTx in restoring QOL for end-stage patients with limited options.

107.3 | Ethical Pursuit of Expanded Xenotransplantation and Potential for Disability Bias in Pediatric Patients

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Introduction: With the development of xenotransplantation (XTx), relevant stakeholders must be involved to address many of the topics (informed consent, risk-benefit analysis, xenozoonosis, xenograft candidacy) in order to develop policy and practice solutions ethically. For pediatric XTx, ethical frameworks and discussions are not as advanced as for adult XTx. Unique ethical challenges exist in XTx for children, and patients who have been excluded historically due to perceived unfavorable benefit-to-risk or benefit-to-resource use ratio may require a novel assessment. If XTx resolves concerns of organ availability, ethical considerations may further support revisiting criteria for patients previously denied from physiological limitations.

Methods: We review whether XTx in children can ethically be pursued before obtaining safety and efficacy data in adults. Specifically, trial design, trial recruitment, organ growth and harm, and psychosocial and moral hazard issues are explored. Arguments for XTx as bridge-to-allotransplantation or as therapy for those with genetic conditions are examined as future directions for XTx, and disability biases are addressed.

Results: Proactive attention to ethical issues may allow pediatric XTx trials prior to obtaining safety and efficacy data in adults, supporting ethical arguments of justice and beneficence to allow potential benefits. Additionally, when considering any patient that may benefit, a subset is denied based on genetic comorbidities due to the presumption of limited benefit rather than poor outcomes. Ethical justification for denial based on resource limitation will lose strength with XTx, and some disease states and syndromes may no longer qualify as absolute contraindications to transplantation (e.g., T21 patients are being considered for allotransplantation with waitlist/post-transplant outcomes comparable to other patients). Patients with other contraindications may warrant individual consideration rather than automatic disqualification (i.e., trisomy 18). Select patients may benefit from prolonged survival and increased QOL, even if QOL remains limited.

Conclusion: In pediatrics, XTx-specific guidance is required to address minimum regulatory terms for an appropriate benefit-to-risk ratio and candidate identification. Successful pediatric XTx may subsequently drive re-evaluation of contraindications, such as genetic syndromes, driving individual assessment rather than automatic disqualification.

Acknowledgments: The authors would like to thank Dr. César Palacios-González for the thematic discussion of the topic.

107.4 | Public Attitudes Toward Xenotransplantation: A National Survey

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Introduction: Xenotransplantation (XTx) presents a promising clinical alternative to address the current shortage of human organs. Recently, XTx has been performed using decedent models and under expanded access approval as a prelude to human clinical trials. Before such trials can occur, public assessments are crucial to understand potential barriers to acceptance, as recommended by key regulatory bodies. This study aimed to elucidate attitudes toward XTx in the United States and identify characteristics associated with barriers to acceptance.

Methods: A survey assessing demographic characteristics and attitudes toward XTx was distributed to a nationally representative sample of adults ≥18 years old in the United States via the distribution platform, Cint. Multivariable regression analysis was employed to identify characteristics associated with attitudes toward XTx.

Results: From May 25, 2023 to June 14, 2023, a total of 5008 respondents completed the survey. Respondents were evenly distributed across US regions, aged 18–65, 47.6% male, 50.9% female, with 61.7% identifying as White, and half with a college degree or higher. Approximately 25% reported close transplant experiences as recipients or as waitlisted candidates, or were closely connected to a recipient or someone in need of an organ. Overall, <20% indicated they were familiar with XTx, yet >40% of respondents had heard of recent XTx experiments via the media. Approximately 40% expressed reservations about using pigs for human transplants and reported discomfort with the idea of receiving a pig organ for themselves, their child, or a loved one. Notably, 38% reported being more comfortable with a pig organ after learning during the survey that the source pig was genetically modified. If a pig organ were as effective as a human organ transplant, over 50% would opt for the pig organ if it meant a faster transplant. Even in the absence of xenotransplant outcome data, 36% expressed willingness to participate in experimental XTx if in need of an organ. However, 57% rated lack of current evidence of success or fear of rejection/complications as their top concerns. Just over half of survey respondents (54%) were concerned about potential xenozoonosis. Regression models consistently associated being younger, female, or a racial minority with lower acceptance of XTx.

Conclusion: This study is the largest survey to date across the United States that explores public attitudes toward XTx. Despite overall acceptance, concerns persist. Identifying opportunities to increase public acceptance will be key as the field progresses toward human clinical trials and eventual translation to clinical practice.

107.5 | Patient Perspectives of Kidney Xenotransplantation: A Survey by the National Kidney Foundation (NKF)

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Introduction: Transplantation is the best treatment for kidney failure. Approximately 100 000 people are on the US kidney allotransplant (alloTx) waitlist, yet only ~25 000 receive an alloTx annually. Dialysis is an alternative to alloTx yet carries a reduced quality of life (QoL). Kidney xenotransplantation (KXTx) is a promising alternative. The FDA has approved the initiation of first-in-human clinical trials in 2025.

Methods: A 32-item survey was completed by 522 kidney patients recruited via NKF's patient database and social media channels. Respondents were largely female (56%) and aged 50–79, with a range of kidney function and treatment experiences. The survey evaluates motivators for considering a KXTx, consideration of perceived risks, and perspectives on clinical trials. The survey provides a snapshot of patient attitudes toward KXTx given recent developments; it does not encompass all possible risks or scenarios.

Results: *Treatment Satisfaction: Comparison of Dialysis and AlloTx Recipients:* Among those with a functioning alloTx, 81% reported being either very or completely satisfied with their alloTx. Meanwhile, 49% of dialysis patients reported only moderate satisfaction. *Sources of Information:* Patients reported learning about KXTx primarily through news (68%) and social (30%) media. However, 49% prefer to receive KXTx information from medical professionals. *Support:* Key motivators for considering a KXTx included avoiding time on dialysis (55%), improving QoL (46%), and reducing time on the alloTx waitlist (41%). Advancement of science was also cited (27%). Overall, patients were strongly (46%) or somewhat (33%) supportive of KXTx as a treatment option. *Risk Perceptions:* Zoonotic infection was the most concerning risk; 36% reported they would not likely accept a KXTx due to this. Perceptions of other risks—such as catastrophic events, graft rejection, and potential ineligibility for future alloTx—were mixed, with responses split across levels of concern. *Clinical Trials:* Patients expressed mild concern about potential clinical trial monitoring requirements. Weekly monitoring was the most acceptable (32%), followed by inpatient (23%) and daily (20%) monitoring. Urgency of need for alloTx was the leading factor influencing willingness to enroll (55%). Twenty-five percent reported being somewhat likely and 24% moderately likely to accept a KXTx. Support for KXTx as a treatment option remained high (46% strongly; 32% somewhat supportive).

Conclusion: Patients view KXTx as a promising alternative to alloTx, with strong overall support despite concerns. Zoonotic infection was a key concern, while rejection and monitoring were less so. Negative feelings about dialysis may contribute to a patient's decision to accept a KXTx. Urgency was the most significant factor in deciding on clinical trial participation. These findings offer valuable insights into patient priorities and may inform trial design, risk communication, and education strategies.

Acknowledgments: The survey was disseminated via the National Kidney Foundation's patient database and social media channels.

110.1 | Differential Pig and Human Protein Abundance in Plasma of Patients Exposed to Pig Hepatocytes Following Bioartificial Liver Treatments

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Introduction: Recent advances in pig-to-human xenotransplantation using genetically modified pigs underline the significant clinical potential. Bioartificial liver (BAL) therapy containing

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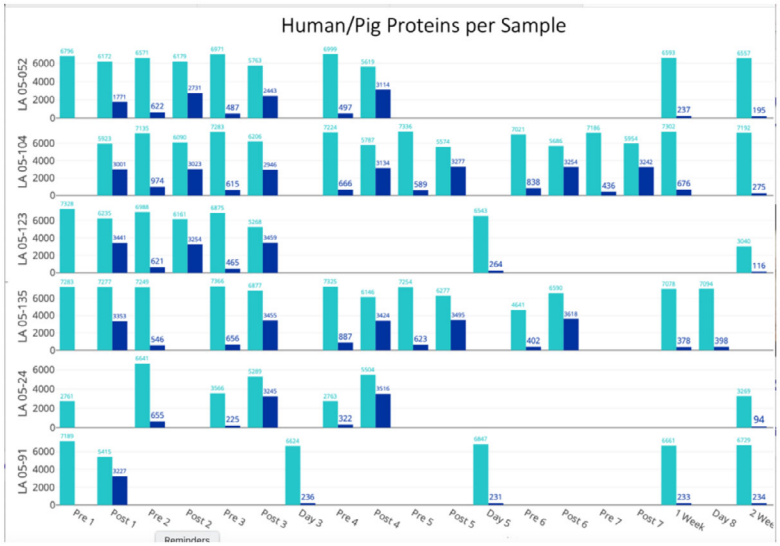


FIGURE 1 | 110.1

wild-type (wt) pig hepatocytes offers a unique clinical model to investigate the xenogeneic immune response in humans. Understanding the dynamics of human and pig protein abundance following BAL treatments is critical for optimizing future pig-to-human xenotransplant strategies.

Objective: Characterize pig and human-specific protein abundance in plasma of patients exposed to pig hepatocytes during BAL treatment, using nanoparticle-based enrichment technology and quantitative mass spectrometry.

Methods: Six patients who received 1 to 7 BAL treatments were included. Each BAL treatment consisted of 6 h of plasma perfusion through a cartridge containing wt pig hepatocytes. For patients receiving multiple treatments, the BAL was repeated within 24 h. Plasma samples were collected pre-treatment ("Pre") and serially for 2 weeks after the BAL treatments. Additionally, plasma samples were obtained from the BAL tubing as plasma returned to the patients after completion of the BAL treatment ("Post"). Controls included normal human and wt pig plasma. A total of 80 plasma samples underwent high-throughput proteomic analysis using the Seer Proteograph Assay (nanoparticle-based enrichment) and quantitative mass spectrometry to identify and quantify human and pig plasma proteins across all time points.

Results: Quantitative mass spectrometry analysis (DIA-NN v1.8.1) using human and pig UniProt databases, identified 159 601 unique peptides at 1% FDR, including 65 093 human-specific and 25 539 pig-specific peptides. These species-specific peptides were reconstructed into 8744 human and 4225 pig protein groups. The graphic shows temporal trends in human (light blue) and pig (dark blue) plasma proteins at different time points. Baseline analysis of the human protein before pig hepatocyte exposure showed inter-individual variability in human protein abundance, indicative of heterogeneous pre-existing immune status. Immediately following BAL treatment ("Post"), an average of 3157 ± 415 pig proteins were detected in the plasma returning to the patient, confirming the significant transfer of pig proteins into patient circulation during BAL treatments. Within 24 h after the last BAL and before the subsequent BAL treatment ("Pre"), pig protein levels markedly decreased to an average of 581 ± 175, suggesting rapid clearance and/or immune-mediated degradation of the xenogeneic proteins.

Conclusions: This study provides the first characterization of the human plasma proteome following exposure to pig hepatocytes during BAL therapy. Pig proteins were transferred to patients during BAL treatment, followed by rapid pig protein decline within 24 h, suggesting efficient clearance and early immune processing, key insights for advancing pig-to-human xenotransplantation strategies.

110.2 | Screening Human Blood Products for Xeno-Reactive Antibodies to Ensure Compatibility in Clinical Xenotransplantation

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Introduction: Clinical xenotransplantation is now a reality, with advances in genetic engineering and xeno-immunobiology enabling FDA clearance of a first-in-human kidney xenotransplantation multi-center trial, including Johns Hopkins. Blood-derived products such as plasma, intravenous immunoglobulin (IVIG), and red blood cells (RBCs) are frequently administered to transplant recipients and may harbor xeno-antibodies capable of triggering complement activation and graft injury. Robust pre-transfusion screening strategies are needed to ensure compatibility of blood products with xeno-organs.

Methods: Ninety plasma units and 24 RBC units were obtained from the local blood supplier, along with 8 IVIG lots (Gamunex and Gammaguard). Samples were incubated with porcine peripheral blood mononuclear cells (PBMCs) from GGTA1 knockout (KO), multi-KO (GGTA1, CMAH, B4GALNT2; for CDC), and

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multi-KO + human transgenes (txg; for antibody binding assay) pigs. IgM and IgG binding and CDC were quantified by flow cytometry as median fluorescence intensity (MFI) and % cell death, background-subtracted and analyzed using Wilcoxon signed-rank test. IgM MFIs against GGTA1 KO cells were indexed to a control, and ROC analysis with Youden index was used to identify a cutoff predictive of CDC ≥20%.

Results: IgM and IgG binding were significantly lower in multi-KO/txg cells versus GGTA1 KO ($p < 0.0001$). CDC showed a similar trend, with greater lysis against GGTA1 KO targets ($p = 0.0004$). IgM binding to GGTA1 KO strongly correlated with CDC ($R = 0.65$, $p < 0.0001$), and an IgM index cutoff of 1.52 predicted CDC ≥20% with high sensitivity/specificity. IVIG lots showed uniformly low IgM binding to all targets but higher IgG binding to GGTA1 KO than to multi-KO/txg. RBCs showed low IgM but high IgG binding to GGTA1 KO, indicating potential to deliver xeno-reactive antibodies despite being considered serologically inert.

Conclusions: Using a 20% cell lysis threshold for CDC, 4% of plasma samples would be excluded for multi-KO/txg compared to 23% for GGTA1. Given the strong correlation with CDC, IgM index-based screening may help preselect compatible products. Multi-KO/txg cells showed reduced immunoreactivity compared to GGTA1 KO across a large panel of blood products. However, despite overall reduced reactivity, outliers with high immunoreactivity remain a concern even in more extensively genetically modified strategies, underscoring the importance of blood product screening in clinical xenotransplantation.

110.3 | Monitoring Donor-Derived Cell Free DNA in Porcine Heart Xenotransplant Recipients

Alesha Luxon[1], Muhammad M. Mohiuddin[2], Bartley Griffith[2], Avneesh Singh[2], Sarah Cipriano[2], David L. Ayares[3], Kimberly M. Clark-Langone[1], Dennis Fu[1], Natalie Gulbahce[1], Evgeniya Vaskova[1], Robert N. Woodward[1], John Morlan[1]

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Introduction: With recent advances in gene editing technology came the first heart transplants from genetically modified pigs into humans. While the recipients only survived for weeks post-transplant, similar technologies will undoubtedly be used in future transplant patients. As xenotransplantation becomes more commonplace, the need for non-invasive diagnostic tools will also grow. Here we describe the development of 2 assays for monitoring donor-derived cell-free DNA (dd-cfDNA) in the world's first xenotransplant patients to receive a heart from a genetically modified pig.

Method: Blood was collected in Streck BCT tubes approximately weekly after transplant. cfDNA was extracted using the QIAamp Circulating Nucleic Acid Kit and quantified by Qubit. Firstly, shotgun sequencing was performed on the Illumina NextSeq or MiSeq, and data were analyzed using an in-house bioinformatics pipeline. Secondly, digital PCR (dPCR) assays were designed to specifically detect exclusively either human or porcine sequences and genome copies of each species measured on the Stilla Naica system. In both cases, percentage of porcine dd-cfDNA was determined as well as the recipient cfDNA.

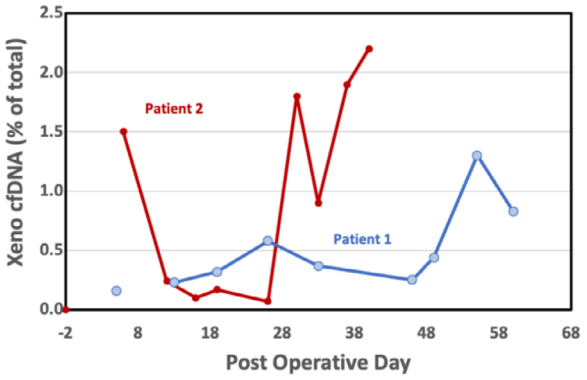


FIGURE 1 | 110.3

Results: Both cPCR and shotgun sequencing assays demonstrated practically zero dd-cfDNA signal in blank samples and showed good linearity with contrived samples. Patient dd-cfDNA results from shotgn and dPCR showed a very similar trend over time. dPCR results illustrated 0.12% dd-cfDNA at Day 6 post-transplant, rising to 0.79% by Day 55 in Patient 1. Patient 2 had a high % dd-cfDNA initially that dropped but spiked again at the time of a major clinical event. This patient also had very high recipient cfDNA levels beginning at post-operative Day 30.

Conclusion: Both shotgun sequencing and dPCR were successfully developed to monitor the world's first Xenotransplant patients to receive a heart from a genetically modified pig. dPCR provides a cheap and rapid result, but accurate quantitation may be hindered by limited porcine genome copies. On the other hand, shotgun sequencing is more expensive but has the potential to reach lower limits of quantitation due to the larger number of targets being measured.

110.4 | Real-Time Intraoperative Quantification of Xenograft Blood Flow Into a Porcine Xenothymokidney in a Living Human With a Left Ventricular Assist Device

Jacqueline Kim[1], Imad Aljabban[1], Aprajita Mattoo[1], Vasishta Tatapudi[1], Edward Y. Skolnik[2], Ian S. Jaffe[1], Karen S. Khalil[1], Molly S. Ratner[1], Elaina P. Weldon[1], David Ayares[3], Bernard S. Kadosh[4], Randal I. Goldberg[4], Tajinderpal Saraon[4], Alex Reyentovich[4], Deane E. Smith[5], Jeffrey M. Stern[1], Adam Griesemer[1], Robert A. Montgomery[1], Nader Moazami[5]

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Introduction: Patients with concomitant end-stage heart and renal disease are not considered for left ventricular assist device (LVAD) implantation as destination therapy due to high mortality rates. Therefore, the fluid dynamics of blood flow through a newly transplanted renal graft, let alone a porcine xenograft, in the setting of a LVAD have not been investigated. In this study, we quantify intra-operative real-time xenograft blood flow through a newly transplanted xenothymokidney renal artery in a living recipient with an LVAD.

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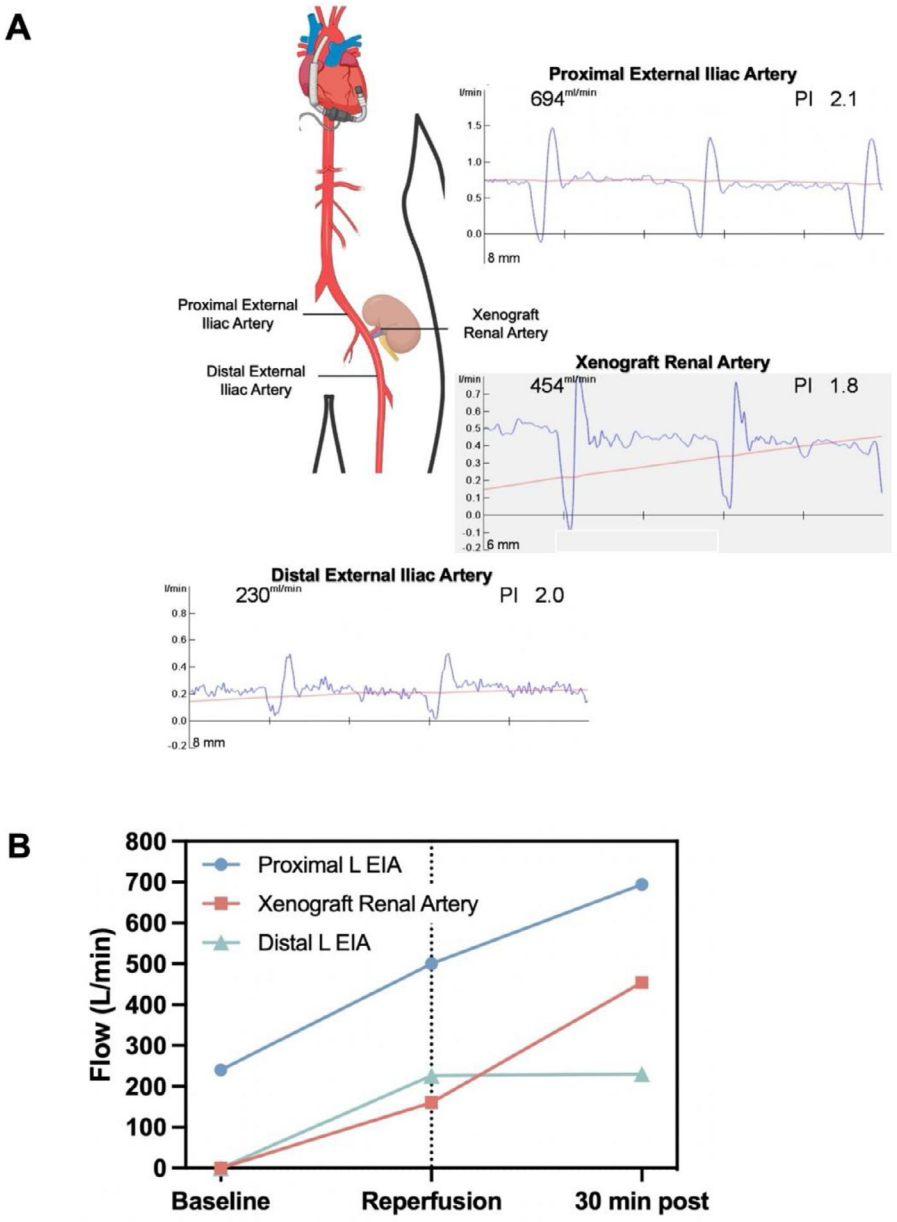


FIGURE 1 | 110.4

Methods: One week after implantation of a continuous flow mechanical circulatory device (Heartmate 3 LVAD), a 200.0-g alpha-Gal knockout xenothymokidney was transplanted into a 170-cm female living human recipient. Intra-operatively, transit time flow measurement was utilized to quantify real-time volume flow through the newly transplanted porcine renal artery. LVAD pump speed was set to 4800 rpm peri-operatively, and systemic hemodynamics were measured continuously during the xeno-transplantation. Approval for this procedure was obtained from the FDA under a single-patient expanded access Investigational New Drug application and the New York University Grossman School of Medicine Institutional Review Board.

Results: Baseline blood flow through the recipient's proximal left external iliac artery (L EIA) just prior to xenograft implantation was 240 mL/min, with a systemic mean arterial pressure (MAP) of 76 mmHg and an estimated systemic flow of 3.4 L/min. Immediately after xenograft reperfusion, proximal L EIA blood flow increased to 500 mL/min (2.1-fold), with 160 mL/min xenograft renal artery blood flow (4.7% of systemic blood flow) and 226 mL/min distal L EIA blood flow. Thirty minutes after xenograft reperfusion, with systemic MAP 73 mmHg and estimated systemic flow of 3.6 L/min, proximal L EIA blood flow further increased to 694 mL/min (2.9-fold), and xenograft renal artery blood flow increased to 454 mL/min (2.8-fold from reperfusion, 12.6% of systemic flow). Distal L EIA flow remained grossly unchanged at 230 mL/min.

Discussion: In this single-patient study of intraoperative xenograft kidney blood flow dynamics, we observed rapid adaptations in real-time blood flow through a newly transplanted xenokidney renal artery, suggesting that the porcine xenograft exhibits brisk compensatory physiologic adaptations in a human recipient even with an LVAD.

Funding: This work was supported by United Therapeutics, PBC.

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110.5 | Correlation of Left Ventricular Global Longitudinal Strain With Porcine Cell-Free DNA in the First and Second Genetically Modified Porcine to Human Xeno Heart Transplant

Javier Galindo[1], Muhammad M. Mohiuddin[1], Andy A. Tully[1], Alison Grazioli[1], Susie Hong-Zohlman[1], Brian Barr[1], Timm Dickfeld[1], Albert Hicks[1], Mohammed Asadi[1], Anuj Gupta[1], Erika Feller[3], Avneesh K. Singh[1], Dave Ayares[2], Bartley Griffith[1], Manjula Ananthram[1]

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Background: Our institution performed two genetically modified porcine-to-human cardiac xenotransplants (CXT). Patient 1 (P1) & Patient 2 (P2) were declined for allotransplantation & mechanical circulatory support at multiple centers. Left ventricular global longitudinal strain (GLS) is predictive of major adverse cardiac events & all-cause mortality in cardiac allograft recipients. We observed progressive declines in GLS in both CXTs. We hypothesized that GLS would correlate with biochemical markers of xenograft dysfunction (XGD), specifically, xenograft-derived porcine cell free DNA (xcfDNA). We performed a retrospective analysis to address this.

Methods: Serial transthoracic echocardiography (TTE) was performed & cfDNA measurements were obtained (CareDx Inc., Brisbane, CA, USA). TTE was analyzed to calculate GLS. Simple univariate linear regression was performed & Pearson correlation coefficient was calculated using GraphPad Prism 10 (Dotmatics, Boston, MA).

Results: GLS declined to about −10% & xcfDNA rose from negligible to a pre-mortem peak for P1 & P2. Regression analysis showed a modest, non-significant association in P1 &. Pearson correlation coefficient was 0.42 for P1 & 0.66 for P2.

Conclusion: Our results show a modest, non-significant correlation between GLS & xcfDNA in the CXT. A better correlation between GLS & xcfDNA was expected, however the infrequent measurement of xcfDNA is a limiting factor. Both GLS & cfDNA are important in assessing XGD.

110.6 | Pig Kidney Xenograft Size After Pig-to-Human Transplantation – GGTA1 KO Thymokidney Vs. 10 Ge Xenokidney

Tal Eitan[1], Imad Aljabban[1], Jacqueline Kim[1], Ian Jaffe[1], Aprajita Mattoo[1], Vasishta Tatapudi[1], David Ayares[2], Elaina Weldon[1], Karen Khalil[1], Jayme Locke[2], Adam Griesemer[1], Robert A. Montgomery[1], Jeffrey Stern[1]

[1]NYU Langone Transplant Institute, New York, New York, USA; [2]United Therapeutics Corp., Durham, North Carolina, USA.

Introduction: As xenotransplantation studies in humans evolve in both decedents and living recipients, different genetic edits and immunosuppressive regimens are being optimized to overcome immunologic barriers. We compared post-transplant pig

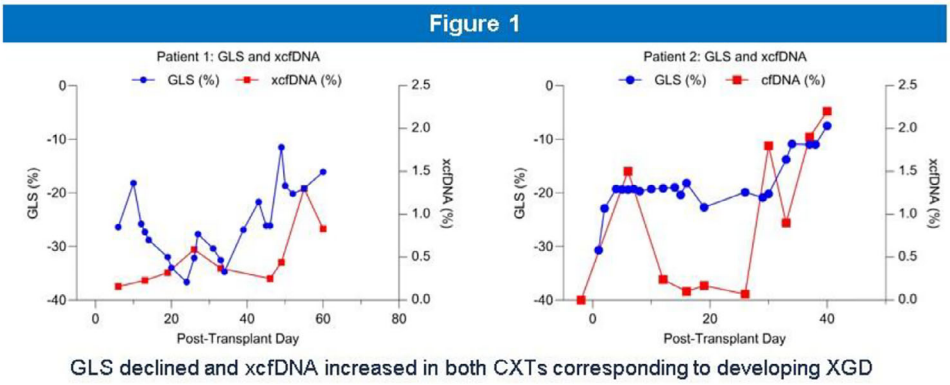


FIGURE 1 | 110.5

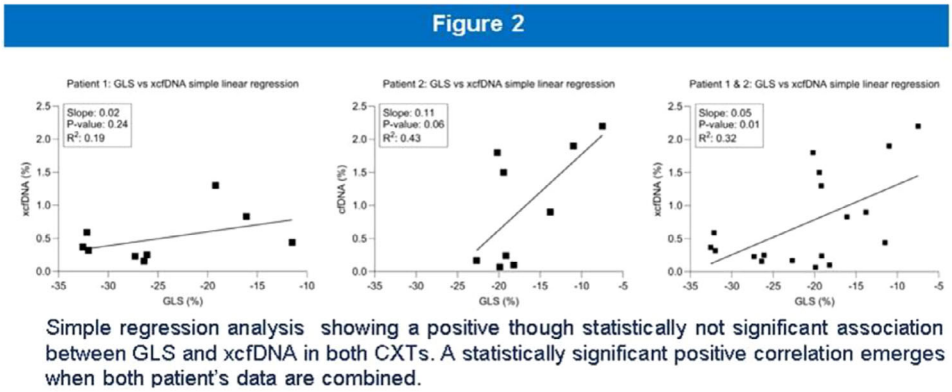


FIGURE 2 | 110.5

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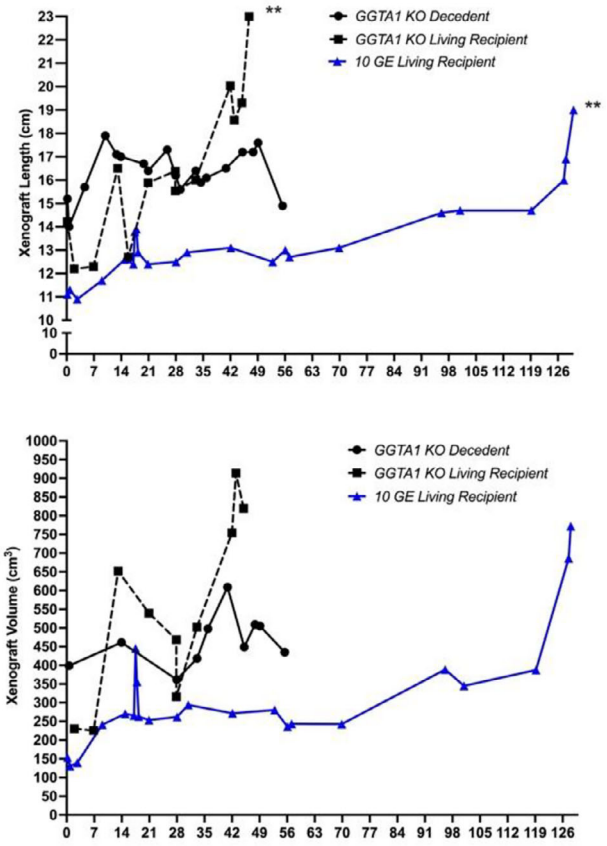


Figure 1. A). Pig kidney xenograft length by US after transplantation between *GGTA1 KO Decedent*, *GGTA1 KO Living Recipient,* and *10 GE Living Recipient*. Final lengths (**) of both living recipient grafts measured grossly on explant not by US. B). Xenograft volume by US after transplantation between *GGTA1 KO* and *10 GE*.

FIGURE 1 | 110.6

xenograft sizes of *GGTA1* KO Thymokidneys, in a decedent and living recipient, and a 10GE Xenokidney in a living recipient.

Methods: A 57-year-old male brain-dead human decedent underwent bilateral nephrectomy and xenotransplant of a 203 g *GGTA1* KO Thymokidney. The source pig was an 80 kg, 6.6-month-old landrace swine. Postoperatively, this decedent was somatically supported for 61 days before elective termination. A 54-year-old female living human with multiple co-morbidities, including end-stage heart and kidney disease, was determined not to be a heart-kidney transplant candidate and underwent xenotransplant with a 268 g *GGTA1* KO Thymokidney 8 days after implantation of a HeartMate 3 LVAD. The source pig was a 200 kg, 11-month-old landrace swine. A highly sensitized living human underwent transplant of a 240 g 10GE Xenokidney, including knockouts of 3 known immunogenic carbohydrates and the porcine growth hormone receptor and human transgene expression of proteins responsible for inhibiting human complement and coagulation pathways. The source pig was a 99.3 kg, 15-month-old landrace swine. Ultrasound (US) measurements were collected during per-protocol and for-cause exams using Seimens Acuson S3000. A combination of direct volumetric scanning and calculation of length, width, and depth from US exams was used.

Results: In the *GGTA1* KO Thymokidney in the decedent, by POD 10, graft length increased by 2.7 cm (Figure 1A). By POD 14, graft volume increased by 60 cm^3. Volume increased by 150 cm^3 during an episode of rejection that began on POD 33. Volume decreased by 320 cm^3 with treatment. In the *GGTA1* KO Thymokidney in the living recipient, by POD 13, graft length increased by 4.3 cm and volume increased by 420 cm^3. From POD 33 to 45, renal function declined without evidence of immunologic rejection, and on POD 45, volume had increased by 167 cm^3 and length had increased by 6.5 cm versus POD 13. In the 10GE Xenokidney, by POD 9, graft length increased by 0.6 cm and volume increased by 110.7 cm^3. During an episode of rejection that started on POD 17, volume increased by 53.3 cm^3 , and length increased by 2.1 cm. By POD 57, volume returned to baseline with treatment (Figure 1B). A second episode of rejection in the last week of the study yielded a volume increase of 530 cm^3 and a length increase of 7 cm.

Conclusion: All three xenografts experienced expected early size increases, likely due to hyperfiltration. None of the xenografts had clinically significant volume changes. Volume changes may be more suggestive of immune factors than dysregulated growth, as volumes decreased after rejection treatment. The volume increase in the *GGTA1* KO Thymokidney in the living recipient was likely related to edema from hemorrhagic necrosis.

Funding: Work funded in part by Lung Biotechnology, a wholly owned subsidiary of United Therapeutics Corporation. Tal Eitan was supported by the National Cancer Institute, National Institutes of Health, through Grant Award Number T32 CA193111.

110.7 | How Many Potential Candidates Are There for Clinical Kidney Xenotransplantation in Japan?

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Urology, Hokkaido University Hospital, Sapporo, Japan

Purpose: Kidney transplantation is the only curative therapy for end-stage kidney disease, but a critical donor organ shortage means many appropriate candidates never receive transplantation. Novel gene editing tools have enabled advanced genome engineering of porcine donors, and a few clinical cases have undergone transplantation from gene-edited pigs by compassionate use. Such as those recipients, candidates for xenotransplantation should be somewhat old and most unlikely to receive allotransplantation due to high levels of anti-HLA antibodies. In Japan, the average waiting period for deceased kidney transplantation has lengthened to 16 years. Due to an exceedingly long waiting time, so many patients have declined registration for deceased donor kidney transplantation in Japan. This study was aimed at assessing the number of xenotransplant candidates in Japan.

Methods: Two hundred fifty-three patients who presented to our facility from 2016 to 2023 were enrolled in this study. Exclusion criteria were age under 18 years, indications for simultaneous liver or pancreas transplantation, or those who did not show up before a clinical decision was made. The number of patients aged 55–65 years old who are supposed to be potential candidates for xenotransplantation was evaluated out of the patients who were ineligible for living kidney transplantation.

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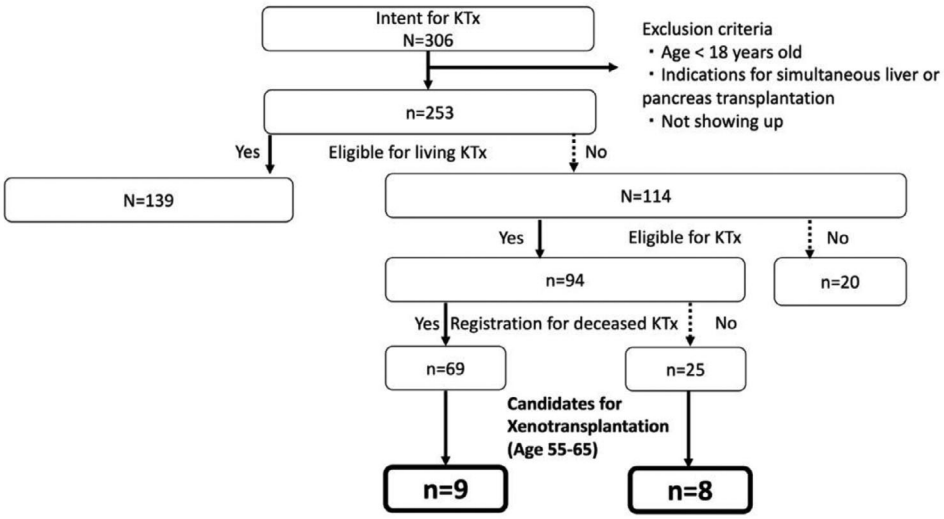


FIGURE 1 | 110.7

Results: Out of 253 patients, 150 were male and 103 were female. The median age was 50 (18–76) years old. The number of those who are eligible for living kidney transplantation (already done or waiting) was 139 (Figure 1). Among the remaining 114 without indication for living kidney transplantation, 94 were found eligible for transplantation, while the other 20 had to abandon transplantation because of their own medical problems. Out of the 94, 69 registered for the waiting list, but the remaining 25 did not. Those who were 55–65 years old were 9 out of 69 (13.0%) in the patients with registration, while 8 out of 25 (32.0%) were in those without registration.

Conclusions: The study suggested that there is a substantial number of candidates for kidney xenotransplantation among those who were ineligible for living kidney transplantation in Japan. The need for clinical application of xenotransplantation is tremendously high in Japan due to an extremely long waiting time.

111.1 | Sequential Genome Editing Enhances Triple-Knockout Efficiency and Human Transgene Expression in Porcine Fibroblasts for Xenotransplantation

Micaela Navarro[1,2], Carolina Alvarez[2], Bianca Estefania Devia[2], Marcelo Perez-Pepe[2], Yasemin Sezgin[3], Genesis Snyder[3], Adrián Abalovich[2], Meisam Naeimi Kararoudi[3], Adrián Mutto[1,2]

[1]Laboratorio de Biotecnologías Aplicadas a la Reproducción Animal, Instituto de Investigaciones Biotecnológicas "Dr. Rodolfo Ugalde", Buenos Aires, Argentina; [2]CrofaBiotech SA, Buenos Aires, Argentina; [3]Center for Childhood Cancer and Blood Disease, Abigail Wexner Research Institute, Nationwide Children's Hospital, Columbus, Ohio, USA

Xenotransplantation using genetically modified pigs offers a promising alternative to solve the global organ shortage. Advances in gene editing have improved the safety and feasibility of pig-to-human transplants by overcoming key immunological and viral barriers. Notably, the triple knock-out (3-KO) of the main genes involved in hyperacute rejection -GGTA1, CMAH, and B4GalNT2- has significantly increased compatibility. In this study, we evaluated which strategy is the most efficient for generating 3-KO pig fibroblasts. Dermal fibroblasts from PERV negative and mycoplasma-free pigs were edited using the CRISPR/Cas9 tool. Two gene-editing strategies were tested: all-at-once editing (AE) and sequential editing (SE). KOs were confirmed by sequencing, and protein expression was assessed by flow cytometry. Next, the 3-KO fibroblasts were transduced with lentiviruses encoding human genes involved in immune modulation and coagulation -CD39, CD46, CD47, CD55, and thrombomodulin (THBD). Successful insertions were validated by flow cytometry, detecting human protein expression. Thereafter, edited fibroblasts were clonally isolated and used as donors for somatic cell nuclear transfer (SCNT). Finally, resulting blastocysts were transferred laparoscopically into synchronized surrogates, with ~150 embryos transferred/swine. As a result, ICE analysis revealed that the SE achieved higher KO-Scores for GGTA1, B4GalNT2, and CMAH (92%, 93%, and 87%, respectively) compared to AE (91%, 47%, and 50%, respectively). These findings were consistent with protein expression levels, where SE resulted in lower residual activity of all three proteins compared to the AE. Subsequently, 3-KO fibroblasts generated by SE or AE were transduced with lentiviral particles encoding the previously mentioned human genes. We observed that AE-derived 3-KO fibroblasts expressed lower levels of CD39, CD46, CD47, CD55, and THBD (39%, 40%, 77%, 74%, and 17%, respectively) than SE-derived cells (60%, 72%, 94%, 92%, and 49%, respectively). Due to regulatory considerations, subsequent experiments were performed using the SE-derived 3-KO fibroblasts. Clonal isolation was performed, resulting in 12/30 clones, of which 8/12 carried heterozygous mutations and 4/12 displayed homozygous mutations. One of the homozygous clones was selected as a nuclear donor for SCNT and resulted in 17% of Day 7—in vitro blastocyst development, compared to 30% when using wild-type donor cells ($p < 0.05$, ANOVA). The resulting blastocysts were successfully implanted and established pregnancies, which are ongoing to date. In summary, our findings demonstrate that the sequential editing strategy is more efficient for generating porcine 3-KO fibroblasts compared to all-at-once. This approach also enhanced the expression of human transgenes and supported successful SCNT

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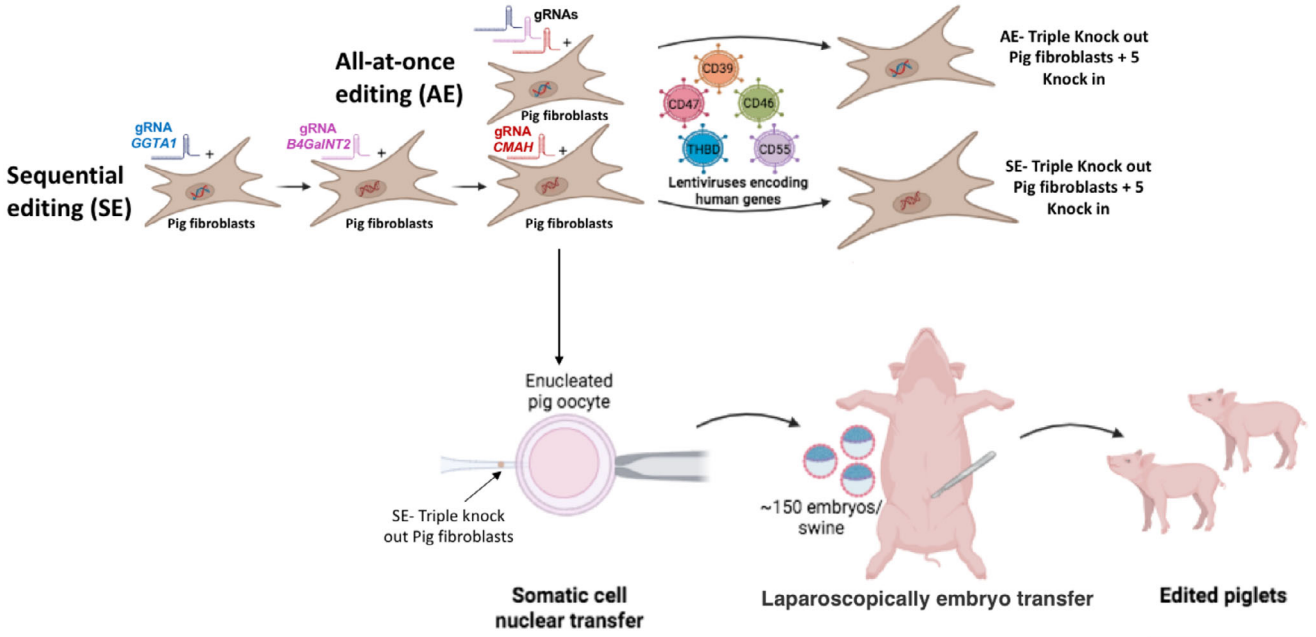


FIGURE 1 | 111.1

and embryo development. Our results represent a promising step toward the generation of genetically tailored porcine donors for xenotransplantation.

111.2 | Biocompatibility of Genetically-Engineered Pig Cornea in Corneal Xenotransplantation for the Use of Human Clinical Trials

Chung Young Kim[1,2], Seung Hyeun Lee[2,3], Jin Suk Ryu[2], Dong Hee Choi[2], Chang Ho Yoon[1,2], Chung-Gyu Park[4,5], Joohyun Shim[6], Hyunil Kim[6], Mee Kum Kim[1,2,4]

[1]Department of Ophthalmology, Seoul National University College of Medicine, Seoul, Korea; [2]Laboratory of Ocular Regenerative Medicine and Immunology, Biomedical Research Institute, Seoul National University Hospital, Seoul, Korea; [3]Department of Ophthalmology, Chung-Ang University Gwangmyeong Hospital, Gwangmyeong, Korea; [4]Transplantation Research Institute, Seoul National University College of Medicine, Seoul, Korea; [5]Department of Microbiology and Immunology, Department of Biomedical Sciences, Seoul National University College of Medicine, Seoul, Korea; [6]Optipharm, Inc., Cheongju, Korea

Introduction: Corneal xenotransplantation, first attempted in the 19th century, was historically hindered by xenogeneic immune barriers. With the advent of alpha-1,3-galactosyltransferase gene-knockout pigs and CRISPR-Cas9-mediated multi-gene editing—including PERV inactivation—xenotransplantation has re-emerged as a viable clinical strategy. Given the persistent shortage of donor corneas in East Asia, porcine corneas are under investigation as alternative graft sources. While early genetically-engineered (GE) models showed limited graft survival without substantial immunosuppression, multi-gene-edited pigs are being developed to address this. However, the impact of extensive immune-related gene editing on corneal endothelial cell function remains unknown. This study assesses the biophysical integrity of endothelial cells in these multi-gene-edited porcine corneas, a critical determinant for their clinical applicability in full-thickness xenotransplantation.

Methods: Triple or quadruple knockout (T[Q]KO) pigs with targeted deletions in GGTA1, CMAH, β4GalNT2, and/or iGb3s were utilized, combined with knock-in (KI) or overexpression (OE) of human CD55, CD39, CD46, and thrombomodulin (TBM) genes. A total of 27 eyes from 14 genetically engineered pigs were analyzed to assess physical and functional suitability as corneal donors. Corneal endothelial cell density (ECD) was monitored over 7 days in storage medium. Endothelial cell proliferative capacity was assessed via doubling time (DT), and immunofluorescence was used to evaluate expression of endothelial pump and tight junction proteins.

Results: The GE pigs had a mean age of 11.9 months, with an average central corneal thickness of 718.2 µm. ECD loss was significantly greater in pigs under 6 months old (55.1%) compared to those over 6 months (8.8%) ($p < 0.001$). T(Q)KO/hCD46OE/hTBMOE corneas exhibited early endothelial apoptosis or necrosis, whereas other GE corneas did not show such changes. Although DT was increased across all GE pigs, there was no age-related difference. Key endothelial markers (ZO-1, N-cadherin, ATPase, SLC4A1, and aquaporin 1) were consistently expressed across all GE variants.

Conclusion: These findings suggest that corneas from both T(Q)KO/hCD55KI/hCD39KI and QKO pigs over 6 months of age exhibit sufficient endothelial cell function, making them suitable donor candidates for human corneal xenotransplantation.

111.3 | Genome-Engineered Porcine Expanded Potential Stem Cells as a Novel Cellular Source for Xenotransplantation

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Kong SAR, China; [3]FLI, Institute of Farm Animal Genetics, Mariensee, Germany

Introduction: Porcine-to-human xenotransplantation offers a promising solution to the global organ shortage. While previous studies relied on somatic cells for genetic engineering, their limited lifespan and low editing efficiency hinder complex modifications. Porcine expanded potential stem cells (EPSCs), derived from preimplantation embryos via modulation of key developmental pathways, overcome these limitations. With unlimited proliferation, high editing efficiency, and the capacity to differentiate into all three germ layers and extra-embryonic lineages, EPSCs provide an ideal platform to generate humanized pigs. Here, we leverage these advantages to engineer immune-compatible porcine cells for clinical xenotransplantation.
Methods: We performed quadruple knockout (QKO) editing of porcine EPSCs, targeting *GGTA1, CMAH, B4GALNT2* (to eliminate xenoantigens), and *GHR* (to prevent organ overgrowth). Human *CD47* was inserted at the *ROSA26* safe harbor locus via Cre-mediated recombination. Differentiated endothelial cells (ECs) from QKO-hCD47-EPSCs were assessed for immune compatibility.
Results: QKO-hCD47-EPSCs maintained genomic stability and differentiation potential. Derived ECs exhibited significantly reduced human antibody binding, complement-mediated lysis, and macrophage phagocytosis compared to wild-type controls.
Conclusion: This robust porcine EPSC-based gene editing system permits sophisticated genetic engineering. Meanwhile, the porcine EPSC-derived endothelial cells provide an in vitro platform for the fast functional assessment afterwards. These research efforts would benefit the eventual generation of ideal xenotransplantation donors in the future.

111.4 | Identification of the Potential Role of Swine Leukocyte Antigen in Xenotransplantation

Nayoung Ko, Hyoung-Joo Kim, Yongjin Lee, Jun-hyeong Kim, JaeKyung Park, Kyungmin Kwak, Byunghyun Kim, SungHun Park, Hyunil Kim, Joohyun Shim
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Introduction: Xenotransplantation is a feasible substitute to resolve shortage of organs for transplantation. To success pig-to-primate xenotransplantation, genetic engineering of donor pig is essential for overcoming pig-to-primate immune rejection. As various genetically modified donor pigs, such as Knock-out (KO) of xenoantigens, and expression of genes to modulate immunological rejection have been advanced, the survival of pig-to-nonhuman primate xenotransplantation has been prolonged significantly. However, a deeper understanding of the biological barriers between pigs and primates is still needed to accomplish pig-to-primate xenotransplantation. Although swine leukocyte antigen (SLA) has been suggested to be associated with human T cell response and antibody-mediated rejection, the effect of SLA remains unsubstantiated in pig-to-primate xenotransplantation. Therefore, we generated SLA I knock-out pigs and SLA null cells to identify the response to SLA antigen in human or nonhuman primate T cells and pig-to-nonhuman xenotransplantation.
Methods: SLA I KO pig was generated by disruption of beta-2-microglobulin(β2m) gene based on QKO (quadruple KO, GGTA1/CMAH/A3GalT2/B4GalNT2 KO) using somatic cell nuclear transfer technology. Generation of SLA null fibroblasts was achieved by knock-out of CIITA gene based on PKO (penta KO, GGTA1/CMAH/A3GalT2/B4GalNT2/β2m KO). Expression of SLA I and SLA II was identified by flow cytometry analysis on cells from PKO pig and SLA null fibroblasts. Aorta endothelial cells (AECs) from WT (wild type), QKO, and PKO were cocultured with human or cynomolgus peripheral blood monocyte cells (PBMCs), and proliferation of lymphocytes and CD8 T cells was estimated using cell proliferation kit. The antibody response to SLA antigen was evaluated by incubation of either SLA-positive or SLA-negative fibroblasts and serum from recipient following pig-to-primate kidney transplantation.
Results: Various cells from PKO pig did not express SLA I. SLA-null fibroblasts did not express SLA I and SLA II antigen. The proliferation of human CD8+ T cells was significantly reduced against AECs from PKO pigs compared to AECs from WT and QKO. In contrast, cynomolgus CD8+ T cells showed reduced proliferation against AECs from both QKO and PKO pigs compared to WT. The donor-specific antibody related to SLA antigen, especially SLA II, was increased in several cases of pig-to-primate kidney transplantation recipients.
Conclusion: Knock-out of β2m and CIITA gene was sufficient to eliminate SLA. Although pig-to-primate xenotransplantation using SLA KO pig has not been performed to assess immune response in this study, our findings imply that SLA may play a potential role in both human T cell response and memory response, leading to delayed immunological rejection.
Funding: This research was supported by a grant from Korea Health Technology R&D Project through the Korea Health Industry Development Institute (KHIDI), funded by the Ministry of Health & Welfare, Republic of Korea (grant number: RS-2023-KH136747).

111.5 | Human Thrombomodulin-Expressing Transgenic Pigs for Islet Xenotransplantation: Potential Implication for Pancreatic Function and Disease

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Introduction: Human thrombomodulin (hTBM) is frequently introduced into genetically engineered pigs to reduce coagulation-related complications in xenotransplantation. While hTBM expression improves vascular compatibility in solid organ transplantation, its impact on endocrine organs such as the pancreas remains unclear. This study aimed to evaluate the metabolic and immunological consequences of hTBM expression in pigs engineered for islet xenotransplantation.
Methods: Transgenic pigs expressing hTBM (QKO-hTBM) were produced by genetic engineering system. Total pancreatectomy was followed by comparative analysis of gross morphology, histology, serum biochemistry, and cytokine profiles among wild-type (WT) and four xeno-antigen (GGTA1, CMAH, b4GalNT2 and A3GALT2) knockout (QKO) pigs. Human serum was also analyzed for comparison. Fat accumulation was assessed by Oil

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Red O staining, and insulin-positive islet area was measured via immunofluorescence.

Results: Gross and histological examination revealed extensive pancreatic fat infiltration in QKO-hTBM pigs, while WT and QKO pancreas appeared normal. QKO-hTBM pigs showed significantly larger islets but exhibited elevated fasting blood glucose and reduced porcine insulin and C-peptide levels. Serum hTBM levels in transgenic pigs were also significantly higher than in healthy humans. Cytokine profiling indicated increased IFN-γ, IL-1β, IL-17, and TNF-α, alongside reduced IL-10, suggesting a shift toward a pro-inflammatory state.

Conclusion: Although hTBM expression regulates coagulation-related complications in xenotransplantation, its systemic overexpression may impair pancreatic function and immune balance, posing risks in islet transplantation. Tight regulation of hTBM expression, potentially through tissue-specific or inducible promoters, may be necessary to optimize metabolic outcomes in future clinical islet xenotransplantation.

Funding: This research was supported by a grant from the Korea Health Technology R&D Project through the Korea Health Industry Development Institute (KHIDI), funded by the Ministry of Health & Welfare, Republic of Korea (grant number: RS-2023-KH136747).

111.6 | Overexpression of Human CD55 in Tripple Knockout (TKO) Porcine Red Blood Cells (pRBC) Can Further Reduce Human Antibody-Induced Hemolytic Reactions

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Introduction: The contradiction between global blood supply and demand is growing, and the shortage of clinical blood has become a key public health challenge. Previous research indicates that the use of TKO-pRBC can mitigate immune rejection in pig-to-monkey blood transfusions study, but with limited effect. In this study, we aimed to test the effect of combined approach by over-expressing human-derived complement inhibitor (CD55 transgene) on TKO-pRBC in preventing human natural antibody-mediated hemolysis.

Methods: The knockout of xeno-antigens and expression of transgene were designed using CRISPR-Cas9 system, the gRNA targeting GGTA1, B4GALNT2, CMAH, Rosa26 locus, Cas9 protein, and the expression construct vector was transferred to pig fibroblasts by electroporation. Positive clones were obtained through PCR identification and then used for obtaining cloned piglets. The red blood cells of cloned pigs were isolated, and expression was identified by flow cytometry. In vitro experiments, blood agglutination reaction, and IgM/IgG antibody binding level were detected after incubation with human serum. The complement-dependent cytotoxicity assay was used to measure the level of hemolysis.

Results: The TKO/hCD55 porcine red blood cells demonstrated successful knockout of α-Gal Neu5Gc, Sda, and exhibited about 10 times higher expressions of hCD55 than human red blood cells. In vitro studies demonstrated that IgM and IgG binding to TKO pRBCs was significantly lower than WT and GTKO pRBCs ($p < 0.001$), and the binding of TKO/CD55 pRBCs was only slightly higher than human ABO-C RBCs ($p < 0.05$). There was no difference in complement-dependent cytotoxicity between TKO/hCD55 and the human ABO-C group, demonstrating that after knockout of three major antigens, there are still other xeno-antigens which can induce IgG/IgM antibody binding, but the over-expression of human CD55 can effectively reduce human antibody-mediated hemolytic reactions in vitro.

Conclusion: Over-expression of human CD55 on the basis of TKO porcine red blood cells can further reduce the antibody-mediated hemolysis reaction with human serum. Future in vivo study, will be done to demonstrate the potential of such combined strategy to further improve the viability of porcine red blood cells in human blood transfusion, which could relieve the shortage of human blood supply.

111.7 | A Novel Transgene to Regulate Oxidative Stress and Mitochondrial Damage

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Background: Oxidative stress plays a key role in various conditions, including cancer, cardiovascular and neurodegenerative diseases, diabetes, chronic inflammation, and organ transplantation. In transplantation, ischemia-reperfusion (I/R) injury poses a major threat to graft survival. During reperfusion, the sudden return of oxygen triggers a surge in reactive oxygen (ROS) and nitrogen species (RNS). These overwhelm cellular antioxidants, causing mitochondrial dysfunction and oxidative damage to DNA, proteins, and lipids, leading to lipid peroxidation and inflammation via pathways like NF-κB. Elevated Fe^{2+} levels further drive ROS production through Fenton reactions, intensifying mitochondrial damage and respiratory failure. This cascade results in tissue injury, reduced cell viability, and impaired organ function.

Methods: We developed transgenic pigs and conducted preliminary in vitro studies to assess the function of a novel transgene in protecting against oxidative stress-induced cell death pathways, including apoptosis, necrosis, and ferroptosis. Additionally, we evaluated its effects on mitochondrial membrane integrity, superoxide production, respiratory capacity, and recovery following oxidative stress.

Results: Our analyses showed reduced cell death responses, including lower apoptosis marked by decreased caspase-3 and -7 activity, inhibition of mitochondrial permeability transition pore (MPTP)-mediated necrosis, and reduced lipid peroxidation, the terminal step of ferroptosis. Additionally, mitochondrial superoxide production was decreased, while respiratory function and recovery following oxidative stress were notably enhanced.

Conclusion: This novel pig model demonstrates that novel transgenes can effectively reduce oxidative damage in vitro by modulating intracellular antioxidant defenses, mitochondrial function,

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and ROS production. These transgenes could play a key role in improving long-term outcomes in future xenotransplantation.

Funding: This study was supported the Deutsche Forschungsgemeinschaft (DFG; CRC-TRR 127), the Swiss National Science Foundation (SNSF; Sinergia grant CRSII5_198577/1), and the Leducq Foundation (23CVD01 "Xenogenic and exogenic pig organs for transplantation into humans"). We thank the Animal Research Center for providing infrastructure.

112.1 | Early Xeno-Derived Cell-Free DNA Elevation as a Noninvasive Biomarker of Kidney Xenograft Rejection in Pig-to-Nonhuman Primate Xenotransplantation

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Introduction: Kidney xenograft biopsy is a standard diagnostic method for xenograft rejection. Recently, donor-derived cell-free DNA (cfDNA) has emerged as a promising early, non-invasive biomarker for allograft rejection; however. its utility as a screening test for xenograft rejection remains unclear. This study investigates whether xeno-derived cfDNA levels can predict xenograft rejection and have an association with the severity of histological and immunological injury in kidney xenograft tissues.

Methods: Kidneys from multiple genetically-engineered pigs (knock-out for GGTA1, CMAH, iGb3s, or B4GalNT2; knock-in for human CD39, CD46, CD55, or thrombomodulin) were transplanted into cynomolgus monkeys under a few immunosuppressive regimens, which consisted of thymoglobulin, rituximab, anti-CD154, abatacept, or anti-ICAM-1 (MD-3), anti-C5 inhibitor (crovalimab), and triple maintenance immunosuppressants including prednisolone, tacrolimus, and mycophenolate mofetil. Kidney xenograft biopsies at 2, 4 weeks, and later timepoints were diagnosed based on Banff pathologic classification 2022, including transcriptomic analysis and immunohistochemical staining (CD3, CD68, IgG, C4d, membrane attack complex [MAC], CD61). Recipients were categorized into two groups according to a composite score of g + ptc + v at xenograft biopsy: mild (score <3) versus severe (score ≥3) injury groups. Pig-derived cfDNA was serially measured using a random next-generation sequencing technology at pre-transplant timepoints and post-transplant 2-week, 4-week, and later timepoints.

Results: Among nine recipients, eight monkeys experienced antibody-mediated rejection. Four monkeys belonged to the mild injury group, while the remaining five belonged to the severe injury group at 4 weeks post-transplantation. Overall, cfDNA levels were already elevated at post-transplant 2 weeks, when the composite scores of xenograft biopsies were still low. cfDNA levels remained high at 4 weeks, when the composite scores increased. Then, cfDNA levels decreased beyond 4 weeks post-transplantation, when the composite scores were still elevated. These data suggest that cfDNA levels precede changes in the composite scores in xenografts, highlighting its potential as an early biomarker of rejection. Furthermore, cfDNA levels in the severe-injury group (mean, 4.89%) showed a higher tendency than those in the mild-injury group (mean, 2.53%), while cfDNA level in the case of no-rejection was only 0.39%. There was a strong correlation between cfDNA levels and the composite scores ($R^2 = 0.809$, $p = 0.038$). In parallel, immunohistochemical staining showed that cellular infiltration (CD3+, CD68, CD61+) and deposition of MAC and IgG were markedly elevated in the severe-injury group.

Conclusion: Serial levels of xeno-derived cfDNA are promising as an early, noninvasive biomarker of xenograft rejection, which could be confirmed by subsequent xenograft biopsy.

112.2 | Immunopeptidomic Identification of SLA-Derived HLA Class II Ligands Recognized by Human T Cells, Using a Strategy Adapted for Xenotransplantation

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Background: Recent advances in genetic engineering have drastically extended xenograft survival in NHPs, facilitating the advancement of xenotransplantation toward clinical application.

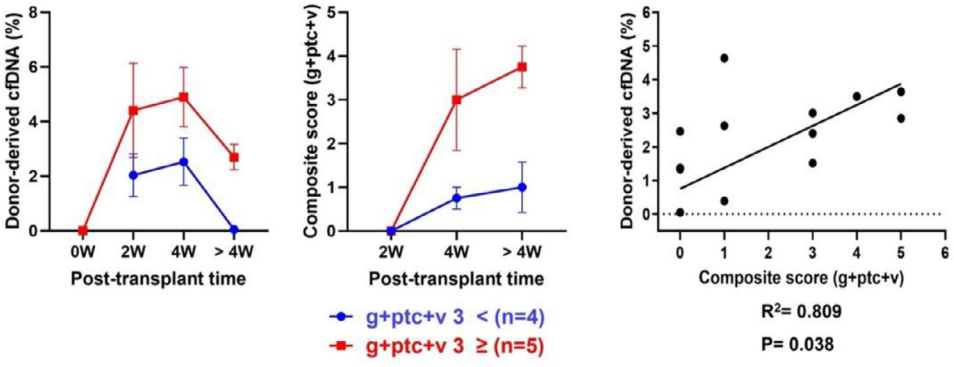


FIGURE 1 | 112.1

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However, long-term graft acceptance remains a major barrier in humans, and the genetic targets required to mitigate human anti-xenogeneic immune responses have not been fully defined. This study aimed to identify peptide epitopes derived from porcine antigens that are presented by human dendritic cells via HLA class II, as potential targets for future genetic modifications to reduce T-cell-mediated rejection.

Methods: $CD14^+$ monocytes isolated from human peripheral blood were cultured with irradiated porcine liver-derived endothelial cells lacking GGTA1, CMAH, and β4GalNT2 for 4 days with IL-4 and GM-CSF, followed by two additional days with IL-1β and TGFα to induce differentiation into dendritic cells. After cell lysis, HLA class II-bound peptides were immunoprecipitated using anti-HLA-DR antibodies and analyzed by nanoLC-qTOF/MS/MS. Peptide sequences were matched to the Immune Epitope Database for immunogenicity prediction. Selected peptides were synthesized and evaluated by ELISPOT assays using PBMCs from healthy donors.

Results: Peptides derived from swine leukocyte antigen (SLA) class I and II molecules, along with other porcine proteins, were consistently identified in all three donors. Several predicted immunogenic peptides elicited cytokine production in ELISPOT assays, indicating T-cell recognition of these epitopes.

Conclusion: Donor-specific anti-HLA antibodies (DSAs), including those cross-reactive to SLA, are associated with xenograft rejection, yet reliable immunological markers for recipient stratification are lacking. In allotransplantation, the presence of shared T-cell epitopes has been associated with de novo DSA formation. Our findings demonstrate that SLA-derived peptides can be presented by human HLA class II molecules and are recognized by human T cells, suggesting their relevance in the adaptive immune response to xenografts. These epitopes represent potential targets for donor genome editing to enhance long-term xenograft survival in clinical settings.

112.3 | Establishment of a Designated Pathogen-Free (DPF) Donor Pig Population of Genetically Edited Pigs in Neijiang, China

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Introduction: As xenotransplantation progresses toward clinical translation, cross-species transmission of porcine-derived pathogens, particularly porcine cytomegalovirus (PCMV) and porcine lymphotropic herpesviruses (PLHV), represents a critical biosafety challenge in clinical use. Based on our newly established designated pathogen-free (DPF) pig facility in Neijiang, China, this initiative aims to establish and develop China's first self-sustaining DPF Bama miniature pig population as a biosafe donor source for clinical xenotransplantation.

Methods: From our Phase I conventional breeding cohort (n = 425), 64 representative individuals (15% stratified sampling) underwent comprehensive microbial screening for xenotransplantation-relevant pathogens (PCMV, PLHV-1/2/3), and vertically transmitted pathogens.

Selected pathogen-negative sows underwent aseptic cesarean section in a DPF barrier facility. Derived piglets received sterilized colostrum to establish the foundation (F_0) generation under strict bio-surveillance, including HEPA-filtered air handling, irradiated feed, and filtered water systems. Longitudinal pathogen monitoring encompassed biweekly blood, fecal, and nasal swab analyses during barrier maintenance.

Results: Initial screening revealed complete absence of vertical transmission pathogens (pseudorabies, parvovirus, circovirus, PRRSV), enabling successful DPF derivation. Xenozoonotic risk profiling identified PCMV (6%), PLHV-2 (22%), PLHV-3 (50%), with notable absence of PERV-C. Through a 13-month surveillance period, 42 F_0 DPF pigs across 8 production batches demonstrated sustained pathogen-free status (90% hysterectomy survival rate), achieving complete elimination of target pathogens (PCMV, PLHV-2/3, parvovirus, Actinobacillus, Streptococcus). The breeding area currently maintains 15 genetically modified (6-gene edited) founders aged 6–12 months, with F_1 progeny successfully generated. In clinical studies, including a renal xenotransplantation trial, the living patient survival exceeded 50 days with maintained pathogen-negative status (unpublished data).

Conclusion: This study establishes China's first DPF Bama miniature pig population for xenotransplantation through integrated pathogen eradication and advanced biocontainment strategies. Our multilayered approach combining hysterectomy derivation, sterile nutritional protocols, and pathogen surveillance demonstrates the feasibility of generating biosafe donors for clinical xenotransplantation, being able to address critical safety barriers in cross-species transplantation, and advance the clinical application of xenotransplantation technology.

112.4 | SLA Antibody Screening Using Auckland Island Pig Cells: Toward Standardized Diagnostics for Xenotransplantation

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Introduction: The Auckland Island pig (AIP) breed is of particular interest in xenotransplantation due to its complete homozygosity across the swine leukocyte antigen (SLA) loci, a result of inbreeding and natural selection. This genetic uniformity offers a major advantage over cloned pigs by providing a stable background and functionally uniform expression of introduced human genes. With the primary carbohydrate xenoantigens (Ags) effectively eliminated by genetic modifications, SLAs have emerged as the next major immunologic barrier, capable of inducing antibody (Ab) responses upon sensitization. Additionally, the generation of de novo Abs following transplantation remains a

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Pathogen	Sample	Method
Porcine respiratory and reproductive syndrome virus	serum	RT-qPCR
Classical swine fever virus	serum	RT-qPCR
Pseudorabies virus	serum	qPCR
Porcine parvovirus	serum	qPCR
Porcine circovirus type 1/2/3	serum	qPCR
African swine fever virus	Oral and nasal swabs	qPCR
Japanese encephalitis virus	serum	RT-qPCR
Porcine rotavirus	anal swabs	RT-qPCR
Foot-and-mouth disease virus	Oral and nasal swabs	RT-qPCR
Hepatitis E virus	serum	RT-qPCR
Porcine cytomegalovirus	serum	qPCR
Porcine lymphotropic herpesvirus 1/2/3	serum	PCR
Streptococcus suis	Oral and nasal swabs	qPCR
Actinobacillus pleuropneumoniae	Oral and nasal swabs	qPCR
Pasteurella multocida	Oral and nasal swabs	qPCR
Lawsonia Intracellularis	anal swabs	qPCR
Mycoplasma hyopneumoniae	Oral and nasal swabs	qPCR

FIGURE 1 | 112.3

major barrier to long-term xenograft survival. Post-transplant Ab levels against donor Ags are monitored for early signs of rejection or graft injury. The study aimed to test a large cohort of human sera, from both healthy individuals and highly sensitized patients, against cells from AIP, wild-type (WT), and genetically modified animals. The ultimate goals were to support SLA matching at the time of xenotransplantation and to enable point-of-care or at-home monitoring through the development of a rapid screening method.

Methods: Peripheral blood mononuclear cells (PBMC) from AIP homozygous for SLA-1*23:03, SLA-2*13:02, SLA-6*10:01, SLA-11*01:01, SLA-DRA*01:01:02, SLA-DRB1*05:01, SLA-DQA*01:03, and SLA-DQB1*08:01. Both wild-type (WT) and triple knockout (TKO) animals (lacking carbohydrate Ags) were included in the study to assess Ab binding. The preparation of a human negative control serum was necessary. A human negative control serum was prepared by pooling sera from multiple donors and immunoadsorbing them first with red blood cells from WT pigs, then with PBMCs from TKO animals to remove anti-carbohydrate, anti-SLA, and other minor Ag-specific Abs. Human sera from individuals on the organ transplant waiting list and the general German population were tested using standardized porcine PBMC-based crossmatch assays, including antibody binding and complement-mediated cytotoxicity, analyzed via flow cytometry.

Results: We successfully adapted and standardized existing allotransplantation crossmatch protocols for use with porcine PBMC to detect anti-pig antibodies in human sera. Strong IgM and IgG binding was consistently observed with WT-derived PBMC. In contrast, most sera showed no reactivity against TKO-derived PBMC, with only a few highly sensitized samples displaying weak or borderline positivity. The use of the AIP breed facilitated compatibility testing by presenting a single, consistent SLA haplotype, thereby reducing screening complexity.

Conclusions: SLA homozygosity in the AIP breed reduces antigenic diversity, aiding compatibility assessment in xenotransplantation. Future work will standardize assays using porcine endothelial cell monolayers and fluorescence microscopy to better reflect xenograft antigens.

Funding: This work was supported by the Deutsche Forschungsgemeinschaft (CRC-TR 127), the Swiss National Science Foundation (Sinergia grant CRSII5_198577/1), and the Leducq Foundation (23CVD01). German Heart Foundation.

112.5 | Standardization of Multiplex qPCR Assays for Detection of PCMV and PCV2 in Organ Donor Pigs

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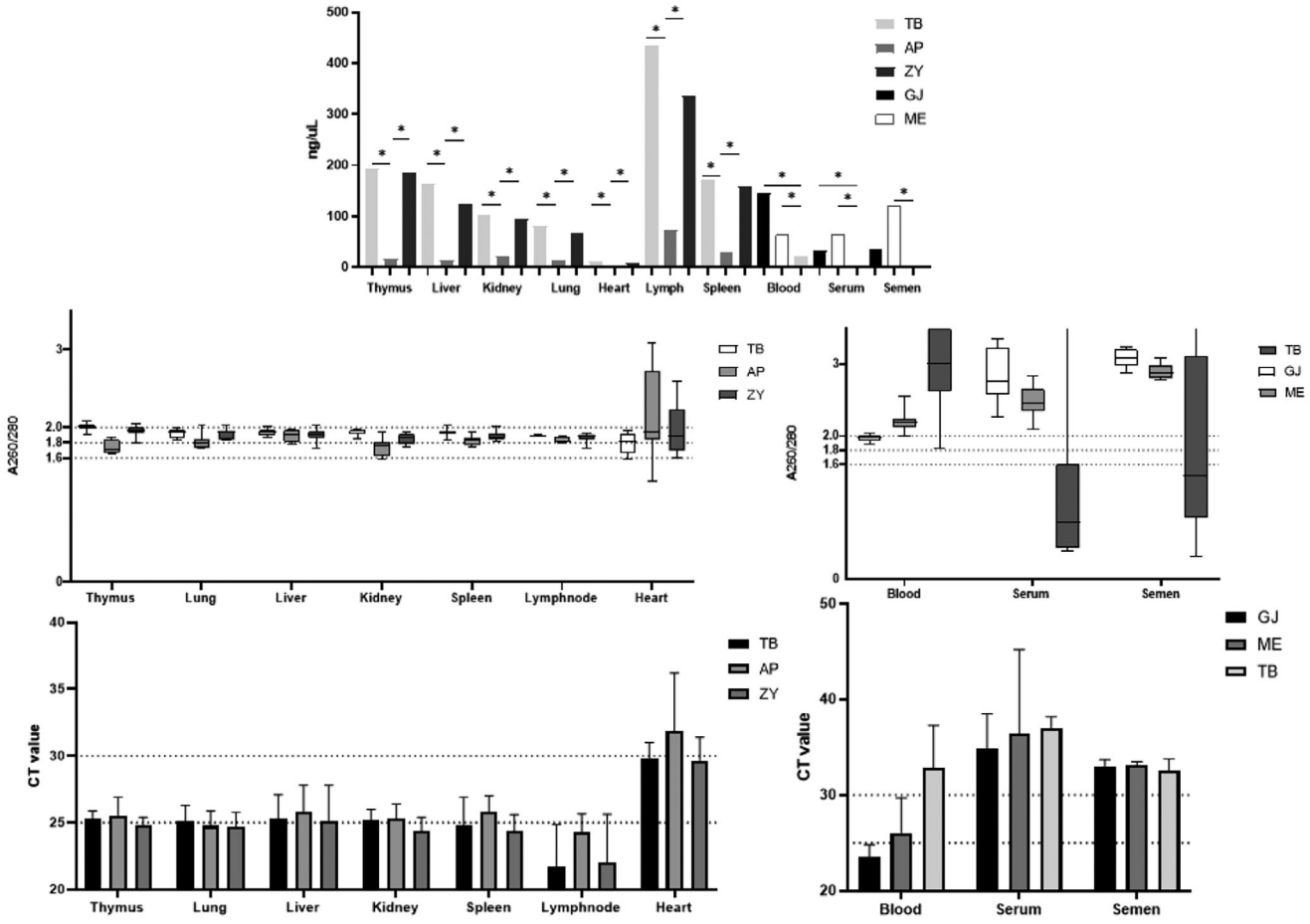
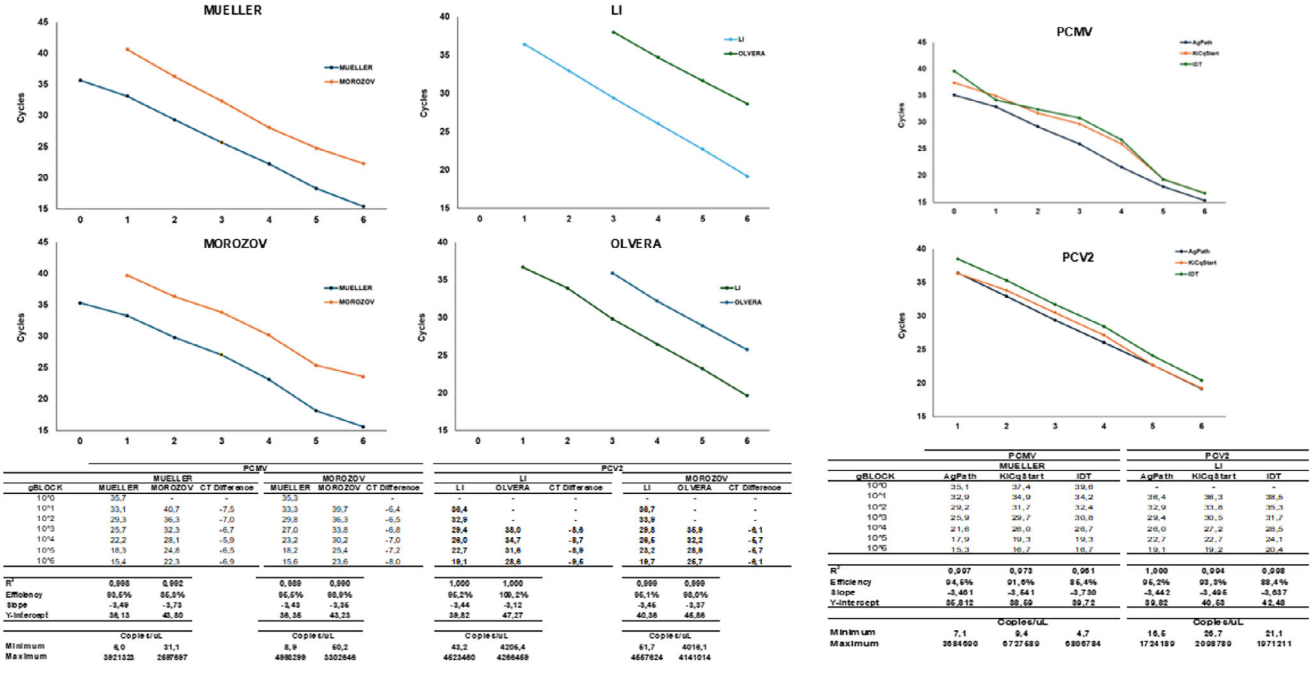


FIGURE 1 | 112.5



PCMV	MUELLER			MOROZOV		
qBLOCK	MUELLER	MOROZOV	CT Difference	MUELLER	MOROZOV	CT Difference
10^0	35,7	-	-	35,3	-	-
10^1	33,1	40,7	-7,5	33,3	39,7	-6,4
10^2	29,3	36,3	-7,0	29,8	36,3	-6,5
10^3	25,7	32,3	-6,7	27,0	33,8	-6,8
10^4	22,2	28,1	-5,9	23,2	30,2	-7,0
10^5	18,3	24,8	-6,5	18,2	25,4	-7,2
10^6	15,4	22,3	-6,9	15,6	23,6	-8,0
R^2	0,998	0,992		0,999	0,999	
Efficiency	96,6%	86,9%		96,6%	98,9%	
Slope	-3,49	-3,73		-3,43	-3,36	
Y-Intercept	36,13	40,80		36,36	43,23	
	Copies/uL			Copies/uL		
Minimum	8,9	31,1		8,9	60,2	
Maximum	3821333	2687697		4698299	3302846	

PCMV	LI			MOROZOV		
qBLOCK	LI	OLVERA	CT Difference	LI	OLVERA	CT Difference
10^0	-	-	-	-	-	-
10^1	30,4	-	-	30,7	-	-
10^2	32,9	-	-	33,9	-	-
10^3	29,4	38,0	-8,6	29,8	36,9	-8,1
10^4	26,0	34,7	-8,7	26,6	32,2	-8,7
10^5	22,7	31,6	-8,9	23,2	28,9	-8,7
10^6	19,1	28,6	-9,6	19,7	25,7	-8,1
R^2	1,000	1,000		0,999	0,999	
Efficiency	96,2%	100,2%		95,1%	98,0%	
Slope	-3,44	-3,32		-3,46	-3,37	
Y-Intercept	38,82	47,27		40,36	46,86	
	Copies/uL			Copies/uL		
Minimum	43,2	4206,4		61,7	4016,1	
Maximum	4623460	4266469		4667824	4141014	

PCMV	MUELLER			PCV2	LI		
qBLOCK	AgPath	KiCqStart	IDT	qBLOCK	AgPath	KiCqStart	IDT
10^0	35,1	37,4	39,6	-	-	-	-
10^1	32,9	34,9	34,2	10^1	36,4	36,3	38,5
10^2	29,2	31,7	32,4	10^2	32,9	33,6	35,3
10^3	25,9	29,7	30,8	10^3	29,4	30,5	31,7
10^4	21,6	26,0	26,7	10^4	26,0	27,2	28,5
10^5	17,9	19,3	19,3	10^5	22,7	22,7	24,1
10^6	15,3	16,7	16,7	10^6	19,1	19,2	20,4
R^2	0,997	0,973	0,961		1,000	0,994	0,998
Efficiency	94,6%	91,6%	86,4%		96,2%	93,3%	88,4%
Slope	-3,461	-3,541	-3,730		-3,442	-3,495	-3,637
Y-Intercept	36,812	38,69	39,72		39,92	40,68	42,48
	Copies/uL				Copies/uL		
Minimum	7,1	9,4	4,7		16,6	26,7	21,1
Maximum	3584600	6727580	5806784		1724189	2098789	1971211

FIGURE 2 | 112.5

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Introduction: Ensuring non-contamination by xenozoonotic pathogens in organs intended for xenotransplantation is crucial. Real-time PCR (qPCR) remains the most widely used method for active infection diagnosis, offering high sensitivity and specificity. Given the wide variety of pathogens to be tested, several types of material are required to ensure reliable results. Consequently, the development of an "in-house" multiplex molecular panel capable of detecting pathogens deemed high-risk for xenotransplantation emerges as a cost-effective and expeditious alternative to the conventional testing methods involving animals and pig facilities. This study presents the progress made in optimizing a multiplex qPCR protocol for the simultaneous detection of Porcine Cytomegalovirus (PCMV/PRV), Porcine Circovirus type 2 (PCV2), and the endogenous gene pGAPDH, evaluating different DNA extraction kits, primer sets, and biological sample types to identify the most reliable combination.

Methods: To compare the extraction efficiency, blood, serum, semen, and tissue samples collected from 10 animals were subjected to DNA extraction using five different commercial kits and assessed nucleic acid yield, purity, and the sensitivity by the endogenous gene pGAPDH amplification. The qPCR limit detection and assay efficiency were evaluated between two primers sets for PCMV (Mueller, 2002; Morozov, 2016) and PCV2 (Olvera, 2004; Li, 2018) using gBLOCK with 10-fold serial dilution in triplicate, additionally with widely used commercial master mixes. Thus, the most reliable extraction kit, primer set and mastermix combination were used to extract and amplify 37 samples of blood, serum, and tissues in singleplex and multiplex, to measure possible sensitivity loss. Samples with CT values near 38–39 were retested multiple times to ensure reproducibility.

Results: The GeneJET viral extraction kit associated with Mueller (2002) and Li (2018) primers and AgPath-ID mastermix showed most reliability and detection of less copy numbers. No sensitivity loss was found when comparing multiplex assays. Among the 37 samples evaluated, 5 (13,5%) and 12 (32,4%) were considered positive, with a reproducibility rate for low number copies samples of 78.9% and 100% for PCMV and PCV2, respectively.

Conclusion: To date, our study has successfully standardized multiplex qPCR for detecting PCMV and PCV2 with limits of 10^0 and 10^1 copies, respectively, with no sensitivity loss, even in materials difficult to amplify. Our next step is monitoring the presence of these pathogens in nursery animals and expanding the standardization to 11 pathogens in a panel format.

Funding: This work was supported by the São Paulo Research Foundation (FAPESP) – grant number 21/11872-5, the National Council for Scientific and Technological Development (CNPq) – grant number 444075/2023-2, and the Água Branca Pig Farm, Itu, São Paulo, Brazil.

112.6 | Successful Somatic Support of Brain-Dead Decedents for Xenotransplantation Research: A Systematic Review and Case Series

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Introduction: The brain-dead decedent model has emerged as a novel approach to support de-risked xenotransplant research under human physiologic and immunologic conditions. However, the ability to provide somatic support for a decedent beyond approximately 1 week has been questioned, as has the physiologic and immunologic fidelity of the model.

Methods: A systematic review (PROSPERO: CRD42023452561) was conducted by two independent reviewers and coders to identify cases in which somatic support of a brain-dead decedent had been conducted for ≥7 days. Xenotransplant decedent studies conducted after the systematic review were identified. Additionally, we report on two cases of prolonged somatic support for xenotransplant research conducted by our group. Decedent research was conducted under the approval and procedures of the NYU Research on Decedents Oversight Committee.

Results: For the systematic review, 26 articles reporting on 25 cases of somatic support, generally to support the gestation of a fetus (88%), were identified (Table 1). Consequently, most decedents were female (92%). The cause of death was most commonly intracerebral hemorrhage (72%). Duration of somatic support was a median of 68 days. Replacement of hypothalamic-pituitary driven hormones, the use of vasopressors for hemodynamic instability, and temperature instability were commonly reported. Since the systematic review, at least 7 cases of somatic support ≥7 days have been conducted: two by our group (providing 63 and 37 days of somatic support), as well as studies providing at least 10, 12, 12, 12, and 22 days of somatic support by groups in China and the United States. In the two cases we performed, decedents were maintained through planned study termination points. In both cases, traditional intensive care, pharmacologic blood pressure management, and repletion of thyroid hormone (either oral or intravenous, guided by thyroid studies), antidiuretic hormone, and glucocorticoids maintained hemodynamic stability within target mean arterial pressure range (65–100 mmHg). Clinically significant alterations in coagulation were not observed, although prolonged clot reaction time and decreased clot maximum amplitude were present at several points in both cases. Infections requiring antibiotic therapy were present in both cases as a result of conditions present at the time of death; in both cases, systemic infections were able to be cleared or controlled, even in the context of systemic immunosuppression. Hypothermia requiring active warming was present in both cases.

Conclusion: Somatic support of decedents is rare but has been successfully performed for the past few decades (typically for fetal gestation), requiring critical care, replacement of brainstem-driven hormones, and often management of hemodynamic and temperature instability. Successful somatic support for xenotransplant research has been replicated by several groups for periods of time ranging from 10–63 days. Further data on inflammatory and immunologic responses after brain death are forthcoming.

112.7 | Optimized Cryopreservation of Porcine Pancreatic Islet Cells: A Novel Protocol for Single-Cell Biobanking

Hany Abdelgawad[1,2], Christina Blechinger[3], Martin Kratzel[3], Carolin Heller[1,2], Undine Schubert[1,2], Janine Schmidt[1,2], Charline Rosenbürger[1,2], Sussan Lehmann[1,2], Peter Jones[4],

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Table 1: Cases from systematic review of somatic support ≥ 7 days

Article (Author, Year)	Age	Gender	Cause of Brain Death	Reason for Somatic Support	Duration	Hypotensive Episodes	Vasopressors Required	Temperature Instability	Glucocorticoid Replacement	Thyroid Replacement	Insulin Replacement	ADH Replacement
I.M. Bernstein, 1989	30	F	Head Trauma	Fetus	110 days			Yes	Yes	Yes	Yes	AVP
ÖF Boran, 2020	21	F	ICH	Fetus	~42 days				Yes			Yes
VA Catanzarite, 1997	25	F	ICH	Fetus	25 days	Yes	Yes		Yes	Yes	Yes	
WP Dillon, 1982	24	F	Meningitis	Fetus	7 days	Yes		Yes			Yes	
DR Field, 1988	27	F	Anoxia	Fetus	63 days	Yes		Yes	Yes	Yes	Yes	
R Gal, 2021	27	F	ICH	Fetus	117 days		Yes		Yes	Yes		DDAVP
A Gopčević, 2017	34	F	ICH	Fetus	64 days	Yes	Yes	Yes	Yes	Yes	Yes	DDAVP
S Holliday, 2017	36	F	Anoxia	Fetus	90 days	Yes	Yes	Yes	Yes	Yes		Yes
IY Hussein, 2006	33	F	ICH	Fetus	14 days	Yes			Yes		Yes	
Y Kinoshita, 2015	32	F	Anoxia	Fetus	1 year				Yes	Yes		DDAVP
M Kredel, 2021	29	F	ICH	Fetus	104 days	Yes	Yes		Yes	Yes		DDAVP
A Lane, 2004	26	F	ICH	Fetus	8 days				Yes	Yes		DDAVP
J Maruya, 2008	43	F	ICH	Family request	165 days			Yes	Yes	Yes		AVP
R Mejia, 2008	29	F	ICH	Fetus	54 days	Yes			ACTH	Yes		DDAVP
JE Heikkinen, 1985 and LS Nuutinen, 1989	31	F	ICH	Fetus	10 weeks	Yes	Yes	Yes	Yes	Yes		AVP
JE Parisi, 1982	49	M	Head Trauma	Unspecified	68 days							
I Pikto-Pietkiewicz, 2019	27	F	ICH	Fetus	89 days				Yes	Yes		DDAVP
AK Reinhold, 2019	28	F	ICH	Fetus	89 days	Yes	Yes		Yes	Yes		DDAVP
A Said, 2013	35	F	ICH	Fetus	110 days	Yes	Yes	Yes	Yes	Yes		DDAVP
L Salazar, 2022	23	F	ICH	Fetus	~27 days	Yes			Yes	Yes		DDAVP
JP Souza, 2006	40	F	ICH	Fetus	25 days	Yes	Yes	Yes	Yes	Yes	Yes	DDAVP
J Wawrzyniak, 2015	30	F	ICH	Fetus	29 days	Yes	Yes	Yes				DDAVP
P Yeung, 2008	26	F	ICH	Fetus	88 days	Yes	Yes		Yes	Yes		
DD Lewis, 1997	20	F	ICH	Fetus	54 days	Yes	Yes			Yes		DDAVP
S Repertinger, 2006	4	M	Meningitis	Family request	20 years	Yes	Yes	Yes	Yes			

ACTH, adrenocorticotropin hormone; ADH, anti-diuretic hormone; AVP, arginine vasopressin hormone; DDAVP, desmopressin; ICH, intracranial hemorrhage

FIGURE 1 | 112.6

Stefan Bornstein[1,2], Eckhard Wolf[3], Elisabeth Kemter[3], Barbara Ludwig[1,2]

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Introduction: Effective cryopreservation of porcine islet cells is crucial for advancing research and enabling their therapeutic use through long-term storage and global distribution. However, traditional methods often compromise cell viability and function. This study evaluates a novel cryopreservation medium optimized to preserve islet cells in single-cell form. Post-thaw, we assessed their viability, reaggregation into pseudoislets, and functional performance.

Methods: Pseudoislet Generation: Native islets were dissociated into single cells using Accumax and seeded into microwell plates. After 5–7 days in culture, pseudoislets (PIs) were harvested for analysis. Cryopreservation: Dissociated cells were washed in D-PBS and resuspended in cryopreservation media, then stored in liquid nitrogen for 6–24 weeks before thawing. Dynamic Glucose-Stimulated Insulin Secretion (GSIS): Islets were pre-incubated in 3 mM glucose for 2 h, then sequentially exposed to 3 mM (basal), 16.7 mM (stimulatory), 60 mM KCl, and basal glucose again. Total insulin content was extracted using RIPA buffer. Insulin levels were quantified using the Revvity HTRF insulin kit and Synergy Neo2 plate reader. Flow Cytometry: Islets were dissociated, stained with a viability dye, fixed, permeabilized, and blocked. Intracellular markers for insulin, glucagon, and somatostatin were used to assess endocrine cell composition. Data were collected using an LSR Fortessa.

Results: Cryopreserved cells in the test medium demonstrated a significantly higher post-thaw viability (87%) compared to traditional "FBS + 5% DMSO" (44%) and other commercial media: NutriFreez D5 (46%), Synth-a-Freeze (39%), and CryoStor CS5 (41%). Pseudoislets derived from cryopreserved and fresh cells were morphologically similar, maintaining dense, rounded structures indicative of intact architecture. Diameter measurements were consistent across both groups. FDA/PI staining revealed similar viability after 5–7 days in culture: 92% in fresh and 87% in cryopreserved-derived pseudoislets. Flow cytometry revealed similar proportions of somatostatin$^+$, insulin$^+$, and glucagon$^+$ cells. Dynamic insulin secretion profiles were also

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comparable between pseudoislets generated from fresh and cryopreserved cells.

Conclusion: We developed a cryopreservation medium that significantly improves post-thaw cell viability compared to conventional and commercial alternatives. Thawed islet cells effectively reaggregated into structurally intact pseudoislets that maintained viability and dynamic insulin secretion comparable to fresh ones. Importantly, cryopreservation had no significant impact on the proportions of key endocrine cell types. These findings support the utility of the developed medium for reliable islet preservation and downstream applications.

Funding: This study was supported by the transCampus S2B 2025 initiative. The authors gratefully acknowledge the financial assistance provided.

203.2 | Longest Surviving Recipient of a Xenotransplantation: ALLO-Sensitized Patient Receiving a 10 Gene-Edited Pig Kidney

Robert A. Montgomery[1], Jeffrey Stern[1], Adam Griesemer[1], Jayme Locke[2], Karen Khalil[1], Imad Aljabban[1], Tal Eitan[1], Ian Jaffe[1], Carl Rosenthal[1], Elaina Weldon[1], Kimberly Sureau[1], Nicole Ali[1], Sapna Mehta[1], David Ayares[2], Megan Sykes[3], Massimo Mangiola[1], Simon Williams[1], Brendan Keating[1], Ming Wu[4], Alexandre Loupy[5], Vineeta Kumar[6], Edward Skolnik[1], Aprajita Mattoo[1], Vasishta Tatapudi[1]

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Introduction: Here we describe the clinical management of the longest living recipient of a porcine xenokidney and the infectious and immunosuppressive complications that eventually led to graft loss.

Methods: A 10-gene-edit porcine kidney (*GGTA1, CMAH, B4GALNT2*, and *GHR* gene inactivations; h*CD46*, h*CD47*, h*CD55*, h*THBD*, h*PROCR*, and h*HMOX1* human transgene insertions) was transplanted into a 53-year-old female prior living donor, on hemodialysis for 9 years, with a cPRA of 99.98%. No experimental immunosuppressive medications were used.

Results: Immediate graft function was evidenced by a mean serum creatine (sCr) and urinary protein-to-creatinine ratios (UPCR) of 1.2 mg/dL and 1.4 g/g, respectively, for the first 2 weeks post-transplant. UPCR increased to 12.7 g/g along with a rise in IgG and IgM on flow crossmatch. A biopsy performed on POD 17 demonstrated glomerulitis, peritubular capillaritis, diffuse C4d staining (Banff g2 t0 i0 ptc0-1 v0 c4d3, cg0 ct0 mm2 ci0 ti0 cv0 ah0 i-ifta0), C3 deposition in the glomeruli, and 90% podocyte foot process effacement. The AMR treatment plan included 10 plasmapheresis sessions, low-dose IVIG, C3/C3b complement inhibitor, and 3 mg/kg rATG. POD 57 and 85 biopsies confirmed the absence of AMR and the resolution of foot process effacement. On POD 73 the patient was administered IL-6 receptor blockade for a monocyte rich transcriptomics signature on POD 57 biopsy. Following the establishment of histologic immunologic quiescence the patient was started on anti-proteinuric agents to lower her still-present nephrotic range proteinuria. Unfortunately, despite these agents the patient remained with proteinuria that averaged 5.8 ± 5.8 g/g daily. She continued to maintain excellent xenograft function, however, with a stable sCr of 1.59 ± 0.32 mg/dL that increased to 2.06 mg/dL once fully optimized on anti-proteinuric agents. The development of two severe soft tissue infections of the skin, the second of which was accompanied

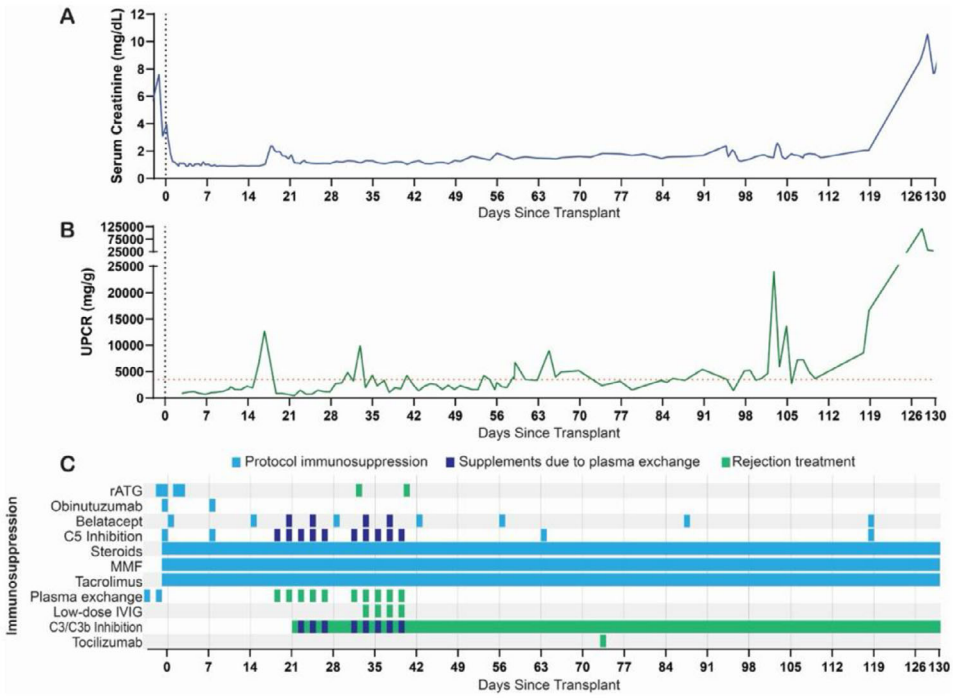


FIGURE 1 | 203.2

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by gram-negative bacteremia, prompted the cessation of IL-6 receptor blockade and a reduction in calcineurin inhibitor goal by 50%. The patient was maintained on mycophenolate mofetil, prednisone, belatacept, and complement inhibition. On POD 125, the patient was noted to have a rise in sCr from a baseline of 2.05 to 3.99 mg/dL. Her kidney function continued to rapidly decline, with sCr peaking at 10.56 mg/dL. POD 128 biopsy demonstrated loss of normal renal architecture and extensive, severe interstitial hemorrhage. The patient underwent explant on POD 130 and returned to dialysis.

Conclusions: Following successful treatment of an early episode of AMR, we were able to maintain a Xenokidney in a living human being for 130 days, establishing the longest surviving recipient of a xenotransplant. The patient's xenograft failed in the setting of reduced immunosuppression due to recurrent infections and the likely loss of monoclonal drug in her urine due to persistent, nephrotic-range proteinuria.

Funding: This data is supported by funding from United Therapeutics Corporation, PBC. Tal Eitan is supported by the National Cancer Institute, National Institutes of Health, through Grant Award Number T32 CA193111.

205.1 | Evidence for Human Thymopoiesis in a Porcine Thymus Graft and T Cell Tolerance to the Source Pig in a Living Human Recipient of a Porcine Thymokidney Xenotransplant

Farshid Fathi[1], Pinar Gur Cetinkaya[1], Yasmeen S. Saad[1], Benjamin Vermette[1], Jeffrey Stern[2], Karen Khalil[2], Jacqueline Kim[2], Ian Jaffe[2], Imad Aljabban[2], Lars Burdorf[3], Adam D. Griesemer[2], Robert A. Montgomery[2], Megan Sykes[1,4,5]

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Study's purpose: Porcine thymic transplantation tolerizes xenogeneic recipients to the porcine source animal in animal models. A 54-year-old woman with complex comorbidities received a composite thymokidney transplant from a GGTA1 knockout pig, achieving initial renal function before xenograft failure for non-immunological reasons on postoperative day (POD) 47.

Methods: Serial peripheral blood mononuclear cell (PBMC) samples were analyzed by spectral FCM, and mixed lymphocyte reactions (MLRs) were performed on purified T cells to assess tolerance. Thymocytes were isolated from the thymic portion of the xenograft explant and analyzed using spectral FCM.

Results: T cell levels in peripheral blood declined to 10/μL post-induction therapy and recovered to a peak of 342/μL by POD28. By POD21, 64% of the recovering CD4 T cells displayed a CD45RA+CCR7+ "naïve" phenotype and expressed CD31, consistent with recent thymic emigrants (RTEs). Pre-transplant CD4 T cells included <10% RTEs (Figure 1A). FCM analysis on cells from the POD47 thymic xenograft revealed human CD45+ leukocytes (0.85%), including double-positive (CD4+CD8+), single-positive, and double-negative thymocytes. The double-negative cells expressed markers such as CD5, CD7, CD1a, and CD34, consistent with human T cell progenitors in the porcine thymus (Figure 1B). MLR results demonstrated progressive hyporesponsiveness toward the source pig with preserved responses to third-party pig and allogeneic human cells,

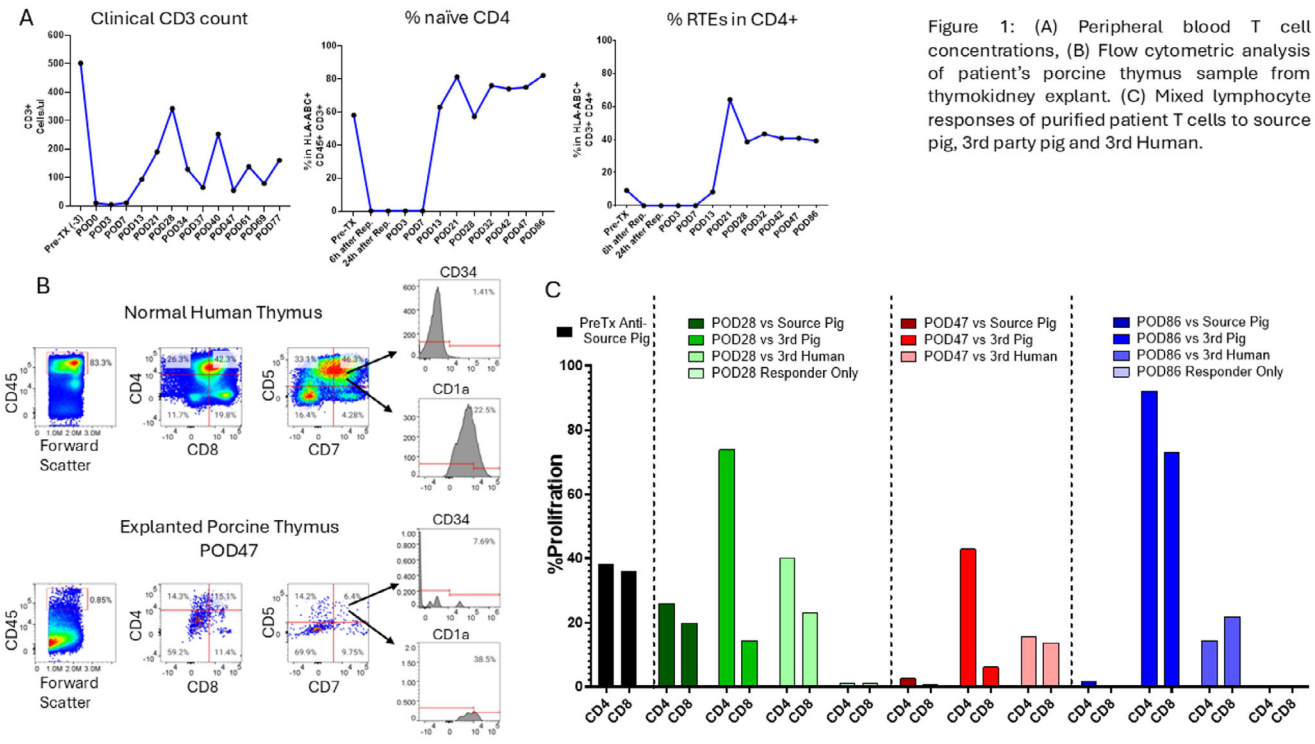


Figure 1: (A) Peripheral blood T cell concentrations, (B) Flow cytometric analysis of patient's porcine thymus sample from thymokidney explant. (C) Mixed lymphocyte responses of purified patient T cells to source pig, 3rd party pig and 3rd Human.

FIGURE 1 | 205.1

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achieving full donor-specific unresponsiveness by POD47 and POD86 (Figure 1C).

Conclusion: These findings suggest that a porcine thymus in a thymokidney xenograft supported human thymopoiesis and the development of T cells tolerant to the pig. Thymic transplantation is a promising approach to achieving T cell tolerance in xenotransplant recipients.

205.2 | Combined Porcine Thymus Transplantation and Mixed Chimerism Induction Achieves Human T Cell Tolerance in the Presence of a Human Thymus

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Introduction: Pig thymus transplantation can tolerize human T cells to xenografts in human immune system (HIS) mice. However, studies in immunocompetent mice have shown that recipient thymectomy is essential for the success of this approach. Avoidance of recipient thymectomy would enhance the appeal of this approach in humans. Additionally, human T cells developing in pig thymus lack tolerance to human tissue-restricted antigens (TRAs) and show reduced responses to antigens presented by HLA and suboptimal homeostasis in the periphery. Pig/human mixed chimerism (MC) can induce central deletional T cell tolerance as well as NK cell and B cell tolerance. We hypothesized that combining MC with pig thymus transplantation in HIS mice in the presence of a human thymus might overcome all of these limitations. Human thymus could delete human TRA-reactive T cells and generate TRA-specific regulatory T (Treg) cells while enhancing HLA-restricted responses and homeostasis. The pig thymus would generate pig-specific Tregs and SLA-restricted T cells to protect the xenografts from infections. MC could assure central deletion in both thymi and allow T cells selected by both human HLA and pig SLA molecules to receive HLA and SLA-restricted antigenic stimulation and homeostatic signals in periphery. In addition, this approach would permit tolerance induction without thymectomy in human recipients.

Method: Thymectomized NSG, pig cytokine-transgenic (PCT) NSG, or PCT-NOD/SCID mice were sublethally irradiated, followed by injection of pig bone marrow cells and implantation of fetal pig thymus and human fetal thymus tissue under the capsule of each kidney. Human fetal CD34+ cells, hematopoietic stem cells (HSCs) were injected intravenously. Control groups received dual pig/human thymus plus human HSCs, human thymus plus mixed chimerism, pig thymus plus human HSCs, and human thymus plus human HSCs. Human and pig chimerism were monitored. At 17–20 weeks post-transplantation, tolerance of peripheral human T cells to donor pig and human antigens was assessed by mixed lymphocyte reaction.

Results: Human T cells from chimeric recipients of human thymus alone and of dual pig and human thymus were specifically hyporesponsive to both donor human and pig antigens, indicating tolerance. Depletion of Tregs led to increased anti-donor responses in animals with pig thymus alone without MC, but tolerance was largely deletional in animals with dual thymus and MC. Human and pig antigen-presenting cells were detected in pig and human thymi of mixed chimeric mice with dual thymus, consistent with ongoing clonal deletion in both thymi.

Conclusion: Transplantation of dual pig and human thymus combined with MC induction leads to tolerance of human T cells to autologous and pig antigens. These data suggest that combined MC and porcine thymic transplantation could achieve tolerance in humans without thymectomy, supporting the clinical application of this approach for xenograft tolerance induction.

205.3 | Urinary Loss of ANTI-CD154 Therapeutic Antibodies: The Impact of Proteinuria on Pig Kidney Xenotransplantation

Kohei Kinoshita[1], Maho Terashita[1], Akihiro Maenaka[1], Ivy A. Rosales[2], David Ayares[3], Seth Lederman[4], Robert B. Colvin[2], Richard N. Pierson III[1], Tatsuo Kawai[1], David K.C. Cooper[1]

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Purpose: Proteinuria is a common complication following pig kidney xenotransplantation. It is well-documented that therapeutic antibodies can be filtered and excreted in the urine in patients with proteinuria. Although blockade of the CD40/CD154 co-stimulation pathway is widely considered the optimal immunosuppressive strategy for xenotransplantation, we report here that therapeutic antibodies targeting these pathways may be lost in the urine under conditions of significant proteinuria. This urinary loss may result in subtherapeutic antibody levels, leading to inadequate immunosuppression and graft rejection.

Methods: Nine baboons received kidneys from gene-edited pigs (either triple-knockout or double-knockout with growth hormone receptor knockout, combined with six human transgenes: hCD46, hCD55, hTBM, hEPCR, hHO-1, and hCD47; Revivicor). The immunosuppressive regimen included induction therapy with anti-thymocyte globulin, an anti-CD20mAb, and C1-esterase inhibitor, followed by maintenance therapy with an anti-CD154mAb (Tonix-1500), rapamycin, methylprednisolone, and anti-IL-6 receptor blockade (tocilizumab). All animals were confirmed cytomegalovirus (CMV)-negative. Post-transplant monitoring included blood counts, serum and urine analyses, ultrasound examinations, and renal biopsies.

Results: Four of nine recipients who survived beyond four months developed persistent nephrotic-range proteinuria (urine protein-to-creatinine ratio >3.5g/g). However, all four maintained normal or near-normal serum creatinine levels for weeks to months. Histopathological analysis of these grafts revealed focal glomerular thrombotic microangiopathy (4/4), transplant glomerulopathy (3/4), focal segmental glomerulosclerosis (2/4), or membranous nephropathy (1/4), without classical features of xenograft rejection. Proteinuria correlated with urinary loss of therapeutic antibodies (Figure 1A), and low serum albumin levels

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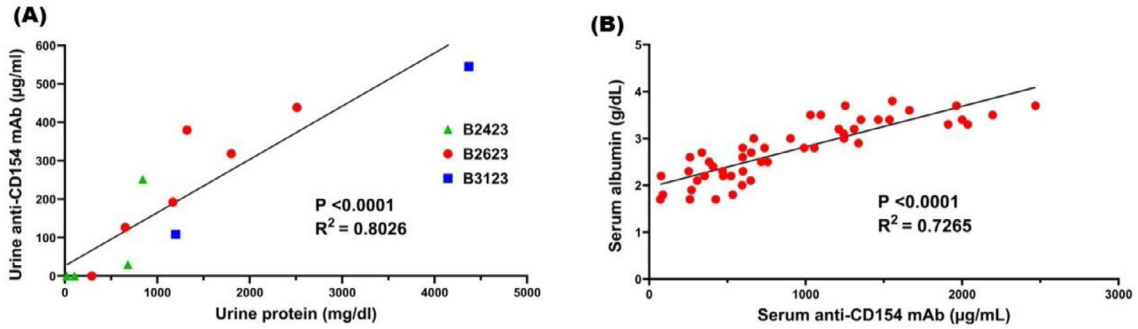


FIGURE 1 | 205.3

were associated with reduced trough levels of anti-CD154mAb (Figure 1B).

Conclusions: Previously, we reported a single case of urinary loss of anti-CD154mAb associated with proteinuria. The present study expands on this finding by documenting nephrotic-range proteinuria in four baboons. We suggest that the development of nephrotic-range proteinuria remains a critical barrier to successful xenotransplantation, as it leads to the urinary loss of therapeutic immunosuppressive antibodies and increases the risk of graft rejection by inadequate immunosuppression. Elucidating the upstream mechanisms leading to proteinuria will be essential for the success of clinical pig kidney xenotransplantation.

Funding: Work on xenotransplantation in DKCC's laboratory is supported in part by NIH NIAID U19 grant AI090959 and in part by a Kidney X Prize from the US DHHS and the American Society of Nephrology.

205.4 | Immunosuppression Weaning in a Pig-to-Baboon Thymokidney Xenotransplant Model

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Introduction. Chronic rejection and immunosuppression (ISP)-associated toxicity and infection are persistent obstacles to successful xenotransplantation. The ultimate solution could be the induction of tolerance. This study investigated thymus-dependent tolerance induction in a pig-to-baboon thymokidney (TK) xenotransplantation model using GalT-KO inbred miniature swine.

Methods. GalT-KO SLAhh inbred miniature swine were used as the TK donor animals. The recipient baboons (n = 2, B9420 and B6521) underwent total thymectomy and induction therapy with rATG and rituximab. On Day 0, they underwent bilateral nephrectomy, splenectomy and received a life-supporting TK graft. Post-transplant ISP consisted of MMF, anti-CD40 mAb, rapamycin, and belatacept. ISP was gradually weaned after post-transplant day 180 in B6521 and day 380 in B9420. Protocol graft biopsies were performed. Donor-specific antibodies (DSA), absolute T cell, B cell, and recent thymic emigrant (RTE) counts were monitored. Mixed lymphocyte reaction (MLR) assays were performed to monitor tolerance development.

Results. Both baboons recovered well from the surgery and maintained a stable clinical course until ISP weaning. RTEs, defined as CD45RA+ CCR7+ CD28+ CD95− CD31+, started to appear 2 months after the transplant (peaking at 33 cells/µL for B9420 and at 55 cells/µL for B6521). After Day 120, consecutive MLR assays indicated pig-specific hyporesponsiveness in both animals. Following 180 days (B6521) and 380 days (B9420) of stable graft function, ISP was gradually weaned. Both animals maintained stable renal functions after withdrawal of MMF, rapamycin, and anti-CD40 mAb. After 61 days (B9420) and 77 days (B6521) of subsequent belatacept monotherapy, the kidney function rapidly deteriorated in both B6521 and B9420, and the animals were euthanized on Days 415 and 502, respectively. B9420 maintained its hyporesponsiveness until the endpoint MLR, while B6521 recovered anti-donor responses during weaning of IS. In B9420, CD25-depletion MLR indicated that hyporesponsiveness was Treg-mediated. IgM and IgG DSA remained unchanged for the duration of the study, while IgA DSA increased 2-fold in B9420 during monotherapy with Belatacept. Before ISP weaning, biopsies in both animals showed mild transplant glomerulopathy and fibrosis with no sign of rejection or immunoglobulin and complement deposition. In contrast, biopsy at the time of euthanasia in both animals showed acute T-cell and chronic antibody-mediated rejection with IgA, IgM, IgG, C4d, and MAC deposition.

Conclusion. Donor-specific hyporesponsiveness was achieved in thymokidney xenotransplantation from a GalT-KO SLAhh swine donor. Although clinical tolerance was not achieved, the kidney grafts survived >1 year, including >2 months on belatacept monotherapy in both animals. Our study suggests that ISP can be markedly reduced but not fully discontinued after TK xenotransplantation, in association with donor-specific hyporesponsiveness.

Funding: This project was funded by NefroHealth and ChoironeX. Research reported in this publication was performed in the CCTI Flow Cytometry Core, supported in part by the Office of the Director, National Institutes of Health, under awards S10OD030282. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

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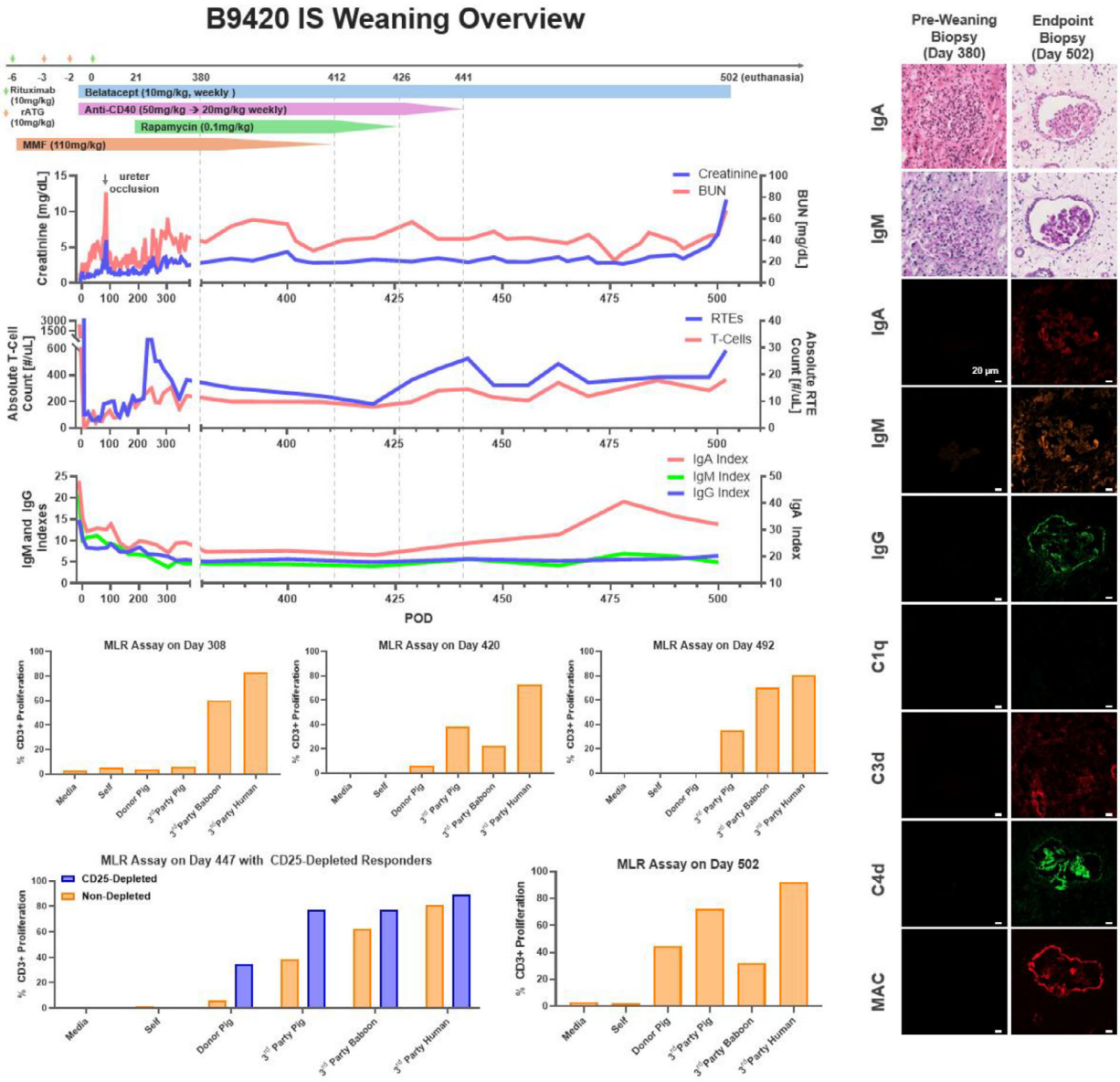


FIGURE 1 | 205.4

205.5 | Engineered MSCS Releasing CCL22 Remodel the Local Immune Microenvironment to Promote Graft Tolerance

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Introduction: Transplantation is essential for patients with severe organ damage or end-stage organ failure. However, challenges like immune rejection, chronic inflammation, infections, and poor graft survival severely limit long-term transplant success. While immunosuppressive therapies prevent rejection, their long-term use often leads to serious side effects. Thus, inducing local immune tolerance without extensive immunosuppression remains crucial. Chemokine CCL22 plays roles in various physiological and pathological processes through chemotactic and non-chemotactic mechanisms. This study explores engineered mesenchymal stem cells (MSCs) that locally release CCL22, examining their ability to reshape the immunosuppressive microenvironment.

Methods: We established a murine allogeneic skin transplantation model, performing graft rejection scoring across different experimental groups. Grafts were analyzed using H&E staining, immunohistochemistry, immunofluorescence, and ELISA. In vitro, the function of CCL22 was assessed through mixed lymphocyte reaction (MLR) assays, flow cytometry, and ELISA. Mechanistic insights were obtained by blocking the CCR4 receptor. Additionally, MSCs overexpressing CCL22 (CCL22-OE MSCs) were generated and subcutaneously transplanted into mice, with subsequent analyses of the transplantation sites.

Results: CCL22 markedly prolonged graft survival, reduced inflammation, enhanced regulatory T cell (Treg) infiltration, and lowered rejection scores in the mouse model. In vitro assays showed CCL22 efficiently recruited Tregs. MLR assays

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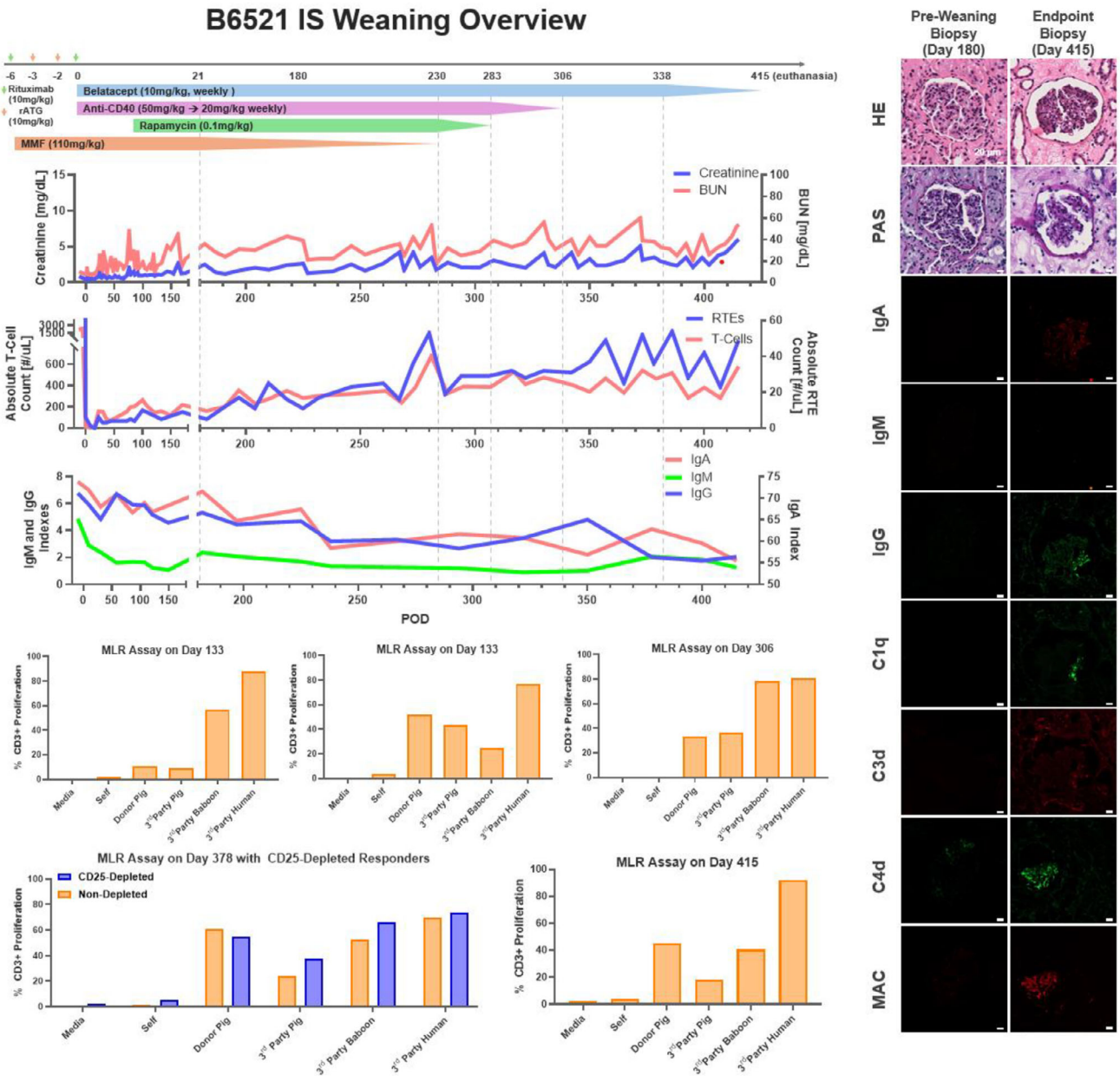


FIGURE 2 | 205.4

demonstrated that CCL22 increased Tregs' suppressive capabilities against T-cell proliferation. Flow cytometry revealed CCL22 upregulated Treg-specific transcription factor FOXP3 and surface markers GITR and ICOS. ELISA indicated that CCL22 treatment elevated production of immunoregulatory cytokines IL-10 and TGF-β, alongside promoting Ca^{2+} influx. Mechanistically, these effects depended on the CCR4 receptor, as demonstrated by the loss of Treg recruitment and function upon administration of CCR4 antagonist C-021. Immunofluorescence analyses showed that subcutaneous transplantation of CCL22-OE MSCs significantly enhanced local Treg recruitment, markedly decreased M1 macrophages, and increased M2 macrophages. Additionally, in vitro coculture experiments revealed that CCL22-OE MSCs induced macrophage polarization toward the M2 phenotype.

Conclusion: Engineered MSCs secreting CCL22 robustly recruited immunosuppressive Tregs and strengthened their suppressive function via the CCL22-CCR4 pathway. Concurrently, these cells promoted local macrophage polarization to the anti-inflammatory M2 phenotype, increased local anti-inflammatory cytokine secretion, reshaped the immune microenvironment, suppressed graft rejection, and significantly prolonged graft survival. This study enhances our understanding of chemokines in transplantation, providing promising insights to reduce immune rejection and graft loss.

Funding: The Key project of the Science and Technology Program of Hunan Province (Grant No.2023ZJ1100). The National Natural Science Foundation of China (Grant No:82372071 and 82272102).

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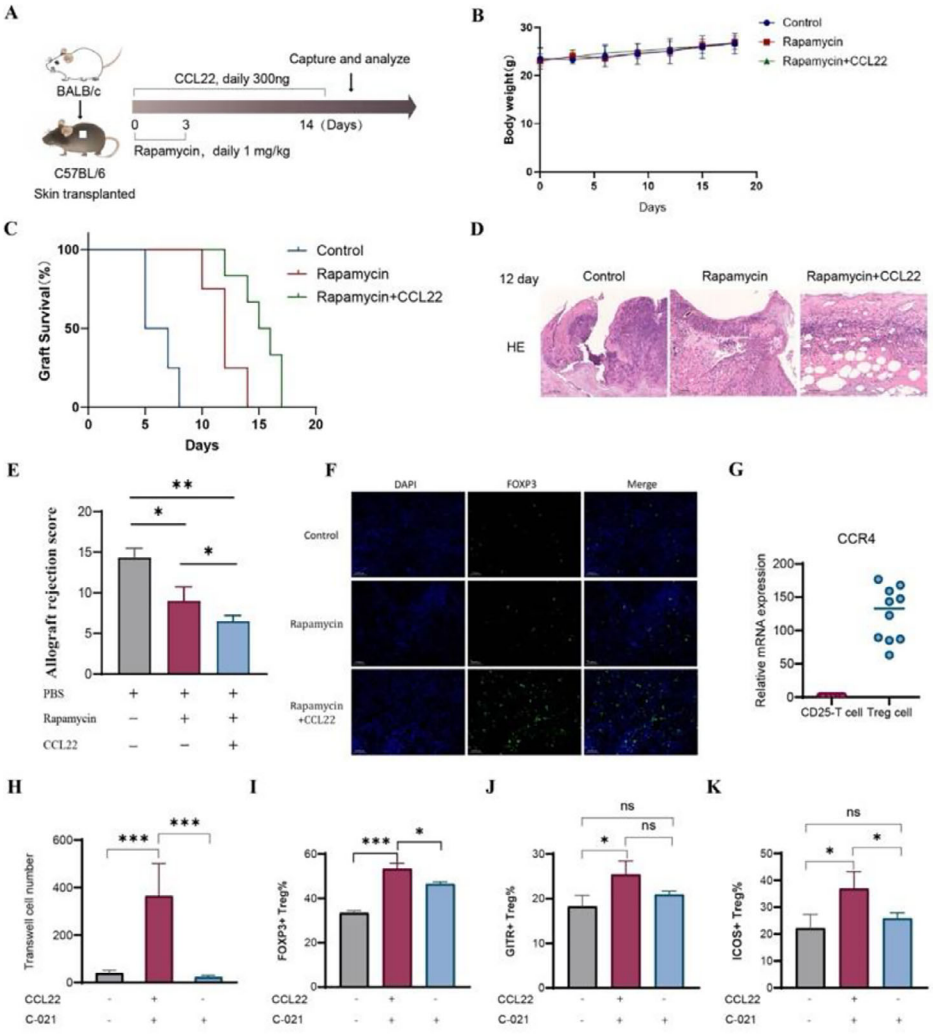


FIGURE 1 | 205.5

206.1 | Development of a Humanized Mouse Model to Assess Neonatal Porcine Vs. Human Islet Engraftment and Rejection

Purushothaman Kuppan[1,2], Joy Paramor[1,2], Mandy Rosko[1,2], Chelsea Castro[1,2], Nerea Cuesta-Gomez[1,2], Arghyadip Bose[1,2], Riley Reid[1,2], Karen Seeberger[1,2], Andrew R. Pepper[1,2], Gregory S. Korbutt[1,2]

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Introduction: T-cell-mediated immune rejection remains a significant translational hurdle in clinical islet transplantation. A comprehensive understanding of how the adaptive immune system responds to an islet graft is essential for developing strategies to prevent islet rejection. Establishing a human immune system in mice through reconstitution with human peripheral blood mononuclear cells (hPBMCs) can provide a valuable model for elucidating the adaptive immune responses to allogeneic and xenogeneic islet grafts. The objective of this study was to assess islet rejection in humanized NSG MHC I/II double knockout (NSG) mice transplanted with either human (HIs) or neonatal porcine islets (NPIs).

Methods: Diabetic NSG mice were transplanted with either 1500 HI ($n = 5$) or 3000 NPIs ($n = 12$) under the kidney capsule. Graft function was assessed by weekly non-fasting blood glucose (BG) measurements. After animals achieved normoglycaemia, they received 40×10^6 hPBMCs intraperitoneally, and islet graft rejection was subsequently assessed by measuring non-fasting BG. Both rejected and non-rejected grafts were collected for histological analysis.

Results: All HI recipients (5/5) became normoglycemic within 2 days post-transplant and maintained normoglycemia for 40 days until administration of hPBMCs. After hPBMC infusion, 100% of the animals reverted to a hyperglycemic state within 11.2 ± 1.7 days. Of the 12 NSG mice transplanted with NPIs, 10 mice achieved normoglycemia (10/12: 83%) within 10 to 27 weeks post-transplant and were administered hPBMCs when normoglycemia was achieved. Following hPBMCs reconstitution, only 10% of NPI recipients (1/10) became hyperglycemic. Survival graft-bearing nephrectomy was conducted on normoglycemic recipients, and the subsequent return to hyperglycemia confirmed graft-dependent function. Immunofluorescence staining confirmed rejection of HIs as shown by significant hCD45

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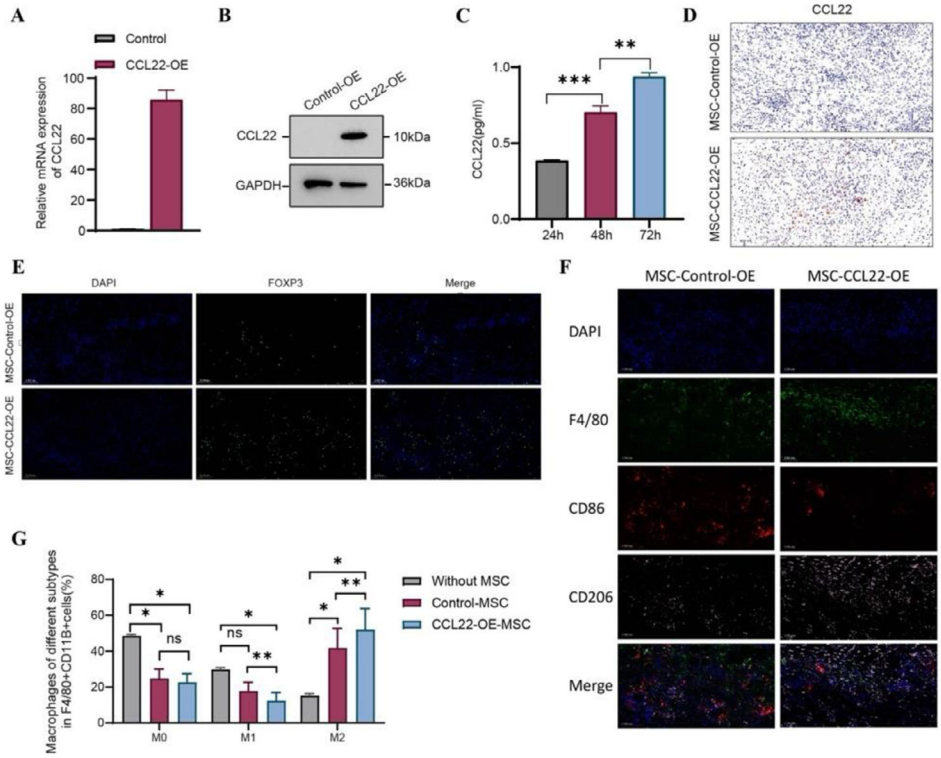


FIGURE 2 | 205.5

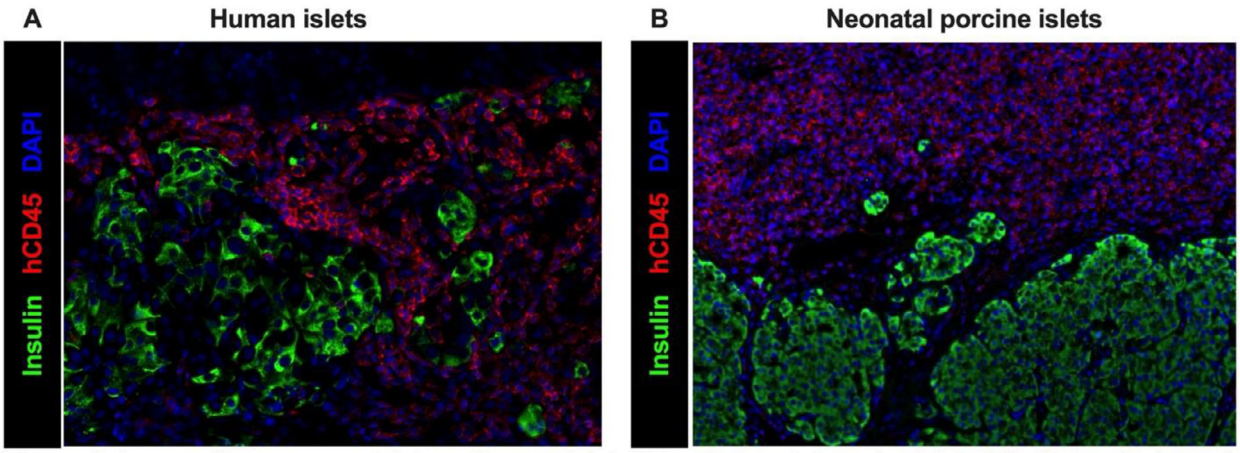


Figure 1: Immunofluorescence staining of human islets [A] and neonatal porcine islets [B] with insulin (green) and human CD45 (red)

FIGURE 1 | 206.1

infiltration and few surviving β-cells (Figure 1A). Similar morphology was observed in the one NPI transplanted mouse that demonstrated rejection. In contrast, NPI grafts in mice that did not reject exhibited extensive hCD45 infiltration and no reduction in β-cell mass (Figure 1B).

Conclusion: These data demonstrate that human islets are highly susceptible to hCD45 cell infiltration and subsequent rejection in a humanized mouse model. In contrast, NPI grafts become infiltrated with hCD45$^+$ cells however, with limited or no islet destruction, thereby suggesting that NPIs could be potentially hypoimmunogenic.

206.2 | Combined Islet and Kidney Xenotransplantation for Diabetic Nephropathy: Islet-After-Kidney Transplantation With Cure of Diabetes and 6 Months of Stable Graft Function in Life-Supporting Pig-to-Baboon Xenotransplantation

Hayato Iwase[1], Weili Chen[1], Daniel Eisenson[1], Yu Hisadome[1], Wanxing Cui[2], Michelle Santillan[1], Alexander Schulick[1], Kasra Shirini[1], Saghar Babadi[1], Du Gu[1], Amanda Maxwell[3], Kristy Koenig[1], Savannah Windley[1], Zhaoli Sun[1], Daniel Warren[1], Andrew Cameron[1], Kazuhiko Yamada[1]

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Introduction: Diabetes is a leading cause of end stage renal disease. Combined islet and kidney transplantation (Tx) through islet-after-kidney Tx may prove curative for patients with diabetic nephropathy. Preclinical studies of pig-to-NHP XTx have demonstrated prolonged survival of porcine kidney or islet xenografts. However, no study to date has described durable function of both porcine kidney and islets in a NHP XTx model. Here we report the first case of combined porcine kidney and islet pig-to-NHP XTx resulting in 6 months life-supporting function of porcine kidney and islet xenografts with stable renal function and normoglycemia in a diabetic baboon with delayed islet-after-kidney XTx.
Methods: Two baboon recipients (MD Anderson) underwent vascularized thymic lobe (VTL) and kidney XTx from a size-matched GalTKO.hCD55 pig (10.7 and 13 kg) on Day 0, followed by islet Tx from a large GalTKO.hCD55 pig (95 and 130 kg) (NSRRC: University of Missouri) on POD 11 and 19. Islet isolation yielded 101K and 254K IEQs (194K and 379K IPN). The baboon recipients underwent induction with rATG and Rituximab prior to XTx and immunosuppression was maintained with anti-CD40mab, CTLA4-Ig, MMF. Streptozotocin was given twice at 100mg/kg on POD 5, 50 mg/kg on POD 9 in case1, 120mg/kg on POD 13, 60mg/kg on POD 16 in Case 2 to induce IDDM prior to islet XTx.
Results: Diabetic induced by high-dose STZ and nephrectomized baboon recipients demonstrated normal serum creatinine and normoglycemia with islet-after-kidney XTx. Both baboons maintained normal blood glucose (BG) levels and normal creatinine levels after XTx. One recipient maintained normal serum creatinine with no evidence of rejection for six months but was euthanized due to sepsis related to pyelonephritis on POD180. Immediately after islet Tx, hyperglycemia was reversed with normalization of BG from >250mg/dL to 80–110 mg/dL without any exogenous insulin treatment. Post-mortem evaluation of liver confirmed presence of insulin-staining islets, and VTL graft was viable. ELISpot assay at necropsy demonstrated pig-specific unresponsiveness. The other recipient also maintained normal BG levels after delayed islet Tx, but developed acute deterioration triggered by post-obstructive nephropathy and was euthanized on POD50.
Conclusion: Although further studies will be required, our findings in islet-after-kidney XTx are promising and represent the first demonstration of successful cure of diabetes and end-stage renal disease with the evidence of donor-specific unresponsiveness in vitro in diabetic and nephrectomized NHP receiving pig kidney, VTL, and islet XTx.

206.3 | Preliminary Evaluation of Local Expression of a Human T Cell Depleting Monoclonal Antibody by Diliximab-hCD55-hCD59-Galtko Pig Islet Xenografts in Diabetic Mice and Baboons

Evelyn J. Salvaris[1], Erin Fuller[2], Thanh Le[2], Nella Fisicaro[1], Stephen McIlfatrick[3], Mark B. Nottle[3], Wayne J. Hawthorne[2,4], Peter J. Cowan[1,5]

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Background: Our preclinical model clearly demonstrates restoration of blood glucose control and long-term islet cell function after transplantation of GTKO-hCD55-hCD59 porcine neonatal islet cell clusters (NICCs) into diabetic immunosuppressed baboons. However, T cell-mediated rejection inevitably occurs following withdrawal of maintenance immunosuppression. We hypothesized that local expression of an anti-T-cell monoclonal antibody by the xenografts may prevent rejection after withdrawal of all exogenous immunosuppression. We previously generated a transgenic (Tg) pig line, using CRISPR to integrate a transgene for diliximab, a chimeric anti-human CD2 monoclonal antibody, into GGTA1. Transgene expression was driven by a mouse MHC class I promoter for ubiquitous tissue expression. Diliximab expression was detected in all pig organs tested, including the pancreas.
Aim: To examine local expression of diliximab produced by Tg pig NICC in pig-to-mouse and pig-to-NHP NICC xenograft models.
Methods: Diliximab-GalTKO and hCD55-hCD59-GalTKOpigs were crossbred to produce diliximab-hCD55-hCD59-GalTKO pigs. Genotyping was performed by PCR. Tg pigs were tested for diliximab expression by ELISA (serum), RT-qPCR, and immunohistochemistry (tissues). Diliximab-GalTKO and control GTKO NICCs were transplanted under the kidney capsule of streptozotocin-diabetic SCID mice, and diliximab-hCD55-hCD59-GalTKO NICCs were transplanted intraportally into two diabetic baboons. NICC xenograft maturation and function and diliximab expression were assessed histologically following biopsy and NICC function by daily blood sugar levels (BSL) and glucose tolerance tests.
Results: Diliximab NICC xenografts restored normoglycemia in diabetic immunodeficient mice and in our diabetic baboons. In the mouse, detectable diliximab expression was seen in situ on Day 85, and from transplanted baboon biopsies at Days 112, 114, and endpoint. Systemic porcine C-peptide was detected from Day 30 onwards. Baboon liver biopsies showed well-preserved morphology with no cellular infiltrate (H&E) and staining for insulin, somatostatin, and glucagon. Moderate to strong expression of diliximab was detected on the islets and in the surrounding biopsy tissues.
Discussion/Conclusion: Islet cell function remains unaffected by the addition of the diliximab transgene in both mouse and baboon models. Preliminary immunohistochemistry data in baboon model demonstrate that islet xenografts express diliximab on the NICCs and in the surrounding recipient liver tissue, confirming local secretion of anti-CD2 by the Tg pig islets. Whether this level of diliximab expression will be sufficient to prevent T-cell-mediated islet xenograft rejection is pending ongoing investigation.

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206.4 | Unlocking the Potential of IL-13 in Islet Xenotransplantation: A Novel Player in Xenotolerance Induction

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Purpose: Interleukin-13 (IL-13) is a pleiotropic cytokine with both pro- and anti-inflammatory effects. While its anti-inflammatory properties have shown promise in type 1 diabetes, its potential role in islet transplantation remains completely unexplored. Therefore, our goal was to determine if IL-13 administration can drive a pioneering advancement in minimal-dose islet transplantation (syn-, allo-, and xenogeneic) by reducing the number of islets required for successful engraftment and accelerating the achievement of permanent normoglycemia.
Methods: 8–10-week-old female C57BL/6 mice were rendered diabetic by a single injection of Streptozotocin (200mg/kg body weight) and used as transplant recipients. Mice were divided into a test group that received islets accompanied by IL-13 treatment, a control group that received islets with saline, and a control group that received IL-13 alone with no islets. 50 C57BL/6 islets, 400 BALB/c islets, or 3000 human islet equivalents were transplanted under the kidney capsule of diabetic mice for minimal dose syngeneic (n = 10/grp), allogeneic (n = 12/grp), and xenogeneic (n = 5/grp) transplantation, respectively. For the syngeneic test group, islets were preincubated with 10ng IL-13/mL media for 3 h before transplantation. For the allogeneic test group, 30ng IL-13/100μL saline was administered to islet recipients via IP injection five times every 48 h post-transplant on Days 2, 4, 6, 8, and10, while for the xenogeneic test group, recipients received 50ng IL-13/100μL saline five times post-transplant on Days 4, 6, 8, 10 and 12. Tail vein blood glucose (BG) was measured daily.
Results: Eighty percent of diabetic recipient mice receiving syngeneic islets pretreated with IL-13 returned to normoglycemia within 16 days, and by Day 20, 100% were rescued. On Day 33, the last day of the observation period, the BG for IL-13-treated mice was 148.4 ± 27.3 mg/dL, while, in contrast, BG was 361 ± 70.9 mg/dL for 55% of saline-treated control mice that remained diabetic. Following minimal dose islet allotransplantation, all control group mice became diabetic on Day 12 ± 8 post-transplantation, whereas all mice receiving islets with IL-13 remained non-diabetic post-transplantation until Day 90, the period of observation. Diabetic recipients receiving xenogeneic islets with IL-13 regained permanent normoglycemia by Day 25 ± 1 with BG 225.2 ± 49.5 mg/dL and remained cured until Day 45, the period of observation, with BG 215.6 ± 60.1 mg/dL, while mice in the control group remained diabetic. Diabetic mice receiving IL-13 with no islet transplantation did not return to normoglycemia.
Conclusions: These findings provide the first evidence that IL-13 therapy significantly enhances islet graft outcomes in syngeneic, allogeneic, and xenogeneic settings. IL-13 reduces the islet dose required and accelerates the restoration of permanent normoglycemia, demonstrating strong translational potential for improving clinical islet transplantation success.
Funding: This work was supported by Manning Family Foundation.

206.5 | Targeting the Redox Balance of Beta Cells by SH/S–S Conversion for Islet Transplantation

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Introduction: Islet graft dysfunction is mainly driven by hypoxia-induced injury. ROS generated under hypoxic conditions oxidize membrane thiol (–SH) groups into disulfides (S–S), disrupting redox homeostasis and aggravating islet damage. However, ROS-targeted therapies have shown limited efficacy, highlighting the need for alternative strategies. In this study, we developed fusogenic liposomes (DA-1) that anchor to the cell membrane and competitively scavenge hypoxia-induced ROS. This preserves surface –SH groups, restores redox balance, and alleviates oxidative injury, ultimately improving islet cell survival and function. Mechanistically, this redox stabilization enhances mitochondrial energy metabolism, supporting β-cell viability and insulin secretion, and improving transplantation outcomes.
Method: Islet cell activity was assessed by flow cytometry, redox status by DCFH-DA fluorescence, and insulin secretion following glucose stimulation by ELISA. Transmission Electron Microscope (TEM) was performed to examine ultrastructural features of the islet. Neonatal porcine islets were transplanted under the renal capsule of NCG mice. Fourteen days post-transplantation, grafts were retrieved for transcriptomic analysis. Graft function was evaluated by insulin immunohistochemistry, viability by TUNEL staining, and redox homeostasis by thiol-specific labeling.
Results: In vitro, DA-1 efficiently bound islet membranes (>95% at 15 min, 10 μg/mL) and was retained >90% for 7 days. Under hypoxia conditions, islet cells exhibited disrupted redox homeostasis, evidenced by reduced membrane thiol (–SH) levels due to excessive ROS accumulation. DA-1 treatment significantly restored membrane thiol expression (45.60% ± 5.51% vs. 59.87% ± 2.53%) and lowered intracellular ROS levels (mean fluorescence intensity: 132.90 ± 3.70 vs. 87.50 ± 6.75), thereby improving islet cell viability (29.33 ± 2.98% vs. 46.20 ± 9.00%) and glucose-stimulated insulin secretion (5.15 ± 2.26 vs. 22.58 ± 6.20 mIU/L). In vivo, DA-1–treated grafts maintained surface thiol expression, preserved insulin function, and reduced TUNEL+ apoptosis at Days 3, 7, and 14 post-transplantation, in contrast to progressive functional decline in controls. Correlation analysis revealed that higher membrane thiol levels are positively associated with better graft viability and insulin function. Transcriptomic analysis revealed enrichment in redox and mitochondrial metabolic pathways, confirmed by GO/KEGG. TEM further demonstrated DA-1–mediated preservation of mitochondrial ultrastructure under hypoxia, supporting its role in maintaining redox balance and mitochondrial energy metabolism to enhance graft function.
Conclusion: This study highlights redox regulation as a key determinant of islet graft outcome under hypoxia stress. By

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stabilizing the SH/S–S redox interface and protecting mitochondrial function, DA-1 offers a promising strategy to enhance islet viability and therapeutic efficacy in transplantation.

Funding: This work was supported by the Natural Science Foundation of China (Grant No. 82272102), the Natural Science Foundation of Hunan Province, China (Grant No. 2025JJ50714), and Natural Science Foundation of Changsha, China (Grant No. kq2403066).

207.1 | Attenuation of Platelet Activation and Thrombosis in a Dynamic Shear Flow Model With GalTKO.hCD55.hTBM Porcine Endothelium

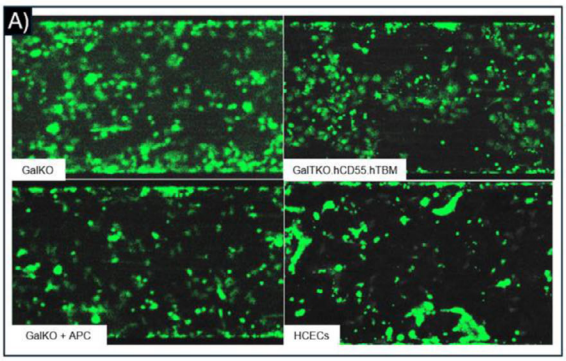
Megan Dufault[1], Seyedamir Sanatkar[1], Zahra Habibabady[1], Victoria Diaz[1], Jonathan Schulz[1], Caroline Gomes Lucas[2], Kevin D. Wells[2], Kristin M. Whitworth[2], Richard N. Pierson III[1]

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Introduction: Long-term xenograft survival is hindered by coagulation pathway dysregulation, marked by the inefficient binding of inactivated protein C (PC) to porcine thrombomodulin (TBM). The TBM/thrombin-mediated cleavage of PC into an active form drives anti-coagulatory, anti-inflammatory, and anti-apoptotic pathways through the endothelial protein C receptor (EPCR). This study evaluates the effect of hTBM inclusion in the porcine genome on platelet activation, adhesion, and aggregation within a dynamic in vitro xenoperfusion model.

Methods: Genetically modified porcine aortic endothelial cells (PAECs) from GalKO (n = 13) and GalKO. β4GALKO.NT2LKO.hCD55.hTBM (n = 7) pigs (GalTKO.hCD55.hTBM) between passages three to six and immortalized human coronary endothelial cells (HCECs, n = 7) were cultured to confluence in microfluidic channels, then treated for 2.5 h with 20ng/mL rhTNF-α. One additional group of GalKO cells was treated with 5.5µg/mL human APC to mimic the intended functionality of hTBM (n = 6). Channels were perfused at 5 dynes/cm^2 at 37°C for 1 h with human whole blood stained with 0.5mM Calcein to visualize thrombosis formation with sequential images generated via Bioflux Montage software (Cell Microsystems) over the duration of perfusion. Blood was collected via slow drip into a sodium heparin tube to minimize platelet activation prior to perfusion. Before and after perfusion, perfusate was collected and fixed in 1% PFA for CD62P analysis via flow cytometry (GalKO n = 8; GalKO + APC n = 6; GalTKO.hCD55.hTBM n = 12; HCECs n = 12). CD62P+ platelets were analyzed as the frequency of the CD41a+ parent population.

Results: Platelet adhesion after 1 h, measured as percent of surface area coverage, was significantly reduced with GalKO + APC (SA 4.43% ± 0.028%, p = 0.0008), GalTKO.hCD55.hTBM (SA 5.30% ± 0.052%, p = 0.0187), and HCECs (SA 3.48% ± 0.25%, p < 0.001) relative to GalKO (7.20% ± 0.45%). Platelet aggregation, measured as total fluorescence, was significantly reduced with GalKO + APC (94 ± 6.2, p = 0.008), GalTKO.hCD55.hTBM (100 ± 7.3, p = 0.015), and HCECs (102 ± 11.4, p = 0.018) relative to GalKO (146 ± 11.5) (Figure 1B). Fold change in frequency of circulating CD62P+ platelets by flow cytometry before and after xenoperfusion was significantly decreased in GalKO + APC (154.1 ± 3.1, p = 0.0097), GalTKO.hCD55.hTBM (158.8 ± 2.7, p = 0.0164), and HCECs (107.6 ± 2.9, p < 0.0001) relative to GalKO (176.9 ± 7.3). CD62P+ frequency was significantly lower in HCECs than GalKO + APC and GalTKO.hCD55.hTBM PAECs (p < 0.0001) (Figure 1C).



B)

Cell Type	GalKO	GalKO + APC	GalTKO.hCD55.hTBM	HCECs
n	13	6	7	7
Adhesion (% SA)	7.20% ± 0.45%	4.43% ± 0.28%	5.30% ± 0.52%	3.48% ± 0.25%
Δ		-38.50%	-26.47%	-51.74%
P		0.0008	0.0187	<0.0001
Aggregation (A.U.)	146 ± 11.5	94 ± 6.2	100 ± 7.3	102 ± 11.4
Δ		-35.77%	-31.46%	-29.94%
P		0.0069	0.0129	0.018

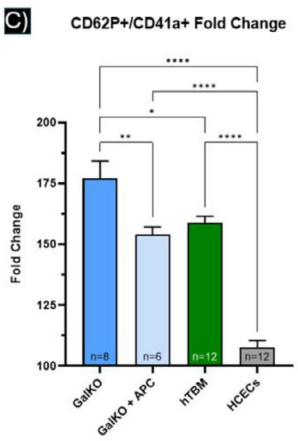


Figure 1: Inclusion of hTBM or APC treatment decreased platelet adhesion, aggregation, and activation when human whole blood was perfused over porcine endothelia.
A) Representative images of thrombosis formation after one hour of whole blood perfusion over porcine endothelia. B) Summary of adhesion and aggregation results following xenoperfusion. Relative change (Δ) and P values are calculated in relation to GalKO. Values are expressed as mean ±SEM. SA, percent surface area coverage. AU, arbitrary units. C) Fold change in CD62P+ expression, indicating CD41a+ platelet activation, compared to whole blood perfusate prior to xenoperfusion. * = p < 0.05; ** = p < 0.01; *** = p < 0.001; **** = p < 0.0001

FIGURE 1 | 207.1

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Conclusion: Conditions mimicking microcirculatory flow reveal an anti-thrombotic role of hTBM when PAECs are perfused with human blood. The inclusion of hTBM in the porcine genome is a promising method to combat dysregulated platelet-driven thrombosis in in vivo xenotransplant models.

207.2 | Anti-Pig Antibodies in Swine Veterinarian Serum: Implications for Clinical Xenotransplantation

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Introduction: Clinical cardiac and renal xenotransplantation and human decedent studies clearly demonstrate that hyperacute rejection of genetically engineered porcine organs, devoid of the major xenogeneic glycans, can reliably be avoided, but that antibody-mediated rejection (AMR) continues to limit graft survival. The clinical immune response to triple knockout (TKO) donor pigs is poorly understood, but under these conditions, AMR may be directed to pig endothelial cells protein antigens, including, but not restricted to, SLA antigens.

Methods: We previously identified porcine glycans and proteins that are immunogenic after cardiac xenotransplantation in nonhuman primates. In this study we use fluorescence barcoded human embryonic kidney cells (HEK) and HEK cell lines expressing porcine glycans (Gal and SDa) or proteins (tetraspanin-29 [CD9], membrane cofactor protein [CD46], protectin, membrane attack complex inhibition factor [CD59], endothelial cell protein C receptor, and Annexin A2) to screen antibody reactivity in human serum (Figure 1A,B). Anti-pig reactivity in serum from 160 swine veterinarians was analyzed as a serum source with potential occupational immune challenge from porcine tissues and pathogens.

Results: Human IgM binding was evident to Gal or Gal and SDa glycans in 84% of serum samples, with no apparent binding to pig proteins (Figure 1C and 2A,B). IgM binding to porcine proteins, primarily CD9 and CD46, previously identified as immunogenic in pig to nonhuman primate cardiac xenograft recipients, was detected in 28 of the 160 swine veterinarian samples (16%). The level of antibody binding to pig proteins was comparable to anti-SDa reactivity and often exceeded 5 standard deviations

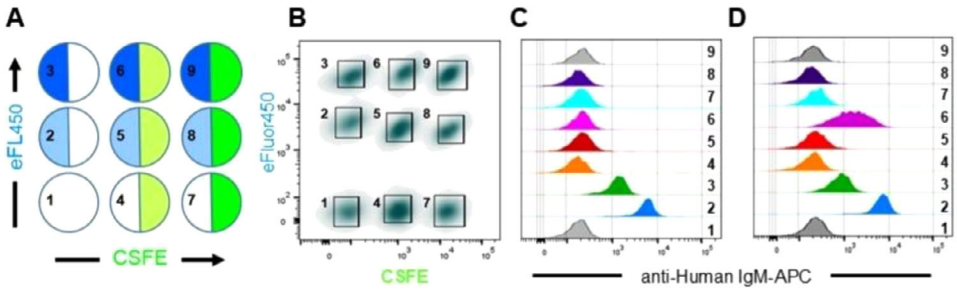


Figure 1. Barcoding of the HEK cell series and screening for human anti-pig antibody reactivity. **A.** Cells, 1; HEK control, 2; HEK-Gal, 3; HEK-SDa, 4; HEK-CD9, 5; HEK-CD59, 6; HEK-CD46, 7; HEK-PROC, 8; HEK-ANXA2, and 9; HEK control are stained with different levels of the vital dyes CSFE (x-axis) and eFluor450 (y-axis) as described in the Materials and Methods. **B.** The mixed barcoded population of HEK cells are stained with veterinarian serum and resolved into 9 distinct cell populations based on fluorescence of the vital dyes. Antibody binding to individual antigens is detected with an APC conjugated anti-human IgM and is evident by gating on each individual cell population **C.** All samples showed anti-Gal reactivity and 83% of veterinarian serum was reactive with Gal or with both Gal and SDa glycans with no apparent reactivity to porcine proteins. **D.** Approximately 17% of serum samples showed reactivity to one or more porcine proteins. In this example positive reactivity is evident to cell lines 2; Gal, 3; SDa, and 6; CD46.

FIGURE 1 | 207.2

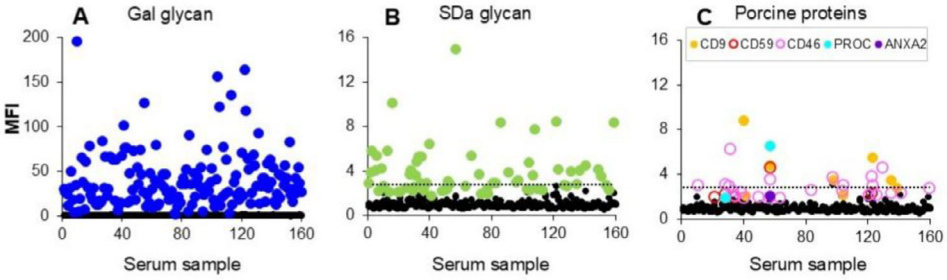


Figure 2. A summary of swine veterinarian serum IgM binding to glycan (**A** and **B**) and protein antigens (**C**). **A.** Shows anti-Gal IgM reactivity for 160 swine veterinarian serum samples. All samples had anti-Gal reactivity. **B.** Shows IgM binding to SDa for 65 serum samples (41%) with antibody binding greater than 3 standard deviations above background. **C.** Shows IgM binding to protein antigens for 28 serum samples (17%) with antibody binding greater than 3 standard deviations above background. Black filled circles in panels **A - C** are IgM binding to control HEK cells. The dotted line in **B** and **C** represents 5 standard deviations above the average MFI of all HEK control cells.

FIGURE 2 | 207.2

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above background binding to control HEK cells (Figures 1D and 2C).

Conclusions: These results suggest that the human non-Gal immune response to clinical xenotransplantation will not be restricted to swine SLA but may extend to a range of porcine endothelial cell membrane proteins. Sera from clinical xenotransplant recipients should be comprehensively analyzed to define a panel of commonly immunogenic porcine antigens. Displaying such an array of porcine non-Gal protein antigens on cells or at high density on solid substrates will create novel and highly sensitive assays to support clinical xenotransplantation by screening clinical blood products, monitoring patient immune responses to detect AMR, and assessing the effectiveness of therapies to reverse AMR.

207.3 | Induced Anti-Swine Leukocyte Antigen (SLA) Class I and Class II Antibodies Play Important Roles in Gene-Edited Pig-to-Rhesus Monkey Xenotransplantation

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Introduction: In gene-edited (GE) pig-NHP xenotransplantation, the induction of de novo antibodies after surgery is still an important factor affecting the long-term survival of the xenograft. However, it is not clear whether the porcine SLA antigen can induce significant anti-SLA antibody production in primate recipients after kidney xenotransplantation.

Methods: We performed 14 pig-rhesus monkey kidney xenotransplantation using kidneys from GTKO/hCD55 ($n = 7$) and GTKO/βGalNT2KO(DKO)/hCD55/TM ($n = 7$) pigs. Five recipients (GTKO: 2 cases, DKO: 3 cases) developed high levels of circulating antibodies against non-Gal antigens and experienced acute humoral xenograft rejection on Days 14 to 29. To investigate the role of anti-SLA antibodies in xenograft rejection, we used serum samples collected before transplantation and at the time of rejection to measure IgM/IgG antibody binding against the following cells by flow cytometry: (1) PBMCs from donor and GTKO/SLA-I/II KO pigs (to exclude interference from anti-Sda antibodies, serum from DKO group was adsorbed with WT pig RBC); (2) DKO/SLA-I^{+}/SLA-II^{-} and DKO/SLA-I^{++}/SLA-II^{++} pAECs (stimulated with pIFN-γ). Finally, a xenogeneic MLR system was established using PBMCs isolated from SLA^{+} (WT, GTKO, GTKO/βGalNT2KO, TKO) and GTKO/SLA-I/II KO pigs as stimulator cells and human PBMCs as responder cells.

Results: In the GTKO group, the binding of IgM and IgG antibodies against GTKO pig PBMCs was significantly (5 to 10 times) higher in the serum of two monkeys at the time of rejection than in serum collected before transplantation. The levels of IgM and IgG binding decreased significantly after the additional deletion of SLA-I/II (IgM decreased by 45% and 83%, IgG decreased by 54.4% and 57.7%, respectively). In the DKO group, the serum IgM/IgG antibody levels against DKO pig PBMCs were also elevate significantly at the time of rejection. After SLA-I/II deletion, the IgM binding levels in 2 cases decreased by more than 50%, and the IgG level in 3 cases decreased by more than 60%. The above results were further confirmed by using DKO/SLA-I^{+}/SLA-II^{-} and DKO/SLA-I^{++}/SLA-II^{++} pAECs, as the binding of IgM and IgG antibodies against pAECs was significantly elevated with the increase in SLA-I and SLA-II expression. Moreover, when anti-SLA-I antibodies were adsorbed by DKO/SLA-I^{+}/SLA-II^{-} pAECs, the IgM/IgG binding to DKO/SLA-I^{++}/SLA-II^{++} pAECs was reduced by more than 60%, indicating that the de novo antibodies were mainly anti-SLA-I antibodies. Finally, when the SLA-I and SLA-II were deleted, the average T cell proliferation rates in the xeno-MLR decreased from more than 28% (SLA+) to 5% (SLA^{-}), indicating the strong immunogenicity of the SLA antigens.

Conclusion: We conclude that induced anti-SLA-I and SLA-II antibodies play important roles in GE pig-to-rhesus monkey xenotransplantation, thus providing further evidence for SLAKO pigs as the preferred organ source for rhesus monkeys as a preclinical model of xenotransplantation.

Funding: This study was supported by the National Key Research and Development Program (2023YFC3404304), the Postdoctoral Fellowship Program (Grade C) of China Postdoctoral Science Foundation under Grant Number GZC20230893, and the Natural Science Foundation of Hubei Province (2024AFB581).

207.4 | Assessment of Allosensitization Following Pig-to-Human Xenotransplantation

Imad Aljabban, Sue A. Tiongko, Jacqueline Kim, Ian Jaffe, Karen Khalil, Tal Eitan, Simon Williams, Brendan Keating, Aprajita Mattoo, Vasishta Tatapudi, Edward Skolnik, Jeffrey M. Stern, Adam Griesemer, Robert A. Montgomery, Massimo Mangiola

NYU Langone Transplant Institute, New York, New York, USA

Introduction: Xenotransplantation presents a promising approach to addressing the organ shortage problem and has been proposed as a bridge to allotransplantation for patients at high risk of mortality while on the transplant waitlist. Concerns remain that exposure to pig antigens could increase alloantibody levels, potentially complicating future allotransplant eligibility. In this study, we examine anti-HLA Class I and II response in three pig-to-human kidney xenotransplant recipients with varying degrees of pre-existing allosensitization to assess changes in alloreactivity post-xenotransplant.

Methods: Three pig-to-human kidney xenotransplants were performed at NYU Langone Health from 2023–2024 using clinically approved immunosuppression. Serum was collected from recipients at serial timepoints and tested for antibodies against human leukocyte antigen (HLA) Class I and II antigens using a Luminex-based single antigen bead assay. Reactivity to HLA was quantified by determining the mean fluorescence intensity (MFI). To calculate the panel reactive antibody (cPRA), beads with an

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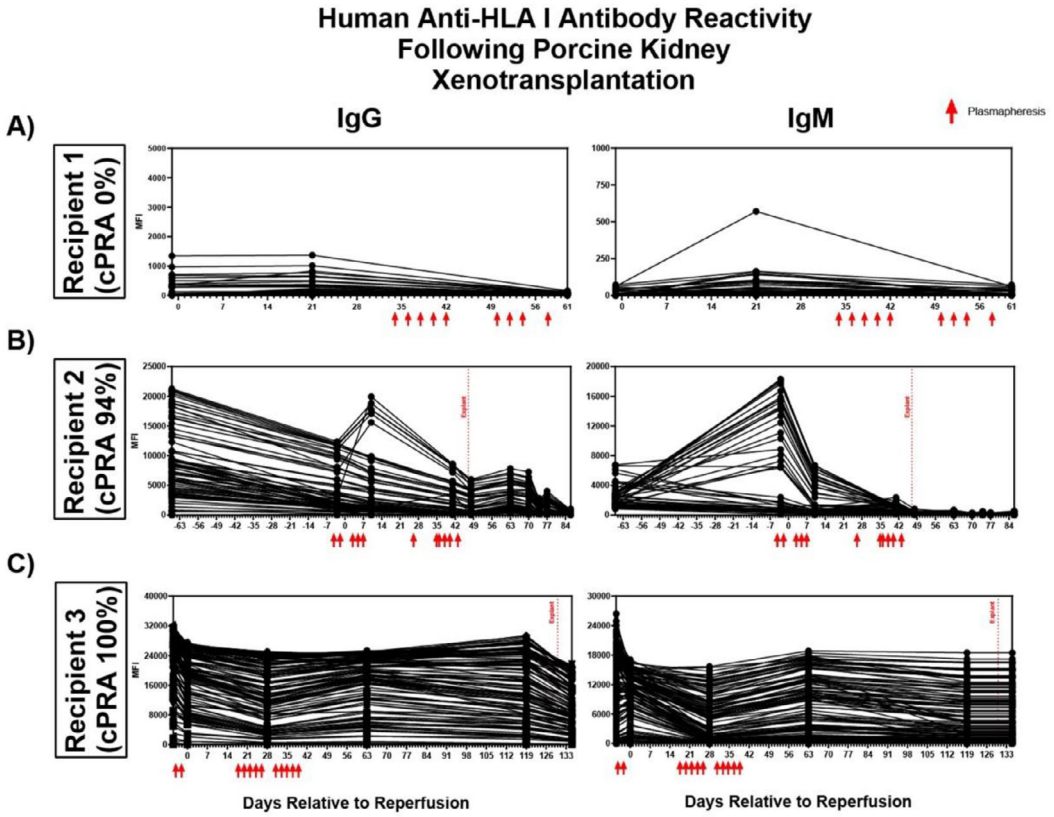


Figure 1. Human IgG and IgM anti-HLA class I antibody reactivity following porcine kidney xenotransplantation. Sera from three human recipients were monitored over time to assess anti-HLA antibody reactivity during the course of xenotransplantation. The timing of xenograft explantation is indicated by a red dotted line, while plasmapheresis sessions are marked by red arrows.

FIGURE 1 | 207.4

MFI ≥1000 were used. cPRA was calculated with the OPTN cPRA calculator.

Results: Recipient 1 was a brain-dead human decedent with a cPRA of 0%, who had previously undergone bilateral nephrectomy followed by implantation of a GGTA1 KO Thymokidney. The recipient was maintained for a planned duration of 61 days. Throughout the study period, both IgG and IgM anti-HLA Class I antibody reactivity remained consistently below the threshold for positivity (Figure 1A). Recipient 2 was a living human with a cPRA of 94% who underwent GGTA1 KO Thymokidney transplantation. By Day 10, an increase in reactivity against HLA-B44, B45, and B82 was observed. However, 37 days following xenograft explantation, anti-HLA Class I reactivity across all specificities declined to levels well below pre-transplant values (Figure 1B). Recipient 3, also a living human, had a cPRA of 100% and received a 10GE Xenokidney transplant. Anti-HLA class I antibody levels remained stable throughout the duration of xenograft implantation. The xenograft was explanted on POD 130, after which anti-HLA class I MFI values fell below baseline pre-transplant levels (Figure 1C). Across all three cases, IgM reactivity was less than IgG, and anti-HLA Class II antibody reactivity remained negative (data not shown).

Conclusion: Although limited by follow-up duration, these data demonstrate no increase in anti-HLA Class I and II reactivity following xenograft explantation.

Funding: Work funded in part by Lung Biotechnology, a wholly owned subsidiary of United Therapeutics Corporation.

207.5 | Investigation of Cross-Reactivity of Rabbit Anti-Thymocyte Globulin With Triple-Knockout Pig Cells and Potential Induction of Anti-Triple Knockout Pig Antibodies in Kidney Allotransplant Recipients

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Introduction: Compared to allotransplantation, it is crucial in xenotransplantation to determine whether administered agents contain antibodies that react with pig antigens or share epitopes with pig cells. Rabbit-derived anti-thymocyte globulin (rATG), commonly used for induction therapy, has been shown to display xenoantigens such as galactose-alpha-1,3-galactose (Gal) and N-glycolylneuraminic acid (Neu5Gc) on its Fab and Fc portions. However, it remains unknown whether rATG (i) contains antibodies that cross-react with triple-knockout (TKO) pig cells, or (ii) induces the production of anti-TKO pig antibodies by sharing antigens with TKO pigs after administration.

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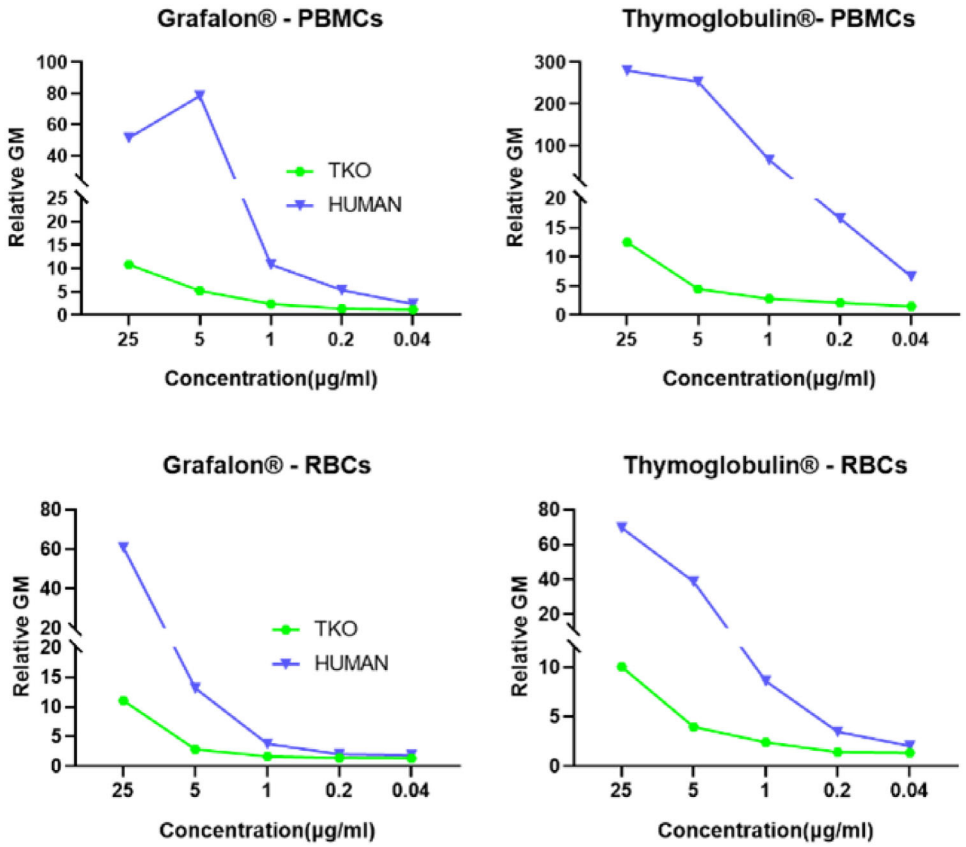


FIGURE 1 | 207.5

Methods: We evaluated the direct IgG binding of two types of rATG (Grafalon and Thymoglobulin) to TKO pig and human peripheral blood mononuclear cells (PBMCs) and red blood cells (RBCs) by flow cytometry. In addition, plasma samples from 31 kidney allotransplant recipients treated with Grafalon ($n = 11$) or Thymoglobulin ($n = 20$) were collected, and anti-TKO pig antibody levels were assessed before and up to 4 weeks after induction therapy. Both groups received a similar maintenance immunosuppressive regimen consisting of tacrolimus, mycophenolate mofetil (with or without rapamycin), and methylprednisolone.

Results: Both Grafalon and Thymoglobulin demonstrated detectable IgG binding to TKO pig cells, although the binding levels were significantly lower than those to human cells (relative geometric mean [rGM]: TKO; 10–15 vs human; 50–300) (Figure 1). Following rATG administration, no consistent overall increase in anti-TKO IgG was observed. However, several recipients exhibited a significant rise in anti-TKO IgM and/or IgG levels compared to their pre-transplant baseline.

Conclusion: This study provides the first evidence that rATG contains antibodies cross-reactive with TKO pig antigens and may induce anti-TKO antibody responses following administration. In addition, our findings suggest that rATG may harbor antigens shared with TKO pigs and could stimulate B cell responses either specifically or nonspecifically. While our findings do not imply that the use of rATG should be avoided in xenotransplantation, they highlight the need for careful protocol design. Specifically, timing strategies—such as administering rATG prior to transplantation rather than post-transplant—should be considered to minimize potential antibody-mediated xenograft injury. Thorough evaluation of induction agents, particularly animal-derived products, is essential to ensure the safety and success of future clinical xenotransplantation trials.

210.1 | Ex Vivo Pig Liver Xenoperfusion With Human Red Blood Cells and Mesenchymal Stem Cells to Enhance the Endothelial Barrier Function and Attenuate Inflammation

Anjali Verma[1], Kamoltip Promnares[2], Aarnav Grover[1], Javier Galindo[1], Avneesh Singh[1], Curt Civin[3], Konstantin Birukov[2], Muhammad Mohiuddin[1], Magali Fontaine[4], Raphael Meier[1]

[1]Surgery, School of Medicine, University of Maryland, Baltimore, Maryland, USA; [2]Anesthesiology, School of Medicine, University of Maryland, Baltimore, Maryland, USA; [3]Pediatrics, School of Medicine, University of Maryland, Baltimore, Maryland, USA; [4]Pathology, School of Medicine, University of Maryland, Baltimore, Maryland, USA

Purpose: Liver xenoperfusion with ex vivo machine perfusion (MP) has emerged as a promising strategy for the treatment of acute fulminant liver failure. MP also enables the application of donor-specific treatments, offering an opportunity to deliver immunosuppressive agents, condition the xenograft, and reduce the immunogenicity before implantation. Emerging evidence suggests that MP can modulate both innate and adaptive immune responses through targeted delivery of anti-inflammatory molecules or cell-based therapies. Red blood cells (RBCs) and mesenchymal stem cells (MSCs) play key roles in reducing immunogenicity, inducing tolerance, and graft endothelial barrier stabilization. Ex vivo perfusion with RBCs has been critical in preventing primary nonfunction in large animal studies

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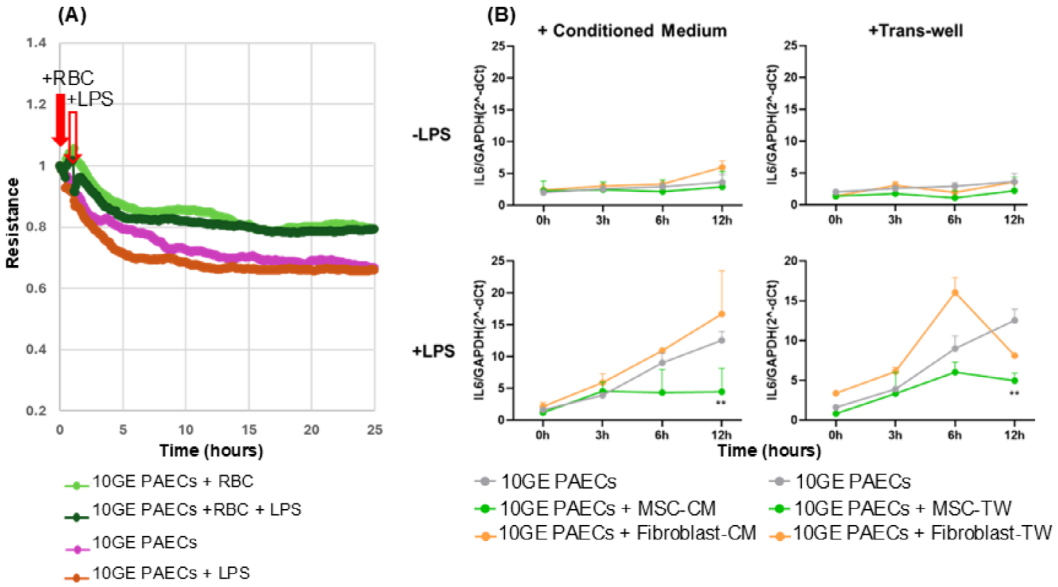


Figure-1: (A) Transendothelial electrical resistance across the 10GE-PAECs when treated with human RBC in the presence and absence of LPS. **(B)** Quantification of pro-inflammatory gene, IL6 by qPCR in 10GE-PAECs when treated with MSC-CM, MSC over transwell, Fibroblast-CM and Fibroblas over transwell in the absence and presence of LPS.

FIGURE 1 | 210.1

and was implemented in first-in-human cases. The objective of our study is to combine RBCs and MSCs perfusion and evaluate their impact on endothelial barrier stability and immune tolerance in the xenogeneic setting.

Method: We tested the effect of RBCs and MSC on 10-gene-edited (10GE) pig aortic endothelial cells (PAECs) in vitro. We measured transendothelial electrical resistance (TER) across 10GE-PAECs when treated with human RBCs using the electric cell-substrate impedance sensing system (ECIS-1600). We then treated 10GE-PAECs with human MSC-conditioned medium (MSC-CM) or human MSCs in transwell (MSC-TW). To stimulate the endothelial response, 10GE-PAECs were concomitantly treated with LPS (200 ng/mL) for 0, 3, 6, or 12 h. qPCR was used to quantify gene expression of inflammatory cytokines, including IL-6 and IL-8.

Results: We established a system using 10GE-PAECs, where the protective effect of anti-inflammatory agents can be studied. The deleterious effects of LPS on endothelial barrier function were partially mitigated by the addition of human RBCs to 10GE-PAECs, as evidenced by TER assay (Figure 1A). This suggests a potential protective role of RBCs in modulating endothelial responses to the pro-inflammatory stimuli. Furthermore, we demonstrated that both MSC-CM and MSC-TW significantly reduced IL-6 expression, a key marker of inflammation, in endothelial cells. These anti-inflammatory effects were consistent both under basal conditions and upon LPS-induced inflammatory challenge (Figure 1B), highlighting the robust immunomodulatory potential of MSC secretome. In contrast, fibroblast cells and their conditioned medium (CM), used as controls, did not mitigate endothelial cell inflammation (Figure 1B).

Conclusions: These data suggest that RBCs have the potential to stabilize the endothelial barrier and mitigate inflammation. Additionally, MSCs reduce endothelial activation and inflammation, which is critical for preventing the initial cytokine storm that provokes accelerated rejection and a pro-thrombotic environment in xenotransplantation.

210.2 | Application of Immunosuppressive Regimen in Xenotransplantation of Gene-Edited Porcine Heart to Rhesus Macaque

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Introduction: With advancements in xenotransplantation, genetically edited pigs are being explored as potential organ donors for primates. This study aims to discuss the immunosuppressive regimen used in the xenotopic heart transplantation from a gene-edited pig to a rhesus macaque, with the goal of providing a reference for future studies.

Methods: A comprehensive immunosuppressive protocol was employed, including preoperative induction therapy (anti-CD20 antibody – rituximab, anti-CD154 monoclonal antibody, antithymocyte globulin, cobra venom factor, methylprednisolone) initiated 14 days before surgery, and postoperative maintenance therapy (anti-CD154 monoclonal antibody, mycophenolate mofetil, methylprednisolone). The recipient's immune status was monitored using flow cytometry and blood cell counts to evaluate the efficacy of the immunosuppression.

Results: The rhesus macaque survived 46 days post-transplant with stable function of the transplanted heart and no signs of hyperacute or acute rejection. The induction therapy effectively reduced the numbers and proportions of lymphocytes, B cells, and T cells, maintaining low levels of immune activity. Postoperative monitoring indicated controlled complement cytotoxicity (3.4%–5.1%), stable IgG and IgM antibody binding, and favorable

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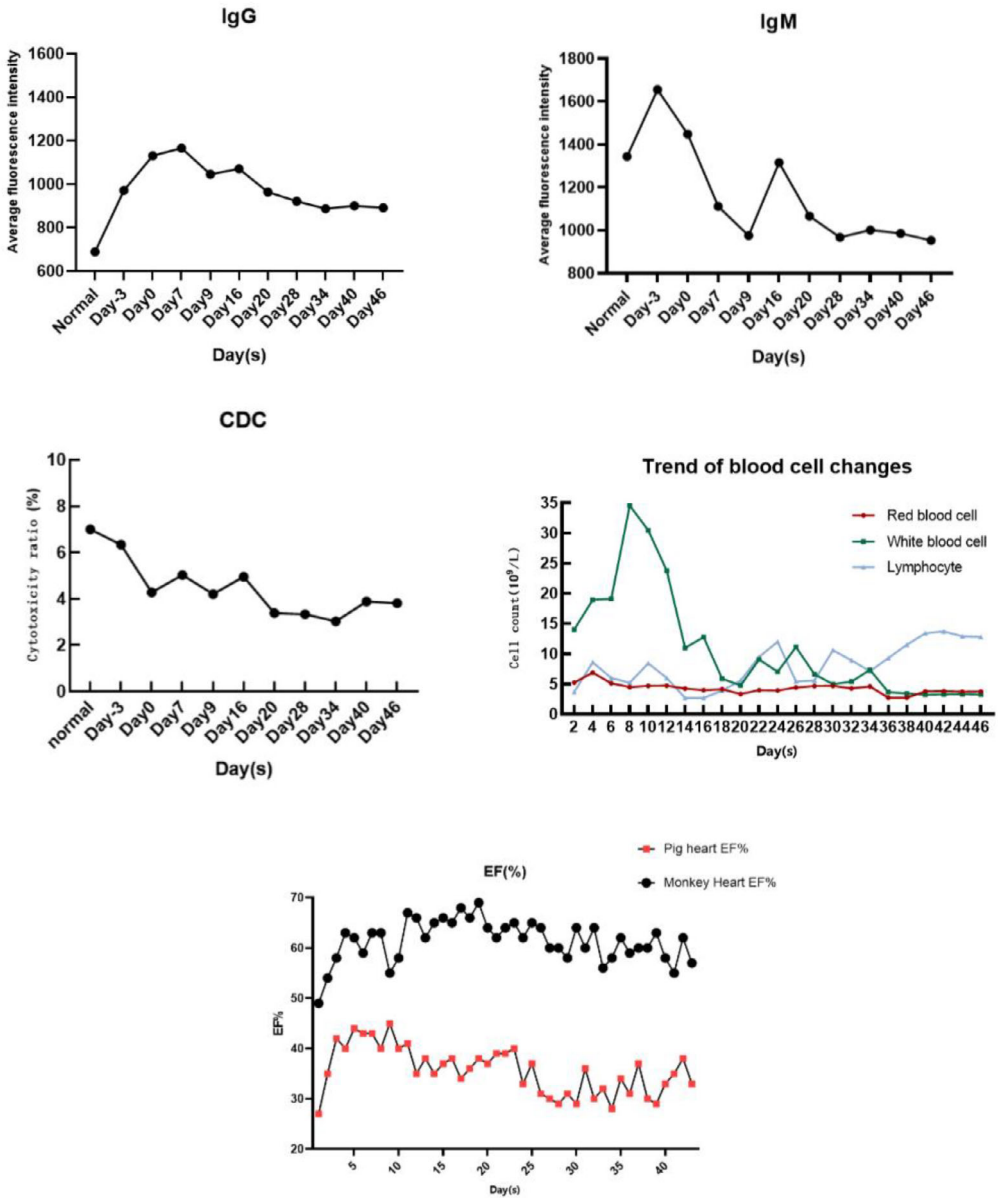


FIGURE 1 | 210.2

absolute values and ratios of B/T lymphocytes. Pathological analysis showed minor myocardial damage in the monkey heart but more severe damage in the porcine heart, suggesting chronic immune rejection.

Conclusion: The immunosuppressive regimen demonstrated significant effectiveness in suppressing hyperacute and acute rejection, providing an important strategy for xenotransplantation of gene-edited pig hearts. However, observed chronic rejection highlights the need for further optimization of immunosuppressive protocols, particularly for managing chronic rejection.

210.3 | Gut Microbiota Metabolite L-Histidine (L-His) Alleviates Tacrolimus-Induced Islet Dysfunction Post-Transplantation Via IRS-2/Akt/mTOR/FOXO1 Signaling Pathway

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Objective: Post-transplantation diabetes mellitus (PTDM) is a key factor affecting the prognosis of transplant patients, with a prevalence of 10%–40%. As a mainstream immunosuppressant, tacrolimus (TAC) is closely associated with the development of PTDM with impaired β-cell function. Our previous study indicated that L-Histidine (L-His), a metabolite of gut microbiota, can improve insulin resistance by inducing inflammation, oxidative stress, and other mechanisms. Therefore, an in-depth Investigation of the glucose metabolism regulatory pathway and molecular mechanism of L-His in the presence of TAC will

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provide novel insight for unraveling the pathogenesis of PTDM and offer a new therapeutic strategy for transplantation.

Methods: We constructed a TAC-induced islet dysfunction cell model and a diabetic mouse model. When L-His was delivered, we collected the cell, the mouse serum, and tissue samples. Insulin secretion was assessed by ELISA, and the histomorphology of islets by H&E staining. RNA and protein in the samples were extracted, gene expression was detected by real-time PCR, and protein expression levels were analyzed by Western Blot. Meanwhile, immunohistochemistry and immunofluorescence were used to assess the effects of TAC and L-His on pancreatic islets, insulin secretion-related signaling pathways to evaluate their molecular mechanisms.

Results: TAC treatment significantly inhibited the insulin secretion of pancreatic β-cells both in vitro and in vivo ($p < 0.01$) and led to histopathological alterations in the pancreas. However, L-His could alleviate the impaired islet function by stimulating the insulin responses under high glucose. Meanwhile, it could restore the normal morphology of pancreatic islets and significantly enhance insulin secretion ($p < 0.001$). Both Western Blot analysis and real-time PCR showed that TAC induced a significant decrease in the IRS-2 and p-mTOR expression ($p \leq 0.05$), while L-His treatment significantly up-regulated IRS-2, p-mTOR, p-Akt, and FOXO1 expression ($p \leq 0.05$), suggesting that the IRS-2/Akt/mTOR/FOXO1 pathway is involved in the L-His-stimulated insulin secretion, and counteracts the toxicity of TAC on islets.

Conclusion: L-His may significantly ameliorate pancreatic β-cell dysfunction induced by TAC by regulating the IRS-2/Akt/mTOR/FOXO1 signaling pathway. These findings provide a new potential drug for the treatment of PTDM.

Funding: This study was supported by the National Natural Science Foundation of China (81802504), the Sichuan Science and Technology Program (2025YFHZ0123), Chengdu Science and Technology Program (2024-YF05-01315-SN), and a grant from Shenzhen Weixin (2024HX0008) for Dr. Yi Wang.

210.4 | Generation and Functional Assessment of Human Cytotoxic T Cells Directed Against Wild-Type and Genetically Modified Pig Endothelial Cells

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Introduction: Efforts are underway to modulate acquired immune responses to prevent chronic cell-mediated xenorejection. Our aim was to develop in vitro assays to evaluate the xenospecific responses of CD8+ T cells (CTLs) to monolayers of porcine aortic endothelial cells (PAECs) from wild-type (WT) and transgenic (TG) animals expressing human PD-L1 and knocked-out for beta-2 microglobulin (B2M-KO).

Methods: Human CTLs were purified from PBMC and stimulated weekly for over 14 days with irradiated WT and TG PAECs. CTL phenotype before and after stimulation was assessed by flow cytometry (FC). CTL specificity was tested by conventional non-radioactive cytotoxicity and FC-based degranulation assays against primary $PAEC_{WT/TG}$ and third-party PEDSV.15 (dd-haplotype) targets in suspension. Live-cell imaging cytotoxicity assays were performed under static (2D, 6 h) and microfluidic (3D, 2 h) cocultures at an effector-to-target (E:T) ratio of 1:1, mimicking physiological endothelial settings. In the 2D assay, images of the endothelial monolayers were captured to quantify apoptotic bodies and necrotic PAECs. In the 3D microfluidic channels, a shear stress of 2 dyne/cm^2 was applied, and readouts included CTL adhesion and Draq7$^+$ dead PAECs on images of the PAEC-coated artificial micro-vessels.

Results: Following stimulation with PAECs, CTLs shifted from a naïve to a memory phenotype. Coculture with $PAEC_{TG}$ resulted in a reduced percentage of CD3+CD8+ T cells and lower overall CD8+ cell yield compared to $PAEC_{WT}$. In suspension assays, CTLs exhibited robust, E:T ratio-dependent cytotoxicity against $PAEC_{WT}$. Specific lysis of $PAEC_{TG}$ was reduced by 18% compared to $PAEC_{WT}$, though, the effect was apparent only at E:T ratios $\geq$ 5:1. In 2D assays, CTLs predominantly induced apoptotic cell death (11.26% $\pm$ 0.55%) with lower levels of necrosis (6.02% $\pm$ 1.73%) in $PAEC_{WT}$ monolayers. In contrast, $PAEC_{TG}$ showed significant resistance, with markedly reduced apoptosis (0.13% $\pm$ 0.12%) and necrosis (1.17% $\pm$ 0.73%). In the 3D system, CTLs adhesion to $PAEC_{TG}$ was significantly diminished; however, Draq7$^+$ dead PAEC frequencies were similar between $PAEC_{WT}$ and $PAEC_{TG}$.

Conclusion: This study demonstrated the generation of human CTLs with robust cytotoxic activity against porcine endothelial cells. Genetic modifications in $PAEC_{TG}$, specifically PD-L1 expression and MHC Class I abrogation, partially reduce CTL-mediated cytotoxicity in conventional and 2D assays. Under flow conditions, killing of $PAEC_{WT/TG}$ was similarly low, though reduced CTL adhesion to $PAEC_{TG}$ suggests altered immune engagement. These findings underscore the immunomodulatory potential of engineered porcine endothelium and support continued efforts toward developing immune-evasive xenografts. Our in vitro platforms offer valuable tools to dissect cellular immune barriers in xenotransplantation.

210.5 | Immortalized Aortic Endothelial Cells From Genetically Modified Pigs for Evaluation of Human-to-Pig Xenoreactive Immune Responses

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Background: Primary porcine aortic endothelial cells (PAECs) are currently the main target cells for studying human-to-pig xenoreactive immune responses, particularly for measuring the levels of preformed natural antibodies (NAbs). However, primary PAECs have a limited life span in culture. After four to five passages, they tend to de-differentiate and eventually reach senescence. The aim of this work was to establish immortalized porcine aortic endothelial cell lines (iPAECs) to facilitate in vitro studies.

Methods: Isolated and cultured primary aortic endothelial cells from different gene-edited pigs (WT, GTKO, TKO:

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GTKO/b4GalNT2KO/CMAHKO): Primary porcine aortic endothelial cells were transfected with lentiviral containing the SV40 large T antigen (SV40LT) and selected on the basis of morphological and phenotypical features. Expression of Gal, Sda, and Neu5Gc on iPAECs from different types of knockout pigs was analyzed using flow cytometry. Anti-pig IgM/IgG binding and complement-dependent cytotoxicity (CDC) to these iPAECs was measured by flow cytometry using pooled rhesus monkey sera ($n = 20$) or human sera ($n = 20$).

Results: In terms of cell morphology, compared to primary cells, iPAECs were rounder in shape, larger in individual size, more transparent, and had indistinct cell boundaries. iPAEC cell lines could be expanded for up to 20 passages without signs of senescence and with preserved characteristics of primary cells, such as cobblestone morphology and staining for CD31 (the purity of CD31 staining is greater than 95%). The expression of αgal, Sda, and Neu5Gc antigens in iPAECs is not significantly different from that in primary aortic endothelial cells. In addition, both the antibody binding (IgM and IgG) and CDC of rhesus monkey and human against WT pig iPAECs were very high (GMean of IgM: ∼8000 and ∼9000; GMean of IgG: ∼200 and ∼600; CDC: >90%). In the case of both humans and rhesus monkeys, the binding of serum IgM and IgG to GTKO pig iPAECs (Gal deletion) was significantly lower than that to wild-type pig iPAECs ($p < 0.01$). The antibody binding (IgM and IgG) and CDC of human serum against TKO pig iPAECs was markedly decreased after the deletion of Gal, Sda, and Neu5Gc in pigs. Unlike the results we obtained for pooled human serum, the binding of rhesus monkey serum IgM/IgG antibody to TKO pig iPAECs was significantly lower than that to pig iPAECs from WT, GTKO pigs. However, the cytotoxicity of monkey serum against TKO pig iPAECs was only slightly lower than that to GTKO pig iPAECs ($p < 0.05$). All the above results were similar to our findings using PBMCs or primary PAEC from the same gene-edited pigs.

Conclusion: In conclusion, iPAECs cells are a newly established porcine aortic endothelial cell line, morphologically, phenotypically, and functionally similar to primary cells. These cell lines should therefore provide valuable tools for in vitro studies of the interactions between the human immune system and the porcine endothelium.

210.6 | KJ047 Is a Next Generation CD40 Ligand Antibody Designed for Safe and Durable Immunosuppression in Combination With IgG Degrading Enzymes

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Introduction: CD40L blockade is a validated strategy to prevent transplant rejection, but prior anti-CD40L antibodies such as Ruplizumab were discontinued due to Fc-mediated thromboembolic complications. Emerging IgG-degrading enzyme therapies further highlight the need for compatible and safer CD40L antibodies.

Methods: We developed KJ047, a novel anti-CD40L antibody incorporating the clinically proven Fab of Ruplizumab and engineered Fc regions to eliminate Fc gamma receptor binding and resist proteolysis by IgG-degrading enzymes. Its pharmacokinetics, immune function, and biophysical properties were evaluated in preclinical studies.

Results: KJ047 maintained potent CD40L binding and functional immunosuppression while demonstrating complete loss of thromboinflammatory Fc activity. KJ047 remained intact and functional when coadministered with IgG-degrading enzymes, preventing degradation and ensuring sustained immunosuppressive activity. KJ047 also showed extended half-life, good physicochemical stability, and suitability for subcutaneous injection.

Conclusion: KJ047 offers a next-generation solution to overcome the safety and compatibility limitations of earlier CD40L antibodies. Its design allows safe, durable, and enzyme-combination–compatible immunosuppression, with significant potential in xenotransplantation and other rejection-prone clinical settings.

210.7 | Early Mixed Chimerism After Pig to Non-Human Primate Liver Xenotransplantation

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Background: Liver xenotransplantation (LXTx) is a promising approach to overcome the shortage of donor organs for end-stage liver failure patients. Despite the liver's inherent tolerogenic properties in allotransplantation, xenotransplantation faces significant immunological challenges. Mixed chimerism, involving the coexistence of donor and recipient cells, can promote transplant tolerance. However, the presence of mixed chimerism following liver xenotransplantation has not been assessed. This study investigates xenogeneic mixed chimerism early after pig-to-baboon LXTx, assessing the liver graft's role in establishing chimerism.

Methods: Four pig-to-baboon LXTx were performed using GalT-KO miniature swine as donors. A non-survival experimental model was established to enable monitoring of peripheral blood and collection of liver samples at multiple time points before and after portal vein reperfusion. Peripheral blood and liver mononuclear cells were isolated and analyzed using flow cytometry. Pretransplant samples from both donors and recipients served as baseline controls. Additionally, peripheral chimerism levels were compared with those obtained from intrabone bone marrow transplantation (IBBMTx) models utilizing human CD47 transgenic and GalT-KO swine. Non-Gal donor-specific antibodies (DSA) were quantified to assess the immune response.

Results: Following portal vein reperfusion, recipients of LXTx exhibited an average peripheral blood chimerism of 12% pig CD45+ cells within 30 min. Peripheral mixed chimerism

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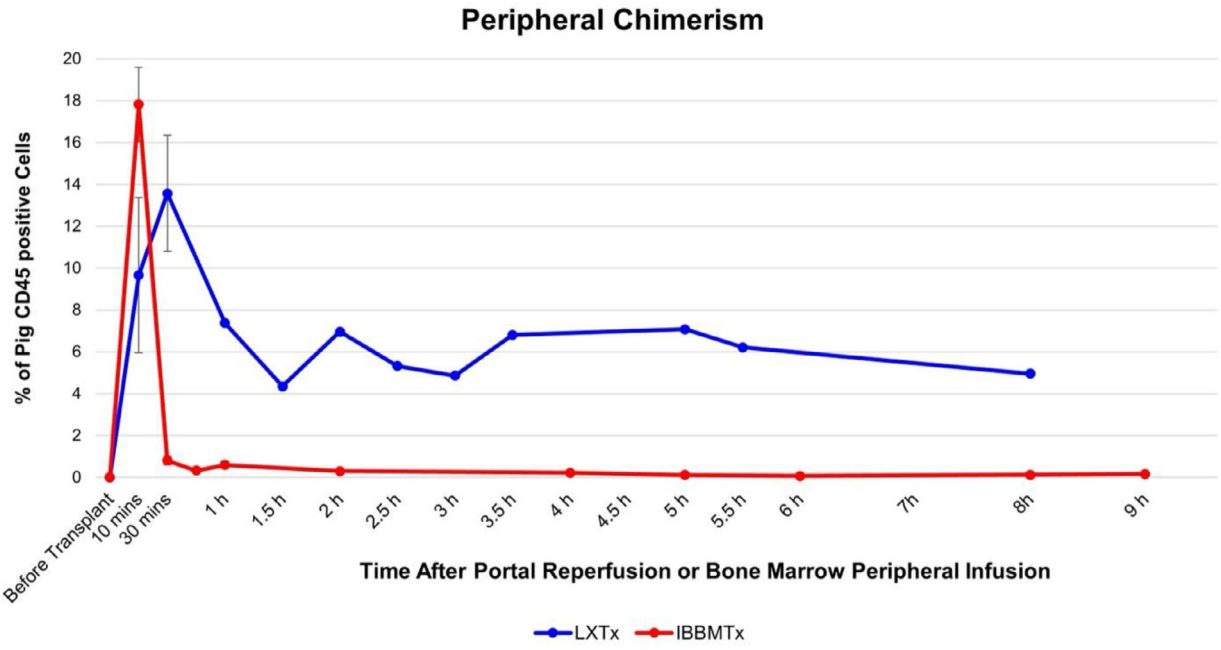


FIGURE 1 | 210.7

subsequently stabilized between 4.4% and 7.1%, maintaining this range for up to 8 h post-reperfusion. Baboon CD45+ cells infiltrated the pig liver graft, peaking at 43.5% at 30 min post-reperfusion. In contrast, the IBBMTx model initially showed higher chimerism, but this rapidly declined within 30 min and remained low (0.1%) thereafter. Peripheral chimerism in LXTx was predominantly characterized by pig CD4+ cells, which peaked at 66.7% at 2.5 h post-reperfusion. DSA assays indicated decreased levels of IgM and IgG post-reperfusion, with IgA also trending downward, in three of four LXTx recipients.

Conclusion: Our study demonstrates that spontaneous xenogeneic mixed chimerism is achievable in a pig-to-baboon LXTx model. Chimerism persisted for at least 8 h following portal vein reperfusion, and from 30 min after reperfusion, continued to be higher compared to that observed in IBBMTx. Immunosuppressive protocols designed to support this spontaneous mixed chimerism could potentially enhance graft survival. Our findings may suggest insights into chimerism in xenotransplantation and highlight opportunities for optimizing immunosuppressive strategies to improve long-term LXTx outcomes.

Funding: This project was funded by the National Institute of Health (NIH) 5P01AI045897 grant and an unrestricted research grant from Nefro Health and ChoironeX.

211.1 | Porcine Expanded Potential Stem Cell Platform for in Vitro Interspecies Chimerism and Cardiac Niche Modeling

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Introduction: Interspecies chimeras using pluripotent stem cells (PSCs) offer a promising strategy to generate humanized tissues in organ-deficient pigs, potentially addressing the global shortage of donor organs. However, human PSCs have so far shown limited integration into porcine embryonic development. Expanded potential stem cells (EPSCs) resemble eight-cell and morula-stage blastomeres, exhibit robust proliferation, allow precise genome editing, and possess enhanced differentiation potential. These features make them strong candidates for improving chimera formation and modeling early development. To investigate interspecies cell competition, we established (1) a porcine-human EPSC coculture system and (2) a porcine EPSC-derived "heart niche" model to study in vitro competitive interactions during early cardiac co-differentiation.

Methods: For the in vitro interspecies coculture assay, GFP-expressing human primed iPSCs were stepwise converted into EPSCs and assessed for the upregulation of pluripotency and core histone genes. Equal numbers of single-cell dissociated porcine tdTomato$^+$ EPSCs and human GFP$^+$ EPSCs were cocultured to evaluate proliferation, cell-cell communication, and apoptosis until confluency. The mixed cultures were then differentiated into cardiomyocytes using a protocol optimized for both human and porcine EPSCs. Additionally, *NKX2.5* knockout porcine EPSCs were generated using the CRISPR-Cas9 system to study cell competition with human EPSCs during cardiac co-differentiation.

Results: We developed an interspecies EPSC coculture system to study in vitro cell competition between porcine and human EPSCs. Under optimized expanded potential conditions, both cell types proliferated efficiently and maintained stable dome-shaped colonies. Time-lapse microscopy and immunostaining for PARP and Caspase revealed no evidence of interspecies competition. During coculture, porcine and human cells

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formed segregated, adjacent colonies connected via E-cadherin at their borders. Upon directed cardiac differentiation, the mixed cultures produced integrated, beating cardiomyocytes. Notably, *NKX2.5* knockout porcine EPSCs displayed impaired cardiac differentiation, leading to fewer cardiomyocytes and dominance of human EPSC-derived cardiomyocytes in interspecies co-differentiation.

Conclusion: Due to their enhanced pluripotency and differentiation potential, porcine EPSCs provide a robust platform for in vitro modeling of interspecies chimerism and for the pre-evaluation of gene candidates aimed at generating niche-enabled pigs for the production of humanized organs.

Funding: This work was supported by Leducq Foundation (23CVD01 "Xenogenic and exogenic pig organs for transplantation into humans"). German Centre for Cardiovascular Research (FKZ 81Z0600601 "Platform Reprograming"). European Research Council (788381 "Deep biomodeling of human cardiogenesis").

211.2 | Development of the Replacement System for Animal Fetal Kidneys With Xenogenic Nephrons: Toward Clinical Application

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Introduction: We are developing a renal failure therapy based on xeno-regenerative medicine, in which human iPS cell–derived nephron progenitor cells (NPCs) are combined with porcine fetal kidneys to generate chimera kidneys. To establish a chassis for chimera kidney generation, a cell-ablation system targeting NPCs is required to create a vacant nephrogenic niche. Several such systems have been developed in mouse models [1]. Furthermore, using an anephric rat model, we demonstrated prolonged survival after transplantation of allogeneic fetal kidneys [2]. That model demands sophisticated microsurgery techniques—creation of an integrated metanephros-bladder composite and anastomosis via a stepwise peristaltic ureter system connecting the fetal bladder to the native ureter. Translating these techniques to the mouse scale is technically challenging; thus, developing a cell-ablation system in a rat model promises to accelerate these studies.

Methods: We generated a "Six2-ATTAC9" BAC transgenic rat expressing FK506-inducible iCaspase9 under the control of the Six2 promoter. Fetal kidneys were harvested and treated with a chemical inducer of dimerization (CID) to ablate NPCs and thereby form a nephrogenic niche. These niche-prepared fetal

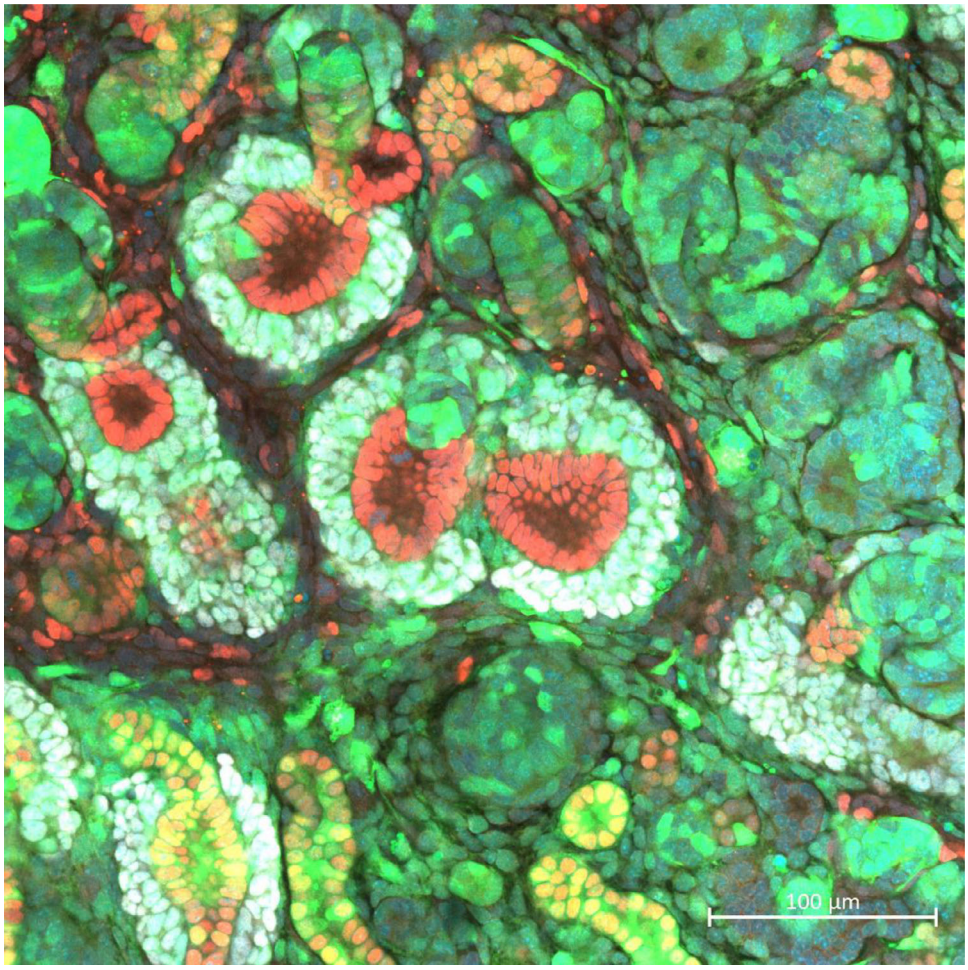


FIGURE 1 | 211.2

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kidneys were then repopulated in vitro by isolating and transplanting exogenous mouse NPCs, and the resulting chimera fetal kidneys were cultured on Transwell inserts.

Results: Fetal kidneys cultured on Transwell membranes with CID showed effective NPC depletion and successful formation of a nephrogenic niche. After transplanting mouse progenitor cells into these kidneys and culturing for 4 days, immunostaining revealed integration of donor mouse cells with rat ureteric buds and the formation of chimeric nephrons (Figure 1).

Conclusion: We have successfully up-scaled our microsurgical platform to a rat model compatible with cell ablation. This advance enables functional evaluation of chimera fetal kidneys and assessment of survival benefit in vivo, moving us closer to clinical application.

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Funding: T.Y. declares support from AMED (grant number JP24bm1223003) and JSPS-KAKENHI (grant number JP21K08288). S. Yamanaka declares support from AMED (grant numbers JP22bm0704049 and JP24bm1123036), JST FOREST Program (grant number JPMJFR2011), and JSPS-KAKENHI (grant numbers JP19K17756 and JP24K11439).

211.3 | Multiplex Gene-Edited Porcine Corneal Xenotransplantation in Tibetan Macaques: Immune Rejection Mechanisms

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Introduction: Full-thickness corneal xenotransplantation from pigs to non-human primates has used wild-type or simple transgenic donors, with few reports on grafts from more extensively gene-edited animals. We investigated immune rejection mechanisms after penetrating keratoplasty (PKP) using porcine corneas carrying triple knockout of α-Gal, Sda, and CMAH antigens (GTKO/SdaKO/CMAHKO) plus human complement-regulatory transgenes (hCD55 ± TBM) in Tibetan macaques.

Methods: Four macaques received standard PKP with gene-edited minipig grafts. Postoperatively, all animals received subconjunctival triamcinolone. Graft status was assessed by slit-lamp microscopy; rejection was defined as stromal edema > 800 μm with neovascularization. Upon clinical rejection, eyes were enucleated and examined histopathologically for inflammatory infiltrates, neovascular density, epithelial and stromal changes, and immunofluorescent markers (IgG, CD31).

Results: Early postoperative course was uneventful: graft clarity and wound integrity were maintained through Day 8. By that time, two monkeys developed keratic precipitates, peripheral stromal vessels, and anterior-chamber fibrin. One graft opacified on Day 19; by Day 28, all grafts showed diffuse stromal edema, opacity, and neovascular ingrowth. Histology revealed dense T-lymphocyte infiltration at graft-host junctions, mixed lymphoplasmacytic and macrophagic infiltrates with abundant eosinophils, and variable angiogenesis (≥ 15 vessels/mm^2 in two cases). Epithelial thickness was irregular (central 45.3 ± 6.2 μm vs. peripheral 62.1 ± 8.7 μm), with posterior stromal adhesions and pigmented tissue invasion. Two macaques exhibited high microvessel density at the graft margin (28.4 ± 3.1 vessels/mm^2), stromal thickness increased to 1024 ± 89 μm, and disrupted collagen architecture. One case showed IgG+ keratic precipitates.

Conclusion: Gene-edited porcine corneas prevent hyperacute rejection but do not avert chronic xenogeneic rejection. Contributing factors likely include donor-recipient corneal thickness mismatch, neovascular invasion, anterior synechiae disrupting the anterior chamber-associated immune privilege deviation (ACAID), and absence of systemic immunosuppression. Histopathology suggests that mechanical mismatch at the graft edge induces neovascularization, enabling direct lymphocyte infiltration and graft attack. While extensive gene editing can reduce antigenicity, overediting may compromise donor viability and expose novel antigens. Optimizing specific combinations of gene edits—rather than maximizing edits—and leveraging the immune-privileged anterior chamber for endothelial grafts may better prolong xenograft survival and offer an alternative to allogeneic donors.

Funding: This research was supported by Shenzhen Science and Technology Innovation Commission, Shenzhen Science and Technology Major Program (No. KJZD20240903103305008).

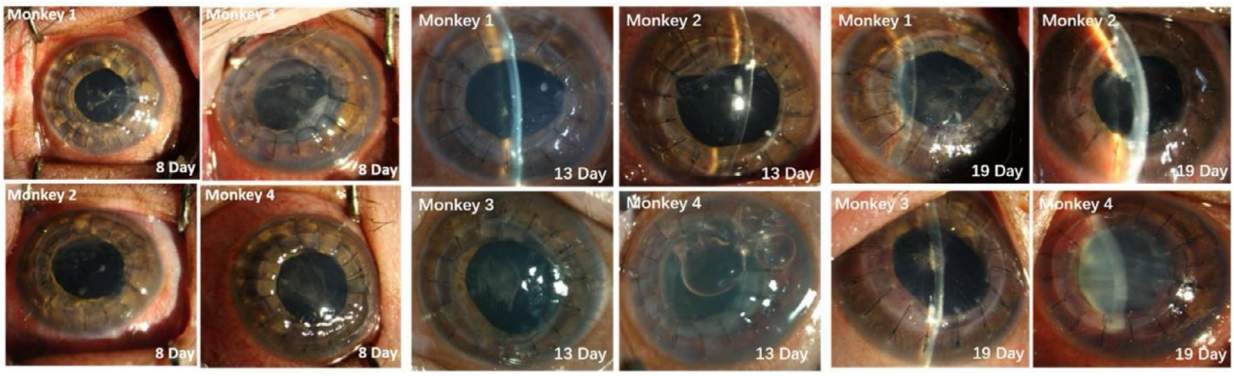


FIGURE 1 | 211.3

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211.4 | Detailed Genomic Analysis of a Highly Inbred Donor Pig Line With Unique Advantages for Clinical Porcine Islet Xenotransplantation

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Introduction: Diabetes mellitus poses a significant global health burden, with islet transplantation recognized as a promising therapeutic strategy. However, the scarcity of human donors limits its widespread application. Porcine islet xenotransplantation offers a viable alternative, yet challenges persist regarding interspecies pathogen transmission and suboptimal glucose-stimulated insulin secretion (GSIS) efficiency compared to human islets. Here, we introduce the Xeno-1 pig line—a highly inbred, PERV-C-negative minipig breed developed through 20 generations of selective breeding. This study aims to characterize the genomic, physiological, and immunological attributes of Xeno-1 pigs and validate their safety and efficacy as donors for clinical porcine islet xenotransplantation.

Methods: Genetic Analysis: Whole-genome sequencing (WGS) of 90 Xeno-1 pigs was performed to assess genetic homogeneity and identify selective sweeps related to insulin secretion and glucose metabolism. Comparative genomic analyses were conducted against Duroc, Yorkshire, and Landrace breeds. PERV Characterization: PERV subtypes (A, B, C) were screened via genome sequencing and transcriptomic profiling across 12 tissues. Functional infectivity of PERV-C-like variants was evaluated using in vitro coculture assays. Islet Function Assessment: RNA sequencing (RNA-seq) and GSIS assays were conducted on islets isolated from Xeno-1 and three control breeds (Duroc, Yorkshire, Landrace). Differential gene expression was analyzed using GO/KEGG enrichment and protein-protein interaction (PPI) network analysis. Microbiome Profiling: 16S rDNA sequencing and RNA-seq were used to compare gut microbial composition and immune-related gene expression in Xeno-1 pigs bred in designated pathogen-free (DPF) versus conventional environments. Clinical Trial: A pilot xenotransplantation trial was conducted in 10 type 1 diabetic patients using Xeno-1-derived islets, with long-term follow-up (up to 10 years) to assess safety and metabolic outcomes.

Results: Xeno-1 pigs exhibited a high inbreeding coefficient ($F_{20} = 0.986$) and genome-wide homozygosity. Selective sweeps identified genes enriched in insulin secretion pathways (PDX1, PAX6, SLC30A8), glucose homeostasis (GYS2, CEBPA), and pancreatic development (NKX6-1). Xeno-1 pigs were PERV-C-negative, and PERV-A/C recombinant formation was inhibited. PERV-unclassified variants lacked intact viral genomes and failed to produce infectious particles in coculture experiments. Xeno-1 islets demonstrated 2–4-fold higher GSIS compared to other controls (Duroc, Yorkshire, Landrace) ($p < 0.001$). Key drivers included upregulated PDX1, PAX6, and SLC30A8 expression. DPF-reared Xeno-1 pigs showed reduced gut microbial diversity, with downregulated genes linked to adaptive immunity (LAG3, CD79A) and upregulated pathways involved in lipid catabolism. No PERV transmission or infections were detected in recipients over a 10-year follow-up. Improved glycemic control was sustained, with reduced exogenous insulin requirements.

Conclusion: The Xeno-1 pig line exhibits genomic stability, enhanced islet function, and negligible PERV-related risks, establishing it as a safe and superior donor source for clinical porcine islet xenotransplantation. This study provides critical insights into optimizing donor pig breeding strategies and advancing the clinical translation of xenotransplantation therapies for diabetes.

Funding: This research was supported by the Natural Science Foundation of China (Grant No. 82272102). Natural Science Foundation of Hunan Province, China (Grant No. 2025JJ50714). Natural Science Foundation of Changsha, China (Grant No. kq2403066). Research Project of Teaching Reform in Colleges and Universities of Hunan Province (Grant No. HNJG-20230110).

211.5 | Advancing Cures for Type 1 Diabetes Using Flow Cytometry as a Guide for Safe and Efficacious Immunosuppression in Xenotransplantation

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Background: Islet xenotransplantation is a promising alternative to allo-islet transplantation to treat Type1 Diabetes. Despite significant advances in transgenic pigs, we still require heavy immunosuppression to prevent rejection. To optimize immunosuppressive protocols, we require a means to more regularly monitor the efficiency of immunosuppression on recipients and prevent graft rejection. Here we present our analysis of the development of a more predictive and more regularly performable method.

Methods: Porcine neonatal islet cell clusters (NICC) from GTKO-hCD55-hCD59 transgenic pigs were transplanted into STZ induced diabetic baboons ($n = 9$). All received anti-CD2 antibody (Abs), tacrolimus, anti-CD154 Ab and Belatacept immunosuppression. Blood samples were collected from recipients before and after transplantation from which counts of blood leukocyte subtypes were monitored using multiplex, multi-panel flow cytometry (Flow). NICC function was assessed by multiple daily blood sugar levels (BSL) and monthly glucose tolerance tests. Additionally, NICC xenograft maturation and function were assessed histologically from biopsies with immune-profiling of NICC graft sites analyzed by multiplex immunofluorescence staining of the 4 months-post transplant biopsy and endpoint tissue collection.

Results: All diabetic baboons ($n = 9$) were transplanted with GTKO-hCD55-hCD59 transgenic pig NICC's and were followed to the endpoint following withdrawal of all exogenous immunosuppression. All grafts demonstrated function and provided normoglycaemia in all animals. Systemic porcine C-peptide

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was detected from Day 30 onwards. Day 120 liver biopsies showed well-preserved morphology with no cellular infiltrate and positive staining for insulin, somatostatin, and glucagon ($n = 7$). Flow using IVD BD true count produced equivalent cell counts compared to standard full blood count (FBC) test, demonstrated by Deming regression analysis (95% CI slope = 0.91–1.031, $p < 0.001$). Noticeably, cell count by Flow was completed faster (50 min ± 15 vs. 150 mins ± 30 for FBC test) and used significantly smaller sample volume (standard 50 μL vs. 500 ± 50 μL blood for FBC test). Using Abs targeting specific markers for different lymphocyte subtypes, the Flow method provided more sensitivity and specificity in real-time monitoring blood immune profiles in xeno-islets recipients. Flow data showed specific T cell-binding and suppression of anti-CD2 Abs with reduction of unoccupied surface CD2 on T cells in recipient's blood after anti-CD2 Abs treatment (mean of 73.21%, 55.26% −91.16%, $p < 0.001$). Long-term NICC survival ($n = 7$) showed significant T cell suppression during the first month post-transplant (36.47%, 13.35–50.75 %) compared to short-term NICC survival ($n = 2$) (176.28%, 171.26 – 181.31%). Early elevated blood lymphocyte levels (40%−142%) were also detected in the two recipients who lost NICC function shortly after the withdrawal of immunosuppression. Qualitative analysis of endpoint NICC grafts by multiplex immunofluorescence demonstrated NICC destruction through cell-mediated killing with increased lymphocyte infiltration and cytotoxic CD8+T cell activation.

Conclusion: This study demonstrates the utility and reliability of Flow to be a more helpful tool to study the immune status of xeno-islet recipients. It is significantly faster to perform than standard FBC, utilizing a much smaller sample, allowing for more regular and easily obtainable samples from bottom pricks rather than formal blood collection.

Funding: This research was supported by Breakthrough T1D of the National Health and Medical Research Council, Australia.

211.6 | Xenotransplantation of Encapsulated Human MMP-9 Enhanced Mesenchymal Stem Cells and Pancreatic Islets in Diabetic Mice

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Purpose: Diabetes is a widespread disease, demanding lifelong insulin dependence with risks including hypoglycemia and kidney complications. Encapsulated pancreatic islet xenotransplant offers hope to broadly treat patients with diabetes, end-stage renal disease (in conjunction with a kidney xenotransplant), and hypoglycemia unawareness. However, limited engraftment of xenogeneic islets remains a barrier. Matrix Metalloproteinase-9 (MMP-9), a key molecule secreted by Mesenchymal Stem Cells (MSCs), has been shown to enhance beta cell function and islet vascularization. We tested whether encapsulated human MMP-9 enhanced MSCs and islets can facilitate and prolong diabetes in mice.

Method: MMP-9 overexpression was achieved in MSCs by two different approaches, GM-MSC1 and GM-MSC2, with 6000-fold and 15-fold increase in MMP-9, respectively. Overexpression of MMP-9 in modified MSCs was confirmed by qPCR and ELISA. We studied the effects of MSCs and modified MSCs on pancreatic islet function and survival in vitro and in vivo. Islet Insulin secretion was evaluated in vitro by measuring glucose-stimulated insulin secretion (GSIS). Islet morphology and viability were assessed over time using FDA/PI staining. Diabetes was induced in C57BL/6 mice by intraperitoneal injection of streptozotocin (STZ) at 220 mg/kg. Diabetes was defined as glycemia levels >360 mg/dL. Encapsulated human islets were xenotransplanted intraperitoneally in diabetic mice within groups: islets alone, islets + MSCs, islets + GM-MSC1, and islets + MSC2. Blood glucose level was monitored twice a week for each mouse group. Islet xenograft failure was concluded when the glucose level was >360 mg/dL for three consecutive measurements.

Results: FDA/PI staining assessed islet viability post-encapsulation, indicating excellent cell viability in all the groups (Figure 1A). Islets cultured with GM-MSC1 demonstrated a significantly increased insulin response compared to other groups in both coculture and transwell settings (Figure 1B). Blood sugar levels of streptozotocin-induced diabetic mice that reversed diabetes after islet transplantation are shown in Figure 1C. Four mice (40%) achieved long-term graft survival in the absence of immunosuppression (i.e., >30 days). Those included one mouse transplanted with islets alone and three mice transplanted with islets + GM-MSCs (Figure 1C). Overall, mice receiving islet + GM-MSC transplants showed a trend toward improved xenograft survival relative to the other groups (Figure 1D). This phenomenon could result from the microenvironment provided by enhanced MMP-9 secretion, which promotes beta cell function and improves xenograft vascularization.

Conclusions: The present study shows that MSCs with upregulated MMP-9 expression can support microencapsulated human islets when transplanted into mice. Future investigations of MMP-9-enhanced MSCs on the encapsulated islets microenvironment will help to better understand the mechanism of their favorable effect on beta cell function.

Funding: This research was supported by the Maryland Stem Cell Research Fund Discovery Grant 2024.

211.7 | Microporous Annealed Particle (MAP) Scaffold Enhances Islet Engraftment and Modulates Inflammation for Beta-Cell Replacement Therapy

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Purpose: Islet transplantation is a promising therapy for diabetes; however, its success remains limited by poor engraftment efficiency and immune rejection. This study evaluates microporous annealed particle (MAP) gel as a supportive scaffold to enhance engraftment and function of dissociated murine

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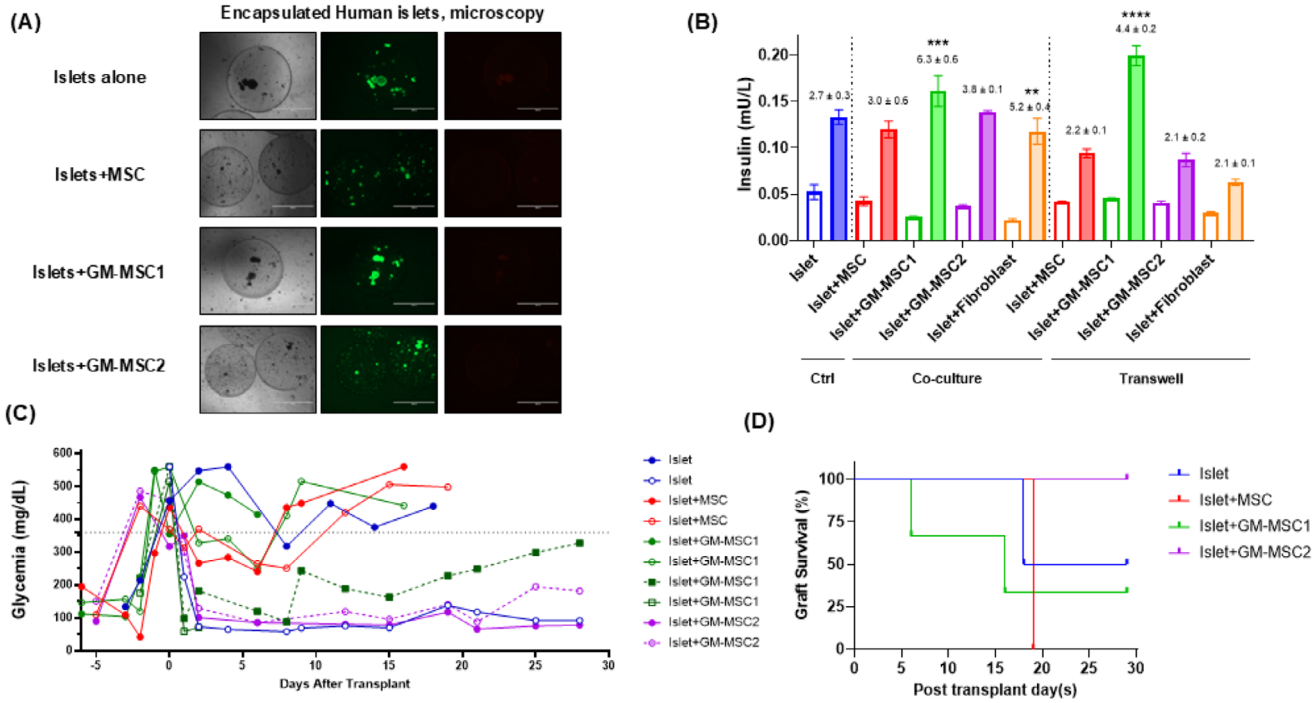


Figure: (A) Encapsulation of Islets, Islets+MSCs, Islets+GM-MSC1, and islets+GM-MSC2. FDA/PI staining indicates cell viability after encapsulation. Both islet and MSCs survived encapsulation (green) with minimal cell death (red). **(B)** Glucose Stimulated Insulin Secretion by human islets when treated with indicated groups in low (2.8 mM) and high glucose (28 mM) condition. *** p<0.01, islet+GM-MSC1 versus all the other groups except for islet+Fibroblast. ** p<0.05, islet+Fibroblast versus islet alone and islet+MSC. **** p<0.001, islet+GM-MSC1 versus all the other groups Average stimulation index±SEM is mentioned for each group in graph. **(C)** Mice were transplanted with encapsulated islets (n=2), encapsulated islets+MSCs (n=2), encapsulated islets+GM-MSC1 (n=4), and islets+GM-MSC2 (n=2). Glycemia was measured regularly thereafter. Dashed black line represents Blood Glucose = 360 mg/dL. **(D)** Kaplan-Meier curve representing the percent xenograft survival.

FIGURE 1 | 211.6

islets in a syngeneic model, with potential applications for stem cell-derived beta-cell therapy.

Methods: Dissociated islet cells from C57BL/6 mice were mixed with MAP gel and transplanted into syngeneic streptozotocin-induced diabetic recipients at the kidney capsule or epididymal fat pad. Blood glucose levels were monitored daily. In additional studies, MAP or nanoporous (NP) gels were implanted with or without Beta TC-6 cells in non-diabetic mice. Cytokine profiles were assessed at Days 7 and 14 post-transplantation. A pilot study with human islets (800 or 400 IEQ) was performed at the subcutaneous (SC) site using MAP gel.

Results: In streptozotocin-induced diabetic C57BL/6 mice, transplantation of 100 dissociated islets combined with MAP gel under the kidney capsule restored normoglycemia (BG <200 mg/dL) within 18 days, with glycemic control maintained for at least 40 days. In contrast, dissociated islets transplanted without MAP gel failed to reverse hyperglycemia. In a syngeneic model using the epididymal fat pad as the transplant site, a pilot study with 400 dissociated islets combined with MAP gel resulted in normoglycemia for up to 44 days in two mice, whereas MAP gel alone, whole islets alone, or dissociated islets alone did not restore normoglycemia. In a more recent cohort ($n = 6$), the same transplant strategy led to rapid glycemic control and prolonged islet function for up to 70 days post-transplantation, further supporting MAP gel's role in enhancing islet engraftment and survival. A pilot xenogeneic study using human islets transplanted at the subcutaneous (SC) site demonstrated MAP gel's potential across species. Mice receiving 800 IEQ human islets with MAP gel exhibited the most significant glucose reduction, while those receiving 400 IEQ showed a moderate effect. In contrast, control groups remained persistently hyperglycemic. Glucose fluctuations in MAP-supported groups indicated active islet function. Beyond engraftment, MAP gel also displayed immunomodulatory effects. Cytokine profiling at 7 days post-implantation of 1 million Beta TC-6 cells mixed with MAP or nanoporous (NP) gels showed that MAP gel significantly reduced pro-inflammatory cytokines, including IFNγ, IL-6, IL-1α, IL-1β, IL-10, IL-17, and TNFα. Notably, IL-10 levels were significantly decreased in the MAP group, suggesting a shift toward a less inflammatory microenvironment conducive to islet survival.

Conclusions: These results highlight MAP gel as a promising platform for beta-cell replacement therapy, capable of improving transplantation efficiency, modulating local immune responses, and enhancing long-term glycemic control.

Funding: The authors are grateful to the Manning Family Foundation and the UVA LaunchPad Grant for supporting our research.

212.1 | A Rapid High Through-Put PCMV Serology Western Blot Assay to Detect Anti-PCMV Antibodies in Pigs

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Introduction: Porcine cytomegalovirus (PCMV), an enveloped DNA virus in the Herpesviridae family (subfamily Betaherpesvirinae), has not been reported to infect humans but raises concerns about transmission via xenotransplantation. In non-human primate studies, PCMV infection has influenced xenotransplantation outcomes. Active viremia typically occurs in pigs between 20 and 90 days of age, after which the virus establishes latency. During latency, viral DNA is no longer shed in nasal secretions, though anti-PCMV antibodies remain detectable. Additionally, latent PCMV can reactivate in peripheral blood mononuclear cell (PBMC) cultures following allogenic stimulation or organ transplant into an immunosuppressed recipient. Current diagnostic options for PCMV are limited. Veterinary diagnostic laboratories (VDLs) offer PCR assays to detect DNA in viremic animals, but PCR cannot identify latent PCMV infection. While serological assays are needed to detect latent infections, these are not currently offered by VDLs. To address this gap, we developed a high-throughput PCMV serology western blot assay to detect anti-PCMV antibodies.

Methods: A portion of the PCMV glycoprotein B was synthesized with a His-tag in E. coli for western blot application using an automated capillary system (Bio-Techne). Serum and nasal secretion samples were collected between May and December 2024 from 78 pigs of varying ages housed in a biosecure facility (without barrier controls for pathogen transmission). All nasal secretion samples were tested for PCMV via PCR, and serum samples for anti-PCMV antibodies via the serology western blot assay.

Results: The western blot assay detected anti-PCMV antibodies in serum samples from pigs at various stages: newborn (~14 days) with maternal antibodies, juvenile (~70 days) in the acute infection phase (PCR positive), and adult (~323 days) in the latent infection phase (PCR negative). Surveillance of 78 pigs in the biosecure facility revealed that 96% of serum samples tested positive for anti-PCMV antibodies, while 97% of PCR samples were negative, indicating successful detection of a latent infection stage.

Conclusion: The PCMV serology western blot assay effectively detects anti-PCMV antibodies at different infection stages in pigs.

212.2 | Screening Donor Pigs and Xenotransplant Recipients for Porcine Viruses

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Xenotransplantation can facilitate the transmission of pathogenic porcine viruses. The recent transmission of porcine cytomegalovirus/porcine roseolovirus (PCMV/PRV) to the first human recipient underscores the need for more effective screening strategies. Although PCMV/PRV does not infect primates, including human cells, it significantly reduced the survival time of non-human primates that received infected pig organs and contributed to the death of the human recipient. To detect PCMV/PRV in both acutely and latently infected animals, a combination of PCR-based and immunological methods is needed. To detect antibodies against PCMV/PRV, a Western blot assay using parts of the recombinant gB protein was established. Similar highly sensitive diagnostic approaches have been developed for numerous porcine viruses, including porcine circoviruses and lymphotropic herpesviruses. These methods are also relevant for the screening of recipients. The effectiveness of these methods was confirmed by screening pigs intended for xenotransplantation, various minipigs, slaughterhouse pigs, and wild boars. While early weaning, colostrum deprivation, cesarean delivery, or embryo transfer can eliminate these viruses from pig herds, porcine endogenous retroviruses (PERVs) pose a unique challenge because they are integrated into the pig genome. PERV-A and PERV-B are found in all pigs and can infect human cells, while PERV-C, which only infects pig cells, is absent in some pigs. Since PERV-C can recombine with PERV-A to form high-titer human-tropic PERV-A/C recombinants, selecting PERV-C-free donor animals is essential. This requires reliable PCR assays capable of detecting all known PERV-C variants. Detecting PERV in xenotransplant recipients is more complex due to microchimerism, also observed in allotransplantation. It is crucial to distinguish between the presence of PERV sequences in disseminated pig cells and a true infection. PCR detection of porcine-specific short interspersed nuclear elements (SINEs), which are abundant in the pig genome, can identify pig cells in the recipient. Using this method, pig cells have been found in all organs of baboons transplanted with pig hearts. Additionally, screening for antibodies against PERV can determine whether infection has occurred. Western blot assays utilizing recombinant core and envelope proteins of PERV, along with corresponding positive control antisera generated by immunizing animals with these proteins, have been developed. To date, no antibodies against PERV have been detected in any of the investigated clinical trials transplanting pig islet cells, in preclinical studies transplanting pig cells and organs into non-human primates, or in experimental infections in small animals and non-human primates. These findings indicate an absence of PERV infection. Since most of these screening methods are unavailable in standard veterinary laboratories, testing must be conducted in specialized virological units.

Funding: This research was supported by Deutsche Forschungsgemeinschaft, TRR127.

212.3 | Establishment and Monitoring of Designated Pathogen-Free Pigs for Xenotransplantation

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Xenotransplantation could offer an alternative source of organs from genetically modified animals, such as pigs, which share many physiological similarities with humans, thus making them a viable option for transplantation. However, successful xenotransplantation requires overcoming significant challenges, including immune rejection, physiological incompatibility, and the risk of zoonotic infections. In particular, optimal donor pigs for clinical xenotransplantation must be managed in a closed herd, bred in a well-controlled environment within Designated Pathogen-Free (DPF) facilities to ensure they are free from pathogens. We have established and are currently operating a DPF facility for breeding miniature pigs for xenotransplantation. In accordance with the guidelines from the Ministry of Food and Drug Safety (MFDS) in Korea, we have developed analytical

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Virus	Methods	Frequency
EMCV (Encephalomyocarditis Virus)	Real-time RT PCR	Quarterly
HEV (Hepatitis E Virus)	Real-time RT PCR	Quarterly
PCMV (Porcine Cytomegalovirus)	Real-time RT PCR	Quarterly
PCV1 (Porcine Circovirus Type 1)	Real-time RT PCR	Quarterly
PCV2 (Porcine Circovirus Type 2)	Real-time RT PCR, ELISA	Quarterly
PCV3 (Porcine Circovirus Type 3)	Real-time RT PCR	Quarterly
PLHV2 (Porcine Lymphotropic Herpesvirus Type 2)	Real-time RT PCR	Quarterly
PPV (Porcine Parvovirus)	Real-time RT PCR, ELISA	Quarterly
PRRSV (Porcine Reproductive and Respiratory Syndrome Virus)	Real-time RT PCR, ELISA	Quarterly

FIGURE 1 | 212.3

methods for a total of 148 pathogens, including 40 species of bacteria, 8 species of fungi, 13 species of parasites, 13 species of protozoa, and 74 species of viruses, to DPF transgenic pigs, the source animals for xenotransplantation. Most exogenous pathogens cannot pass through the placenta and are not transmitted from the sow to the fetus. Therefore, pigs are brought into the DPF facility through cesarean section (C-section). This method of introducing the animals is an effective way to block exogenous pathogens to the production site. Through C-section, most of the bacteria, viruses, parasites, and fungi listed in the exogenous pathogen list can be effectively blocked. Among the exogenous pathogens listed, nine viruses (Table 1) are known to be able to cross the placenta and cause infection. Therefore, we aimed to establish a real-time RT PCR method to detect the nine viruses rapidly and efficiently. For each virus, specific primers and probes were designed from the conserved regions, and gene synthesis was performed on the sequences containing these regions. Positive vectors were then independently constructed for each virus. After confirming the standard curve for the positive control of each virus, blood samples from randomly selected pigs of both commercial farm and DPF origins were tested for viruses. Most viruses were not detected from DPF facility; however, positive reactions were observed in samples from commercial farm pigs. These results demonstrate that we have established testing methods for the nine viruses capable of crossing the placenta and confirmed the ability to detect these viruses.

212.4 | Nursing Perceptions, Motivating Factors, and Potential Barriers to Participating in the Care and a Decedent Xenotransplant Recipient

Juan Esteban Baus Davalos, Grace Hammond, Grant Eickel, Ian S. Jaffe, Katya Kaplow, Cecilia Deterville, Christopher Hickson, Shushaunna Bennett, Jonah Klapholz, Keilee Luo, Nikki Lawson, Jacqueline I. Kim, Rebecca Dieter, Adam Griesemer, Sapna A. Mehta, Robert A. Montgomery, Jeffrey M. Stern, Philip Sommer, Kathleen Zavotsky, Elaina P. Weldon, Karen Khalil
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Introduction: Xenotransplantation offers a potential novel source of organs, and the use of the decedent model allows us to analyze the safety and efficacy of xenografts. This study assessed nursing staff perceptions of xenotransplantation research and identified barriers to their care of a participating decedent, with the aim of encouraging future involvement.
Methods: An IRB-approved anonymous Qualtrics survey was distributed to critical care nurses involved in the care of a brain-dead decedent enrolled in a 61-day xenokidney transplant study at NYULH. Participation in the 25-question survey was voluntary, and all survey questions were optional.
Results: Of the 25 respondents, 16 were included in the analysis, with the remaining excluded for incompletion (7) or ineligibility (2). Among respondents, the most common hesitation for participation was concern for the participant's family (66.67%), ethics of caring for the decedent (60.00%), and risk for zoonosis (53.33%). Responses indicated levels of concern decreased across all areas after caring for the decedent (Figure 1A). The most selected motivating factors were the opportunity to advance science (93.75%) and learn about xenotransplantation (93.75%). Additionally, 87.5% of respondents stated they were motivated by the opportunity to expand the organ donor pool (Figure 1B). Most respondents felt as though they impacted the overall success of the experiment and impacted progress towards implementation of xenotransplantation in clinical practice (68.75%). Moreover, 62.50% of respondents felt their participation impacted their personal/career goals (Figure 2A). Despite noted concerns, 81.25% of respondents reported they would seek out opportunities to be involved in future xenotransplantation cases, and no respondents reported an intent to avoid future xenotransplantation cases (Figure 2B).
Conclusions: After assessing perceptions, potential concerns, and motivating factors, critical care nurses involved in the care of a xenotransplant decedent recipient expressed interest in future involvement, highlighting the potential for expanding personnel participation in the care of xenotransplant recipients.

212.5 | The International Human Xenotransplantation Inventory: Current Data and Future Directions

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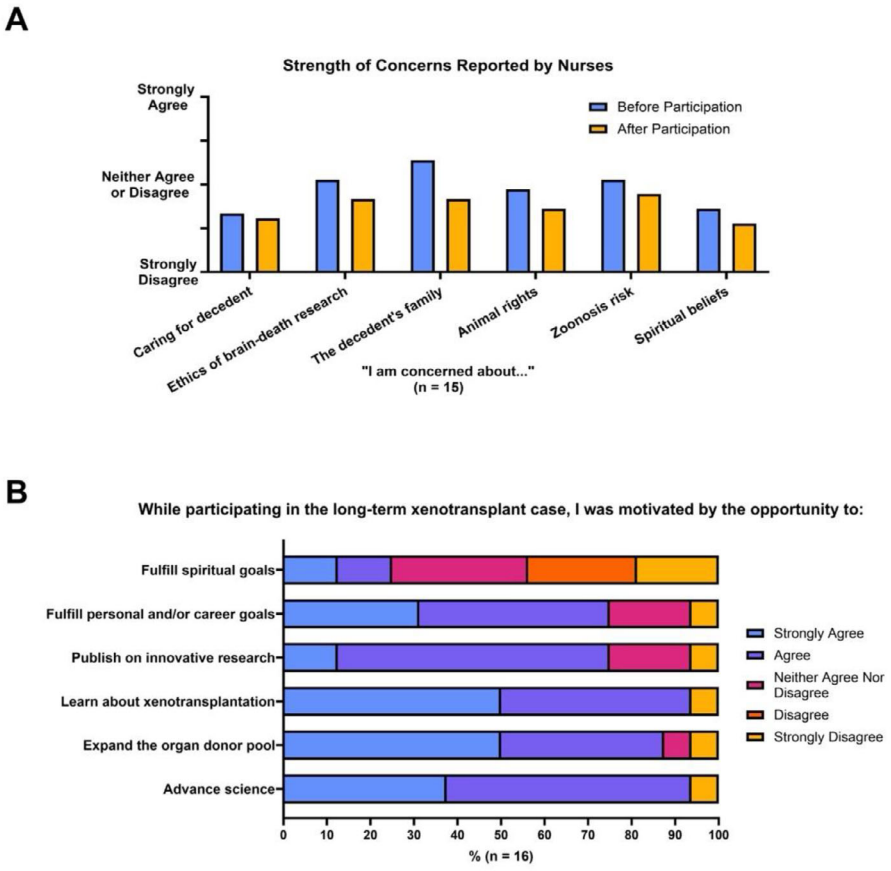


Figure 1.
A. Change in reported strength of concerns after caring for a decedent xenotransplant recipient
B. Motivating factors to participation in the care of a decedent xenotransplant recipient

FIGURE 1 | 212.4

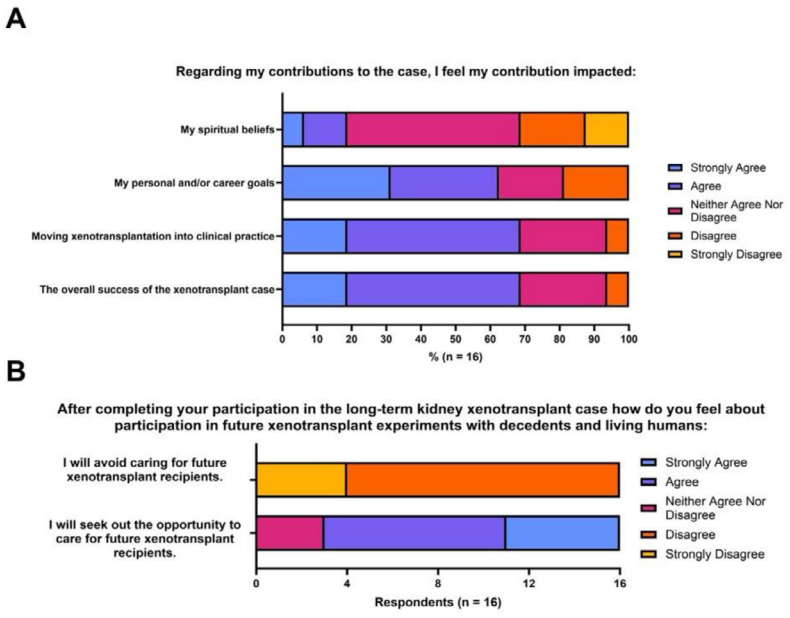


Figure 2.
A. Perceived impact of personal contribution to the case
B. Interest in participation in future xenotransplant experiments after caring for a decedent xenotransplant recipient

FIGURE 2 | 212.4

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Background: The global demand for organ transplantation outpaces supply, necessitating innovative solutions. Xenotransplantation, using animal organs, cells, and tissues, is a promising solution to address the organ shortage. The World Health Organization and the International Xenotransplantation Association collaboratively established an online inventory in 2006 (www.humanxenotransplant.org) to catalog human xenotransplantation practices. The inventory, managed successively by the Geneva University Hospital and the Sichuan Provincial People's Hospital, aligns with the WHO directives for transparency and best practices in the field of transplantation.

Methods: Relevant data have been regularly collected from numerous sources (scientific publications, congresses, press articles, declarations of International Xenotransplantation Association members) by a dedicated team in Switzerland and China, ensuring rigorous verification. The initial information is used to create a first entry in the database, which is then completed when more details become available.

Results: As of February 2025, the inventory contained 58 entries of distinct xenotransplantation procedures undertaken on humans. From these data, various trends can be observed over the last two decades regarding the type of transplantation, their regulation status, and the source animal. Notably, recent high-profile cases of solid organ transplantation involving kidneys and hearts were made feasible through years of progressive xenotransplantation research and ongoing changes to regulations.

Conclusions: Recent clinical applications of solid organ xenotransplantation suggest that more clinical procedures may soon follow for patients with end-stage kidney or heart disease, or diabetes. Future perspectives advocate for increased funding and expansion of the current registry or its potential integration into a larger and more broadly internationally recognized registry, such as the Global Observatory on Donation and Transplantation (GODT).

212.6 | Celebrating the Dead: How Medical Professionals Experience and Interact With Decedents in Xenotransplantation Experiments

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Introduction: Recently, xenotransplantation research has increasingly involved brain-dead donors, a practice known as "decedent studies". This approach holds significant potential to bridge the gap between animal studies and first-in-human clinical trials, thereby reducing both human and animal suffering. However, the practical, ethical, and procedural implications of this work are complex and multifaceted. This paper delves into these pioneering efforts to establish a research model using brain-dead humans as proxies for future xenograft recipients. It examines the ethical considerations, procedural requirements, and organizational dilemmas in the day-to-day tasks when medical staff transform a brain-dead human into a viable model for xenotransplantation. More specifically, we investigate how decedent studies challenge and reshape our understanding of life, death, and dignity, while creating new opportunities for groundbreaking research

Methods: This anthropological study is based on semi-structured interviews with 19 US nurses, xenotransplant surgeons, and researchers involved with decedent studies. Additionally, it incorporates media materials on decedent studies in the United States. All data were transcribed and analyzed thematically. Drawing on the theoretical framework of Scheper-Hughes and Lock, we employ the concepts of "the individual body," "the social body," and "the political body" to understand how xenotransplant professionals perceive and interact with decedents.

Results: The findings reveal that decedent bodies are highly valued for xenotransplantation experiments. Being human but not alive, decedents present unique opportunities for intense scientific investigation. However, they also occupy an ambiguous space for medical professionals, oscillating between categories such as person, patient, deceased, research object, and scientific hero. The results of these experiments generate enthusiasm not only among medical staff but also among the families of the decedents. Nurses, in particular, develop emotional attachments to both the decedent bodies and their families, striving to treat them with respect and dignity. This includes preparing and caring for the individual body, ensuring the success of medical experiments, and acknowledging the personhood of the decedents, sometimes even celebrating their birthdays.

Conclusion: Our study provides insights into the actual practices and dilemmas of decedent experiments and the reflections and emotions of medical professionals caring for decedents. Decedent bodies are ambiguous. They necessitate new language, careful orchestration of clinical care, and both ethical, logistical, and practical considerations. As xenotransplantation advances, we argue that scientific attention to public opinion, medical outcomes, and bioethical principles must be accompanied by in-depth anthropological research on how medical professionals experience and interact with decedents in xenotransplantation experiments.

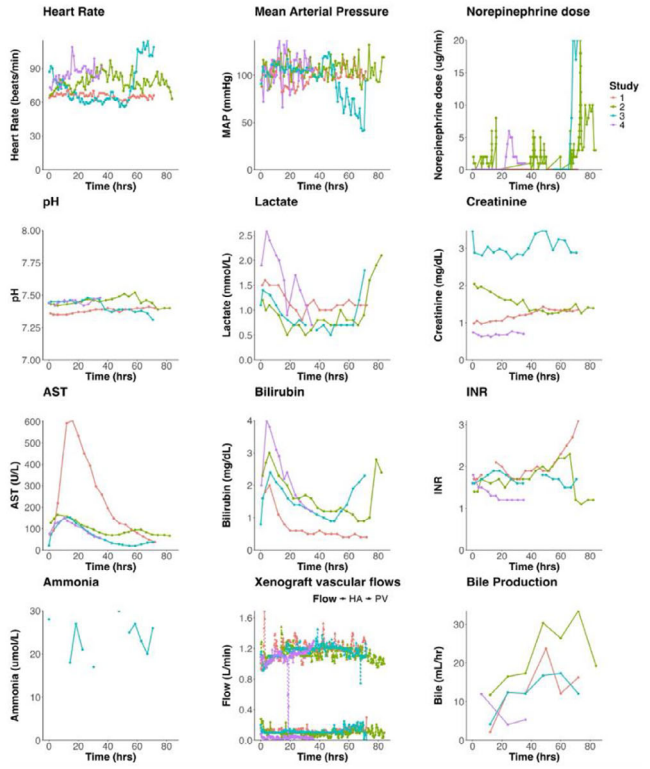
Funding: This study is part of the project "TechnEmotion: The Interaction between Technology and Emotion in Transplant Medicine" supported by a Sapere Aude grant from the Independent Research Fund Denmark (Grant no. 1055-00059B).

305.1 | Extra-Corporeal Liver Cross-Circulation Using Transgenic Porcine Xenografts in the Human Decedent Model

Alex Sagar[1], Abraham Shaked[2], Kim Olthoff[2], Peter Abt[2], Emily Vail[3], Niels Martin[4], Charles Abrams[5], Rick Hasz[6], Mohamed Elzawahry[1], John Fallon[1], Syed Hussain Abbas[1], Christine Radolovic[6], Shannon Kaminski[6], James Markmann[2], Susan Low[7], Leanne Lanieri[7], Kathryn Stiede[7], Kirsten Swenson[7], Danielle Fortuna[8], Rajender Reddy[2], Peter Reese[2,9], Peter Friend[1,10]

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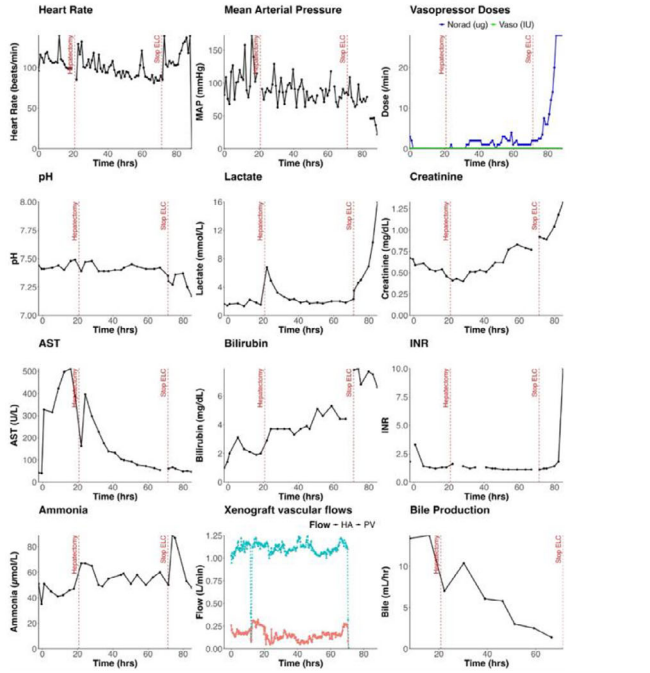


FIGURE 1 | 305.1

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Introduction: There is an unmet need for effective temporary liver support to bridge patients with acute and acute-on-chronic liver failure to native liver recovery or allotransplantation. In extra-corporeal liver cross-circulation (ELC) the patient's blood is perfused through a donor liver maintained by physiological perfusion outside the body. Transgenic porcine livers may be a viable source of livers suitable for ELC.

Methods: Four brain-dead research donors ("decedents") underwent ELC using porcine livers (EGEN-5784) with the following genetic modifications: (i) triple glycan knockout; (ii) hemizygous insertion of seven human transgenes; (iii) functional inactivation of porcine endogenous retrovirus pol gene. Immunosuppression comprised intravenous methylprednisolone.

Decedents 1–3 underwent ELC with their native livers intact for 72–84 h ("ELC 1–3"). Decedent 4 underwent two consecutive sessions of ELC with two EGEN-5784 porcine livers for a total ELC duration of 100 h ("ELC 4 and 5"). 20.8 h into the second ELC session, decedent 4 underwent native hepatectomy with porto-caval anastomosis followed by a further 48 h of ELC. After discontinuation of ELC, Decedent 4 was observed with continued critical care support until cessation of circulation.

Results: During ELC in decedents with intact native livers, the EGEN-5784 porcine livers maintained bile production and augmented native hepatocellular cytosolic, mitochondrial, microsomal metabolism and cholate clearance. Post-native hepatectomy, ELC maintained Decedent 4 with minimal inotrope requirement, and normal lactate, pH, base excess, INR, and ammonia; this persisted until elective termination of ELC 48 h post-hepatectomy. Thrombocytopenia developed within 1 h of commencing ELC in all decedents. The mean platelet count during ELC was 24 000/μL (range of study means: 10–33 000/μL) with only transient response to platelet transfusion. EGEN-5784 porcine liver biopsies demonstrated largely preserved hepatic parenchyma with mild inflammatory infiltration of predominantly CD3-and CD14-postive cells and evidence of IgM deposition.

Conclusions: ELC is feasible for at least 72 h using transgenic porcine livers in a human context and can provide effective temporary liver support for at least 48 h. This approach may provide an effective bridging therapy in acute and acute-on-chronic liver failure.

Acknowledgments: The authors acknowledge the contributions of Emily Blumberg, MD; Greg Everson, MD; Lexi Tumblety; David Wagner, PhD; Mike Duggan, DVM, and the staff at Accuro Farms; Chris Morris; Dan Fower; Shawn Welch; Leah Lambe, Jamie Ann Acero-Webb, and the staff at the Gift of Life Donor Care Center.

305.2 | Multimodal Phenotyping of Pig-to-Human Heart Xenografts' Immune Response

Erwan Morgand[1], Alessia Giarraputo[1], Jeffrey Stern[2], Fariza Mezine[1], Valentin Goutaudier[1], Guillaume Coutance[1], Aurélie Sannier[1], Anais Certain[1], Thierry Hauet[3], Sebastien Giraud[3], Thomas Kerforne[3], Geraldine Allain[3], David Ayares[4], Karen Khalil[2], Jacqueline Kim[2], Sapna Mehta[2], Navneet Narula[2], Alex Reyentovich[2], Deane Smith[2], Renaud Tissier[5], Tajinperdal Saraon[2], Bernard Kadosh[2], Michael Davita[2], Harvey Pass[2], Massimo Mangiola[2], Patrick Bruneval[1], Adam Griesemer[2], Nader Moazami[2], Robert Montgomery[2], Alexandre Loupy[1]

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Introduction: Porcine genome editing has revolutionized xenotransplantation by allowing the first pig-to-human heart transplantations. However, the mechanisms driving the immune response in heart xenografts transplanted to humans remain poorly understood.
Methods: We analyzed two heart xenografts transplanted from 10-gene-edited pigs into brain-dead human recipients. Biopsies at 66-h post-reperfusion were studied using a multimodal phenotyping approach combining: morphological evaluation, immunophenotyping, ultrastructural assessment, automated quantification of multiplex immunofluorescence staining, and gene expression profiling. Xenografts before implantation, wild-type pig hearts with and without ischemia injury, as well as wild-type pig hearts with brain death and ischemia reperfusion, were used as controls.
Results: Both xenografts showed evidence of endothelial activation and mild microvascular inflammation without capillary C4d deposition. Immune infiltrates were mainly composed of CD15+ and CD68+ innate immune cells (A). Ultrastructural assessment showed endothelial swelling with occasional intravascular leucocytes. Deep-learning-based automated multiplex immunofluorescence analysis confirmed that microvascular inflammation was primarily associated with CD15+ and CD68+ innate immune cells (B). Finally, both xenografts showed increased expression of genes associated with monocyte/macrophage activation, neutrophil activation, interferon-gamma response, natural killer cell burden, endothelial activation, apoptosis, and injury repair (C). This phenotype was absent in control pig hearts, in ischemia, and in brain death models.
Conclusion: Multimodal phenotyping of pig-to-human heart xenografts revealed a pattern of mild humoral xenoimmune response, characterized by mild innate microvascular inflammation, endothelial activation, and molecular signature of antibody-mediated rejection. Integration of this approach into future clinical trials could facilitate real-time graft monitoring, mitigate rejection risk, and enhance xenotransplant outcomes.

305.3 | Multiple Human Transgenes Prolong Survival of Triple-Carbohydrate Knockout Porcine Kidney Xenografts in Nonhuman Primates

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Introduction: Porcine xenografts devoid of three major carbohydrate xenoantigens (3KO) are considered the most desirable gene edits for clinical xenotransplantation. However, the necessity of including human transgenes (HTGs) remains controversial, especially in 3KO pigs. We evaluated, in nonhuman primates, immune responses and transplant outcome of 3KO kidney xenografts with or without various combinations of HTGs.
Methods: Kidney xenografts from gene edited pigs with 3KO alone (No HTG) or 3KO with various combinations (Table 1) were transplanted to cynomolgus monkeys. All recipients were treated with an immunosuppressive regimen that included rituximab (20mg/kg on Day 5) and ATG induction, followed by anti-CD154 (20mg/kg weekly) and mycophenolate mofetil (MMF) and 60 day-course of tacrolimus and steroids.
Results: Transplantation of 3KO kidney xenograft without HTG (No HTG) consistently resulted in early graft loss, significantly shorter compared to xenografts in all four groups with HTGs (Figure 1) ($p = 0.0015$). By adding 2 self-recognition pathway regulatory molecules and 3 complement-related HTGs to 3KO in HTG-A, the hazard ratio was reduced to 0.18 ($p = 0.014$) compared to No HTG. Addition of coagulation pathway regulatory molecules (THBD, TFPI and PROCR) in HTG-B and C failed to further extend graft survival. In association with adding the two anti-inflammatory HTGs, TNFAIP3 and HMOX1, in HTG-D, xenograft survival with excellent kidney function beyond 1 year was achieved, with the longest survival exceeding 2 years (Figure 1). Transcriptomic analysis of 1-h biopsy already showed significantly higher expression of genes (HLA-DRA and IGHG3) related to immune activation in No HTG group vs. HTG-D (Figure 2). Protocol biopsies at 3 months revealed enrichment of seven distinct pathway representing rejection in HTG-B, compared to HTG-D, suggesting beneficial effects of adding anti-inflammatory HTGs, TNFAIP3 and HMOX1 (Figure 3).
Conclusions: Multiple combinations of HTGs, particularly those with anti-inflammatory properties, significantly extended the survival of 3KO kidney xenografts, accompanied by more favorable transcriptomic responses.

305.4 | Brain-Dead Human Subjects as Preclinical Models for Xenotransfusion: Feasibility and Safety of Genetically Engineered Pig Red Blood Cell Transfusion

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Objective: Xenotransfusion using genetically engineered (GE) pig red blood cells (pRBCs) offers a potential solution to blood shortages, particularly in emergencies. This study aimed to evaluate the suitability of brain-dead human subjects as a preclinical model for xenotransfusion by comparing their hematological and immunological profiles with those of acute blood loss (ABL) patients and assessing the feasibility, safety, and immunological responses to GE pRBCs in a brain-dead human subject.
Methods: Comprehensive evaluations were conducted on hematological, biochemical, electrolyte, coagulation, and inflammatory markers, arterial blood gas parameters, and immunological profiles in brain-dead subjects ($n = 179$) and ABL patients ($n = 104$) requiring transfusion. In vitro assessments of anti-triple-knockout (TKO; lacking Gal, Neu5Gc, and Sda antigens) pig RBC responses were also performed in brain-dead subjects

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Table 1 Various combinations of HTGs in TKO

			Anti-inflammatory/Immune				Complement			Coagulation		
Group	N	KO	TNF AIP3	HMOX1	CD47	HLA-E/ β2M	CD46	CD55	CD59	THBD	PROCR	TFPI
No HTG	4	3KO										
HTG-A	6	3KO			√	√	√	√	√			
HTG-B	6	3KO			√	√	√	√		√		√
HTG-C	3	3KO			√	√	√	√		√	√	√
HTG-D	16	3KO	√	√	√		√	√		√	√	

Fig. 1

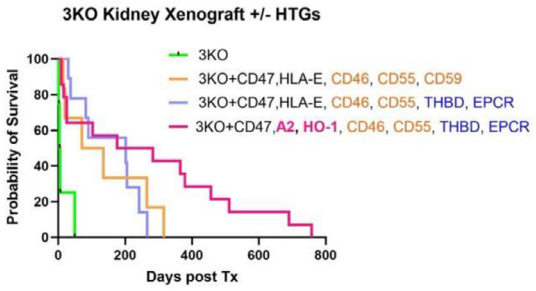


FIGURE 1 | 305.3

Fig. 2 1-hour biopsy No HTG vs. HTG-D

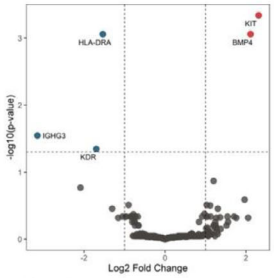
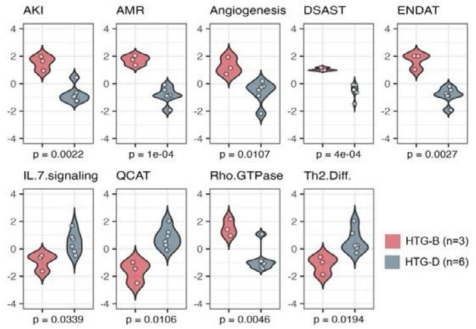


Fig. 3 Bulk mRNA expression in HTG-B vs. HTG-D



(n = 31) and compared to those of ABL patients (n = 102). RBCs from TKO pigs expressing human CD55 (housed under pathogen-free conditions) were labeled with CFSE for tracking. A single brain-dead human subject received a transfusion of GE pRBCs, with pre-transfusion treatments including an anti-C5 inhibitor, corticosteroids, and antihistamines. Monitoring included continuous vital sign tracking, hematological and immunological assessments, complement activation assays, and pathogen screening.

Results: Comparative analysis revealed similar pathophysiological and immunological profiles between DBD and ABL patients, supporting the former's relevance as a xenotransfusion model. GE pRBCs exhibited survival rates of 50% at 24 h, 15% at 48 h, and were cleared by 72 h. Pre-transfusion anti-pig IgM levels were low, transiently decreased post-transfusion, and rebounded by Day 3. Anti-pig IgG levels, initially undetectable, significantly increased by Day 7, accompanied by heightened complement-dependent cytotoxicity and hemagglutination. Both classical and alternative complement pathways were activated but moderated by regulatory mechanisms. No pig-derived pathogens were detected.

Conclusion: Brain-dead human subjects closely replicate the clinical conditions of ABL, providing a valuable preclinical model for xenotransfusion research, particularly in settings where non-human primate models have limitations. This study establishes the feasibility and safety of GE pRBC xenotransfusion, offering critical insights into immune responses and pathogen safety. The absence of zoonotic transmission and the manageable immune profile observed in this first-in-human trial underscore the potential of xenotransfusion as a promising solution to global blood shortages in critical care.

Funding: This study was supported in part by the National Key Research and Development Program (2023YFC3404304: Y.W., 2024YFC3406800: H.J.), National Natural Science Foundation of China (82260154: Y.W., 82460153: H.J., 82400891: T.L.), and Hainan Provincial Science and Technology Talent Innovation Project (Category B) (KJRC2023B08: Y.W.). The funder was not involved in the study design, collection, analysis, interpretation of data, the writing of this article, or the decision to submit it for publication.

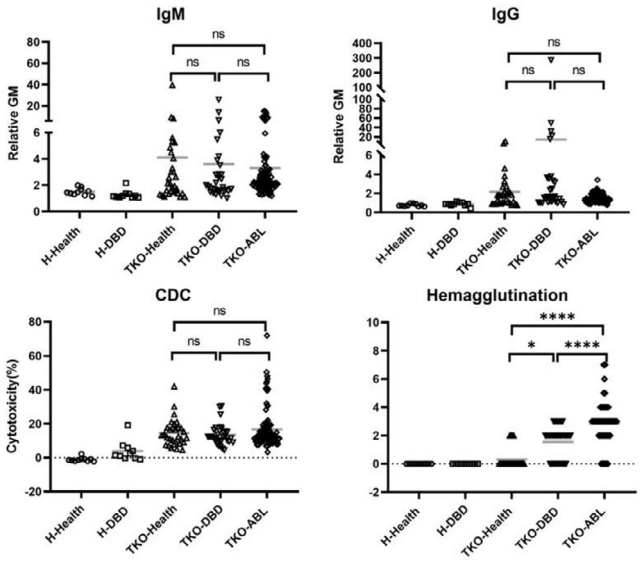


FIGURE 1 | 305.4

305.5 | Combined 11-Gene-Editing With HLA-E Expression and Donor Macrophage Depletion Extends Survival in Pig-to-Baboon Lung Xenotransplantation

Sho Takemoto[1], Seyed Amir Sanatkar[1], Zahra Habibabady[1], Ike Ileka[1], Kohei Kinoshita[1], Victoria Diaz[1], Megan Dufault[1], Lars Burdorf[2], Will Eyestone[2], David Ayares[2], Richard N. Pierson III[1]

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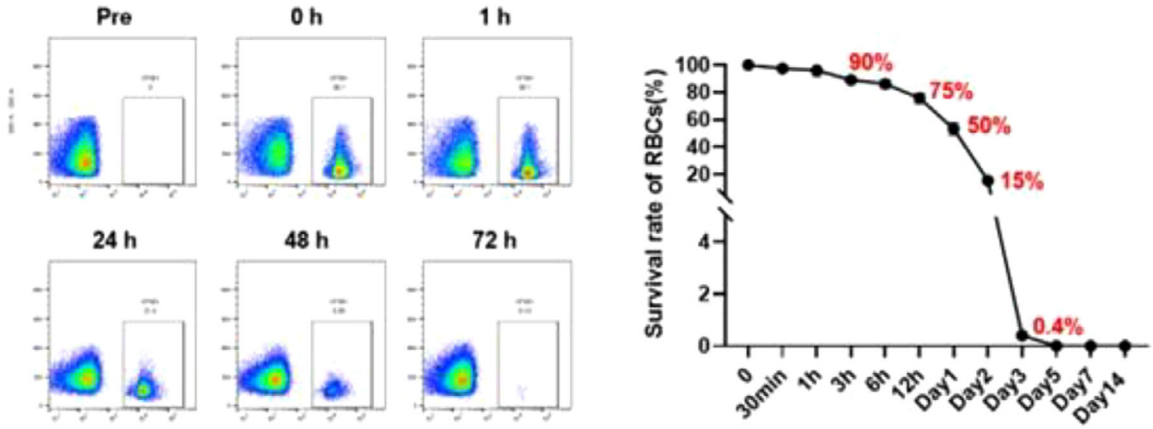


FIGURE 2 | 305.4

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Introduction: Lung xenotransplantation offers a potential solution to the critical shortage of donor lungs, yet significant immunological and physiological barriers remain. Key challenges include maintaining vascular barrier function, preventing coagulopathy, and mitigating innate immune responses, particularly those mediated by macrophages and NK cells. The longest reported survival in pig-to-baboon lung xenotransplant is 31 days, achieved with a double knockout (α1,3-GalT, β4GalNT2) based 7GE pig. We hypothesized that a more comprehensive multigene editing, targeting NK cell activation and donor macrophage depletion, could prolong graft survival. This study aimed to assess the function and survival of 10 and 11GE pig lungs in parallel in vivo (pig-to-baboon) and ex vivo (pig-to-human) xenotransplant models.

Methods: Left single lung transplants were performed in baboons using lungs from 10 ($n = 5$) and 11GE ($n = 1$) pigs. The 10GE pigs had growth hormone receptor and triple carbohydrate gene knockout (TKO) (α1,3-GalT, β4GalNT2, and CMAH), and six added transgenes (hCD46.hCD55.hEPCR.hTBM.hHO-1.hCD47); the 11GE pig additionally expressed human HLA-E. Donor pigs were pre-treated with desmopressin to deplete porcine vWF (pvWF), with three receiving liposomal clodronate (LC) for macrophage depletion. Recipient treatment included splenectomy, αCD20, ATG, CoVF, donor kidney perfusion (3 of 6), selectin/integrin blockers, αGP1b, 1-BIA, α1AT, αCD154, and inhibitors of C1 esterase, IL-6, IL-8, and TNFα. The contralateral right lungs were perfused ex vivo with fresh human blood treated with αGP1b, 1-BIA, and selectin blockade.

Results: The 11GE lung survived 33 days, and 10GE lung survivals were 1 to 3 days in vivo (Figure 1A) and >8 h ex vivo. 10GE lung failure in vivo was primarily due to loss of vascular barrier function despite markedly reduced levels of IL-6 and IL-8 compared to reference GE lungs (Figure 2A,B). LC-lungs had less severe parenchymal damage in vivo (Figure 1B). Histopathology confirmed extensive inflammation, diffuse hemorrhage, and microthrombosis in non-LC 10GE lungs; in contrast, the 11GE+LC lung showed only mild inflammation at POD 1 and preserved alveolar structure and airway epithelium at POD 26 (Figure 1C). Ex vivo, neutrophil and platelet sequestration were delayed, and histamine levels were reduced relative to non-LC lungs (Figure 2C,E).

Conclusion: The 11GE lung, combined with donor macrophage depletion, resulted in a record 33-day survival, surpassing the previous 31-day record achieved with a 7GE lung lacking CMAH-KO and the 7-day survival of humanized pvWF (h*pvWF) expressed lung. Donor macrophage depletion attenuates xenograft injury, in part by impacting neutrophils and platelets, and reducing histamine release. Some combination of omitting the CMAH-KO (9GE), additional expression of h*pvWF and/or HLA-E, and donor macrophage depletion may synergistically extend lung xenograft survival in baboons.

306.1 | Blood Cell Gene Expression in Xenotransplantation of Genetically Modified Pig Heart Into Human Recipient: Comparison to Human Allotransplantation

Corbin Goerlich[2], Muhammad M. Mohiuddin[1], Bartley Griffith[1], Avneesh Singh[1], David L. Ayares[3], Evgeniya Vaskova[4], Robert N. Woodward[4], Alesha I. Luxon[4]

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Introduction: Gene expression profiling (GEP) has been used in the clinic for over 15 years to monitor immune quiescence in human heart transplant recipients. The composite score from 11 genes has been validated to discriminate rejection from quiescence; however, this signature has not been used in other species. In the first human xenotransplant recipient of a genetically modified porcine heart, the GEP score and the results from individual genes were examined in the context of the data from the development and use of the allotransplant GEP.

Method: Collection of GEP samples in the first porcine heart-to-human xenotransplant was the same as in human allotransplantation. Samples were drawn weekly per standard methods, shipped to CareDx, and processed in the CLIA laboratory to generate GEP scores (AlloMap). The standard reported score, as well as the results for individual genes, were exported for investigational analysis in xenotransplantation. Individual genes

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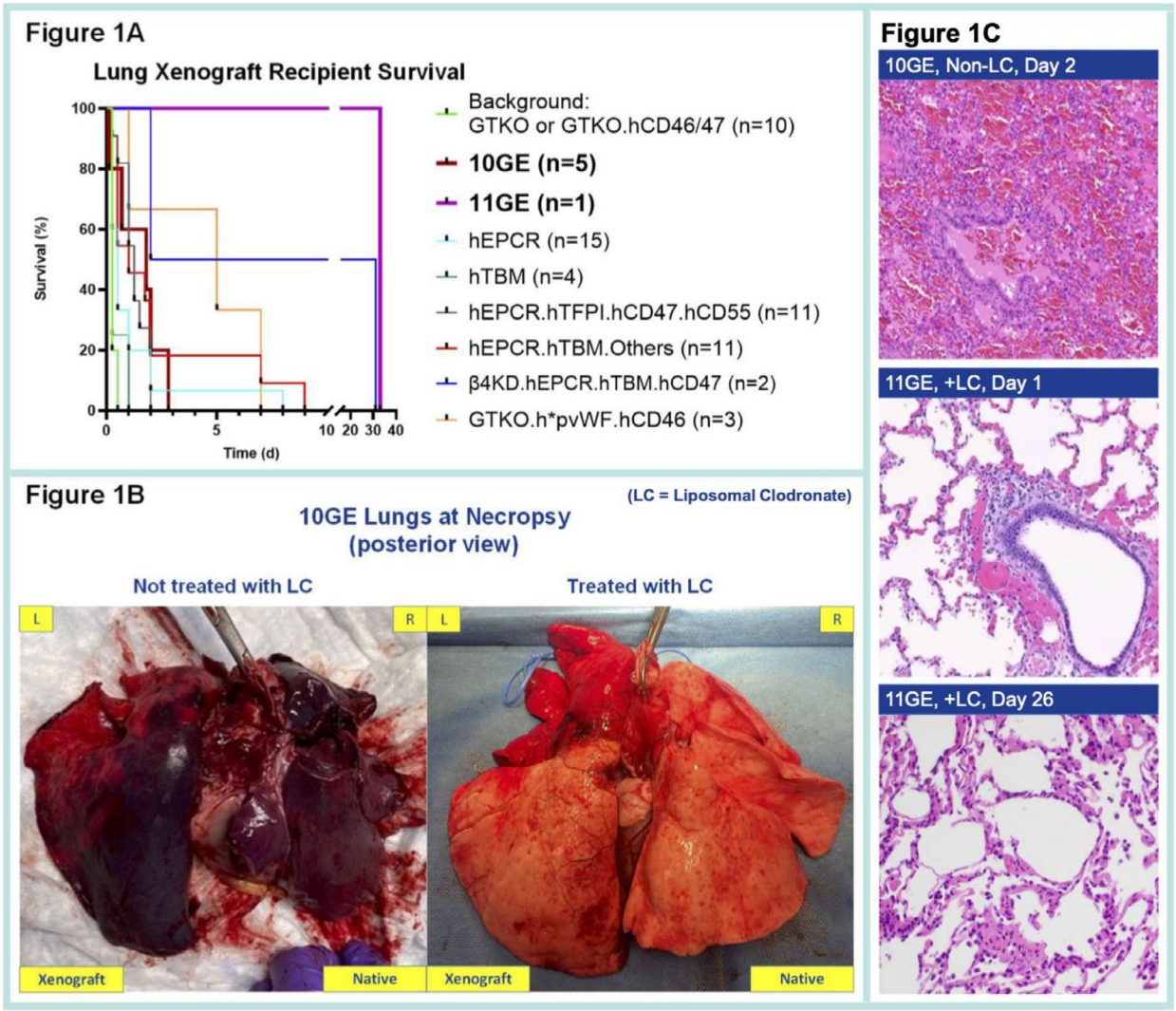


FIGURE 1 | 305.5

(FLT3, IL1R2, ITGAM, ITGA4, PDCD1, RHOU, SEM7A, DCAF12, MARCH8, G6b1, PF4) were evaluated as transcript copy number.
Results: Gene expression from individual genes and the composite GEP score in this xenotransplant experience were all within the range of values observed in human allotransplant. The GEP score was very low early post-transplant, consistent with high levels of corticosteroids commonly used in this timeframe. The GEP score generally followed the established allotransplant trajectory. Individual gene transcript levels were often at extremes, well outside the range of 99% of the >55d allotransplant population. Metagenes clustered together as expected based on allotransplant experience at most timepoints. Deviations from scores consistent with quiescence were attributable to individual metagene clusters, including a novel observation of coordinated elevation of SEMA7A with the DECAF12/MARCH8 metagene cluster.
Conclusion: The established measurement of GEP used to monitor human blood gene expression in allotransplantation appears to be likely applicable to human xenotransplant recipients. Ongoing use of GEP scores established for evaluation of human immune quiescence should continue to be evaluated for use in human recipients of xenotransplants.

306.2 | Correlation of Left Ventricular Global Longitudinal Strain With NT-proBNP in the First and Second Genetically Modified Porcine to Human Xeno Heart Transplant

Javier Galindo[1], Muhammad M. Mohiuddin[1], Andy A. Tully[1], Avneesh K. Singh[1], Sarah Leventhal[1], Marco Oldsman[1], Sarah Cipriano[1], Susie Hong-Zohlman[1], Timm Dickfeld[1], Mohammed Asadi[1], Brian Barr[1], Albert Hicks[1], Anuj Gupta[1], Erika Feller[2], Dave Ayares[3], Bartley Griffith[1], Manjula Ananthram[1]
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Background: Xenotransplantation is emerging as a potential therapeutic modality for the treatment of end-organ failure; however, translation to the clinic will require intensive surveillance of rejection and graft dysfunction. Global longitudinal strain (GLS)1-3 has been shown to be a sensitive marker of rejection and is predictive of major adverse cardiac events & all-cause mortality in cardiac allograft recipients in the heart allotransplantation literature. Our institution performed two genetically modified

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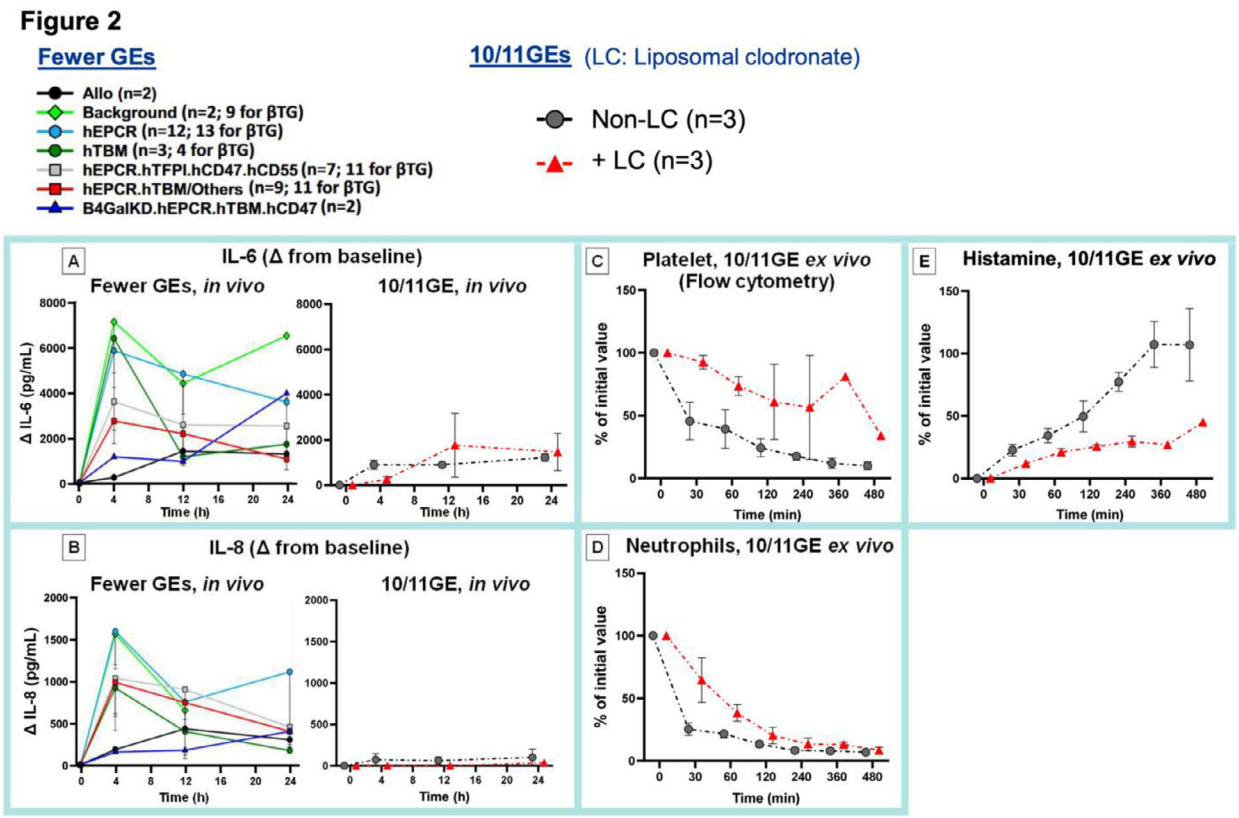


FIGURE 2 | 305.5

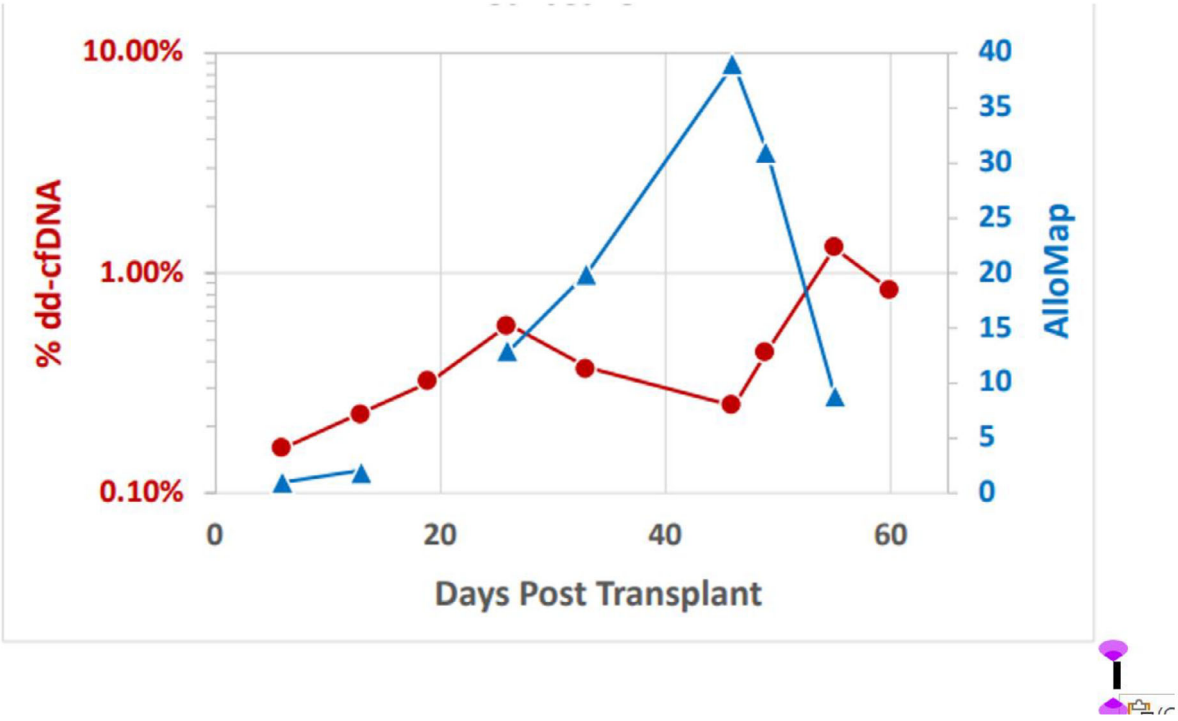


FIGURE 1 | 306.1

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porcine-to-human cardiac xenotransplants (CXT), 6,7. Patient 1 (P1) & Patient 2 (P2) were declined for allotransplantation & mechanical circulatory support at multiple centers. We observed progressive declines in GLS in both CXTs. It was hypothesized that GLS would correlate with biochemical markers of xenograft dysfunction (XGD), specifically serum NT-proBNP values. A retrospective analysis was conducted to investigate this.

Methods: Serial transthoracic echocardiography (TTE) was performed & NT-proBNP measurements were obtained. NT-ProBNP is only available for the first 3 weeks for P1. TTE was analyzed to calculate GLS. Simple univariate linear regression was performed & Pearson correlation coefficient was calculated using GraphPad Prism 10 (Dotmatics, Boston, MA, USA).

Results: GLS declined to about −10% in both patients. In P1, NT-proBNP fell post-operatively from a mildly elevated value of 245 pg/mL. In P2, NT-proBNP fell from a peak of about 6000 pg/mL to 230 pg/mL on Day 19 before rising slightly late in the patient's course. Regression analysis shows a non-significant positive slope ($\beta = 3.355$, $p = 0.7145$) for P1. This corresponds to a weak positive Pearson correlation coefficient of 0.23. In P2, the high starting value of NT-proBNP reflects his pre-transplant condition and results in a moderate and significant negative association between GLS and NT-proBNP ($\beta = -171.8$, $p = 0.0057$). This corresponds to a Pearson correlation coefficient of −0.64. If we exclude the first 10 days of P2's course, this correlation disappears ($\beta = 6.128$, $p = 0.3904$).

Figure 1

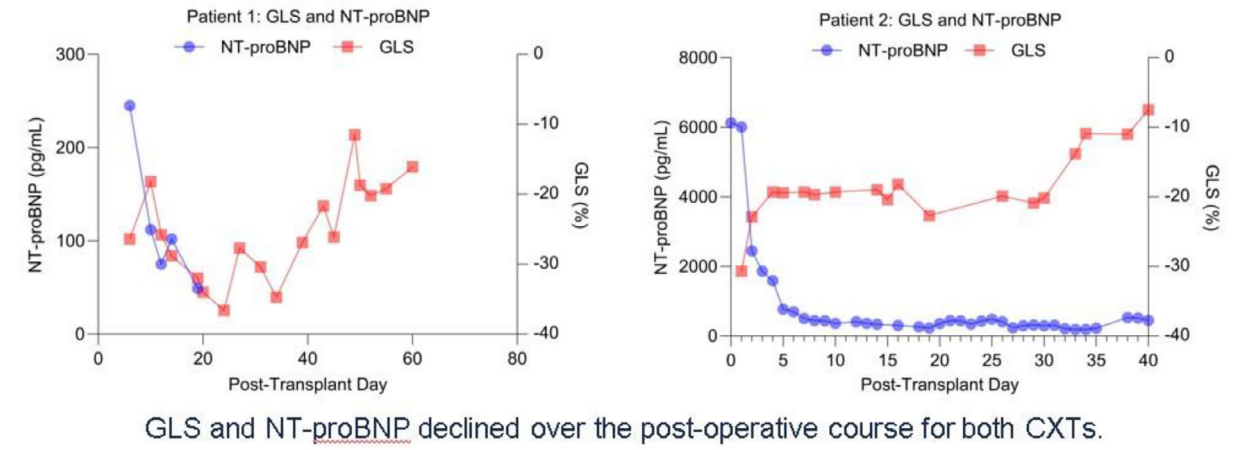
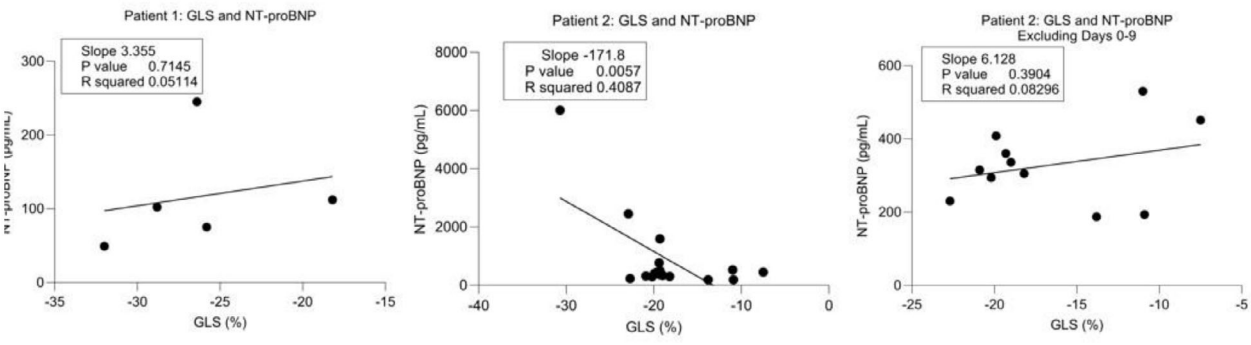


FIGURE 1 | 306.2

Figure 2



Simple univariate regression analysis shows a non-significant positive slope (β = 3.355, p = 0.7145) for P1. This corresponds to a weak positive Pearson correlation coefficient of 0.23. In P2, we find a moderate and significant negative association between GLS and NT-proBNP (β = -171.8, p = 0.0057), corresponding to a Pearson correlation coefficient of -0.64. This association disappears if exclude the first 10 days of P2's course (β = 6.128, p = 0.3904).

	Patient 1	Patient 2
Pearson ρ	0.23	-0.64
P-value	0.7145	0.0057
R^2	0.05	0.41

FIGURE 2 | 306.2

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Discussion: As expected from what is known about its use in cardiac allotransplantation, these data demonstrate that GLS is a sensitive predictor of graft dysfunction in cardiac xenotransplantation. Although we observe a negative and statistically significant correlation between GLS and NT-proBNP, this effect is driven by the high pre-operative NT-proBNP values and disappears when excluding Days 0 to 9. These results are in line with what is described in the allotransplantation literature, where GLS has been shown to predict all-cause mortality in transplant recipients, whereas NT-proBNP is of limited prognostic value. A modest, non-significant correlation between GLS and NT-proBNP was observed in the first two cardiac xenograft recipients. While GLS remains a valuable prognostic tool in assessing xenograft health, NT-proBNP appears to be of limited utility.

306.3 | Establishing Correlation of Non-Invasive Monitoring Techniques in Xenotransplantation

Sarah Cipriano[1], Muhammed Mohiuddin[2], Andrew Tully[3], Javier Galindo[2], Susie Hong[1], Marco Oldsman[1], Albert Hicks[1], Timm Dickfeld[1], Anuj Gupta[1], Alison Grazioli[2,3], Allison Lankford[3], Erika Feller[4], Bartley Griffith[2], Manjula Ananthram[1]

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Introduction: Non-invasive surveillance of cardiac allograft health has included biomarkers, such as NT-proBNP, and donor-derived cell-free DNA (dd-cfDNA). It has previously been shown that these biomarkers, when elevated, indicate graft complications such as rejection, coronary artery vasculopathy, or donor-specific antibody production. As xenotransplantation enters the arena as a potential option for those with advanced heart failure, noninvasive monitoring techniques need to be investigated. Our institution performed the second porcine-to-human cardiac xenotransplant with a 10-gene edited porcine cardiac xenograft under FDA compassionate use authorization. After requiring perioperative transfusions, by postoperative day (POD) 13, endomyocardial biopsy (EMB) revealed antibody and complement deposition without damage. Hemodynamic decompensation on POD 29 prompted EMB, revealing antibody-mediated rejection (AMR).

Research Questions/Goals: The authors sought to investigate whether NT-proBNP and dd-cfDNA positively correlate in patients with cardiac xenografts.

Methods/Approach: NT-proBNP was checked periodically, and dd-cfDNA was assessed once to twice weekly post-transplant. A retrospective review was completed, including Pearson's correlation coefficient analysis.

Results/Data: There was a weak negative correlation between the NT-proBNP and dd-cfDNA ($r = -0.28$). After the initial spike in the perioperative period, NT ProBNP levels declined. NT ProBNP did not rise as dd-cfDNA did at the time of biopsy-proven antibody-mediated rejection (AMR). See the graphic below for details.

Conclusion: Noninvasive surveillance of cardiac xenografts needs further investigation. Biomarkers such as NT-proBNP negatively correlated with dd-cfDNA, which increased as expected at the time of biopsy-proven antibody-mediated rejection. Porcine xenografts are accustomed to a low-pressure circulatory system in contrast to the human recipient milieu. Despite this increased load to the xenograft, NT-proBNP levels decreased significantly after the initial postoperative phase. The NT proBNP values could have been lower than expected due to the effects of continuous renal replacement therapy. It is interesting to also note that the human assay detected xeno NT-proBNP.

Funding: United Therapeutics, Inc. provided funding to the University of Maryland Foundation to help defray the cost of this transplant. No financial support was provided by Eledon Pharmaceuticals or XVIVO. Federal funding was used for the preclinical trial studies leading to this work. DA is an employee of Revivicor Inc., a subsidiary of United Therapeutics, Inc. Members of the Program in Cardiac Xenotransplantation received research funding from United Therapeutics, Inc.

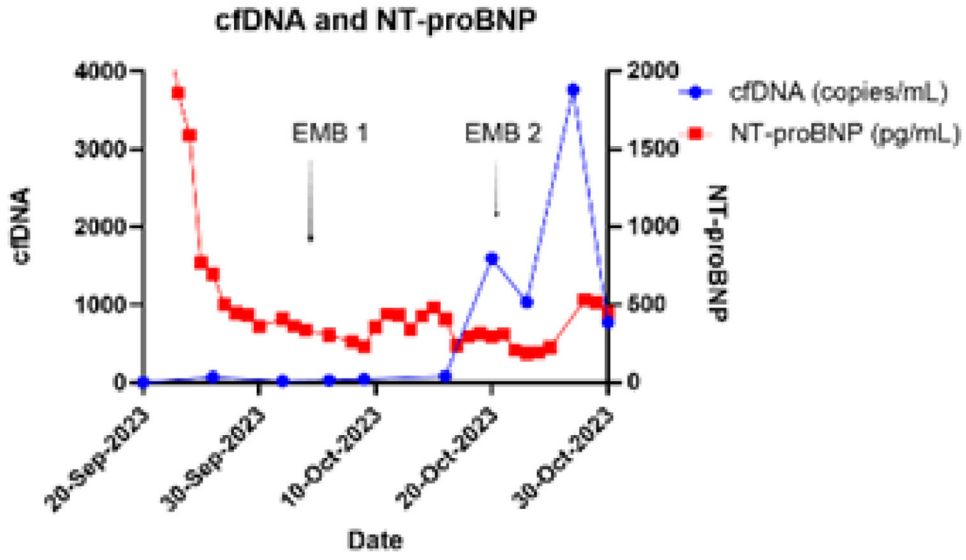


FIGURE 1 | 306.3

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Acknowledgments: The source animal was provided by Revivicor Inc., and the Tegoprubart antibody was provided by Eledon Pharmaceuticals, both in kind. CareDx and Karius analytics were provided in kind.

306.4 | Invasive Versus Echocardiographic Hemodynamics in Two Genetically Modified Porcine to Human Cardiac Xenotransplants

Alice Tang[1], Muhammad Mohiuddin[2], Cullen Soares[1,3], Susie Hong-Zohlman[1], Andrew Tully[2], Javier Galindo[2], Marco Oldsman[1], Sarah Cipriano[1], Timm Dickfeld[1], Brian Barr[1], Sadegh Asadi[1], Anuj Gupta[1], Alison Grazioli[2], Allison Lankford[2], Erika Feller[4], David Ayares[5], Bartley Griffith[2], Manjula Ananthram[1]

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Background: Our institution performed two transgenic porcine-to-human cardiac xenotransplantations. There is limited data with the first cardiac xenograft, suggesting echocardiographic assessment of the cardiac xenograft in humans correlates with invasive hemodynamic assessments. We investigated whether echocardiographic-derived hemodynamic indices would correlate with invasive measurements in both cardiac xenotransplants.
Methods: Serial transthoracic echocardiography (TTE) and right heart catheterizations (RHC) were performed in the cardiac xenografts. Retrospective analysis of TTE and invasive hemodynamics was performed in both the first and second xenotransplant to assess the correlation between various parameters. Invasive hemodynamic measures include right atrial pressure (RAP), pulmonary artery systolic and diastolic pressures (PASP, PADP), Fick cardiac output (CO), cardiac index (CI), right ventricular stroke work index (RVSWI), and cardiac power output (CPO). TTE measurements and calculations included: RAP, PASP calculated via tricuspid regurgitation velocity (TRV), PADP based on end diastolic pressure gradient, tricuspid annular plane systolic excursion (TAPSE), right ventricular outflow time-velocity integral (TVI RVOT), pulmonary vascular resistance (PVR) = [TRV/TVI RVOT × 10 + 0.16], CO, CI, cardiac power output (CPO) = [mean arterial pressure echo-derived CO/451], and right ventricular contraction pressure index (RVCPI) = [4 × (TRV)^2 × TAPSE], as a surrogate of RVSWI.
Results: Pearson correlation using invasive measurements at the time of TTE for cardiac output shows a positive correlation of 0.69 (p = 0.06) with a trend towards significance. Pearson's correlation of RVSWI and RVCPI demonstrated a significant positive correlation of 0.83 (p = 0.04).
Conclusions: In the first two cardiac xenotransplants, there remains a positive correlation in hemodynamic markers between invasive and noninvasive measurements. Given the intense interest in cardiac xenotransplant, continued investigation in correlating noninvasive and invasive hemodynamic markers will provide real-time prognostic and clinical value for future research.

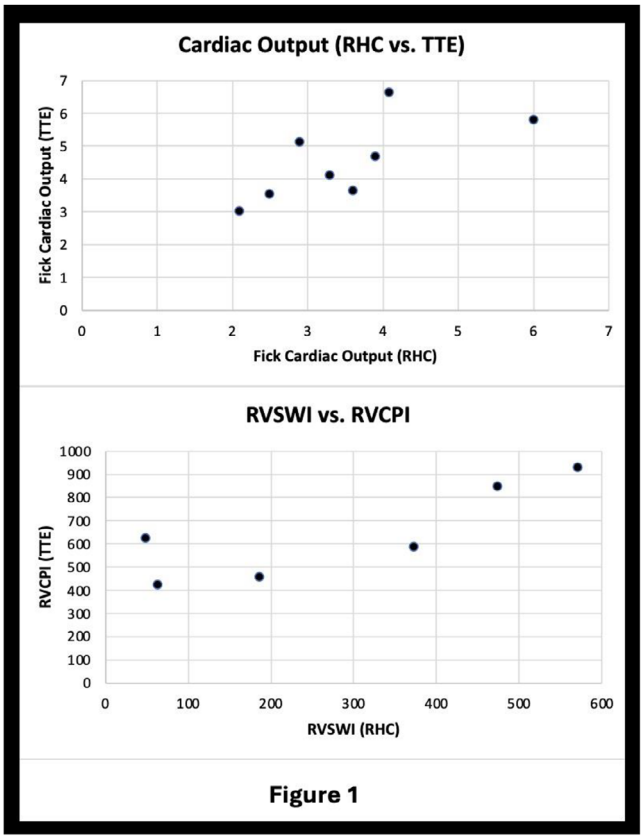


FIGURE 1 | 306.4

306.5 | Nursing Experience: Caring for a Porcine Kidney Xenotransplant Recipient

Alexandra Hawkins, Joy Dixon, Joan McNeil, Kimberly Sureau, Karen Khalil, Aprajita Mattoo, Vasishta Tatapudi, Jeffrey Stern, Adam Griesemer, Robert Montgomery, Elaina Weldon, Margaret Fattal, Dorothy Dojlidko.

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Xenotransplant Research Group.
Introduction: Porcine xenografts can expand the organ pool for patients seeking transplants with end-stage renal disease. This is especially true for those with difficulty finding suitable donors due to high panel reactive antibody (PRA). Our center successfully transplanted a human with a porcine kidney xenotransplant. Here, we detail our bedside care delivery experience for this patient with an interprofessional, collaborative approach, ensuring safety, continuity of care, and favorable outcomes at an academic health system.
Methods: Following an approved study by the IRB and the FDA in collaboration with the Xenotransplant Research Group, registered nurses (RNs) and nurse practitioners (NPs) were educated about the care of the xenotransplant recipients, nurses' responsibilities, and potential known and unknown risks associated with participating with these recipients' care. To prepare the nursing team, education occurred in advance with live meetings,

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video conferencing, and institutional learning modules, which included review of clinical protocols to ethical considerations. Infection prevention precautions consisted of strict hand hygiene before and after patient care. The RN's and NP's assignments excluded other patients with infections requiring isolation precautions. RNs and NPs participated in daily hybrid (virtual and in-person) interprofessional rounds detailing the patient's prior 24-h events, including all clinical data and interventions, culminating in a comprehensive plan for the day. This team also included transplant-specific surgery, nephrology, infectious disease, pharmacy, xenotransplant researchers, and immunogenetics experts. Plans included immunosuppression management, diagnostics, and management of comorbid conditions. RNs and NPs assessed the patient throughout each shift. RNs performed bedside handover to ensure patient-family centered continuity of care. Post-transplant education was provided by NPs, RNs, and pharmacists throughout the hospitalization. Discharge planning encompassed the entire team's input and was executed by NPs, RNs, and care management RNs to arrange follow-up visits, home health nursing, and home infusions.

Results: RNs and NPs were vital to data gathering, assessment of new findings, timely and accurate documentation, medication administration, teaching, and communication with the multidisciplinary team. Nurses were responsible for care coordination to ensure a smooth transition to the outpatient setting. The patient maintained the porcine xenograft for 130 days prior to xenograft nephrectomy. After surgical recovery, the patient was discharged and transitioned back to her primary caregivers.

Conclusions: Nursing care with exquisite attention to detail was at the forefront of the success of our xenotransplant recipient. Our center and others can continue to use this protocol as a model for the care of future xenotransplant recipients.

307.1 | Neutrophil Extracellular Trap (Net) Formation After Xenotransplantation: A Potential Contributor to Inflammation and Thrombodysregulation

Amir Sanatkar[1], Megan Dufault[1], Sho Takemoto[1], Felix Ellett[2], Kirandeep K. Gill[2], Sunil Poudel[1], Lindemberg Da Mota Silveira Filho[1], Victoria Diaz[1], Jonathan Schulz[1], Zahra Habibabady[1], Kristin M. Whitworth[3], Lars Burdorf[4], William Eyestone[4], David Ayers[4], Richard N. Pierson[1]

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Introduction: Platelet and neutrophil sequestration immediately following reperfusion of gene-modified pig organs remains a significant challenge in xenotransplantation, particularly in lung and liver grafts. Evidence suggests that these events contribute to graft dysfunction. Neutrophil Extracellular Traps (NETs)—web-like structures of DNA and proteolytic enzymes released by activated neutrophils—play a critical role in exacerbating thrombodysregulation and cell sequestration in association with systemic infection and ischemia/reperfusion injury. We asked whether xenotransplantation is associated with NET formation in several organ xenograft models.

Methods: Because detecting NETs is challenging due to their transient nature, we employed multiple complementary approaches: (1) dual neutrophil positivity for DNA and neutrophil elastase by flow cytometry; (2) co-localized DNA and myeloperoxidase (MPO) binding to leukocytes during Bioflux ex vivo microfluidic "vascular" perfusion; (3) mechanically trap chromatin fibers from NETs using perfusates (blood) collected during various timepoints of ex vivo Lung perfusions Using an established microfluid device; (4) sandwich ELISA for DNA-MPO complexes in plasma from in vivo heart and lung xenograft recipients.

Results: Human buffy coat leukocytes co-incubated with wild-type (WT) pig endothelial cells (ECs) at a 1:10 ratio demonstrated neutrophil activation followed by NET formation within 30 min (Figure 1A). TNF-α (10 ng/mL) preactivation of either porcine ECs or human neutrophils significantly enhanced NET formation during Bioflux perfusion (Figure 1B), reaching maximal levels when both ECs and neutrophils were pre-activated. Ex vivo perfusion of pig lungs with Galko-based genetic modifications showed a rapid NET release pattern within minutes (Figure 1C). Ex vivo perfusion of pig hearts ($N = 2$) or lungs ($n = 4$) exhibited variable amounts of NET formation and kinetics (Figure 1D). In vivo heart and lung xenotransplant studies revealed a rise in NET markers associated with conditioning regimens used (before and at T_0); within hours after graft revascularization; and in association with clinical events such as during rejection or infectious episodes.

Conclusion: Under experimental conditions where a variety of strategies aimed at reducing neutrophil overactivation were consistently deployed—including anti-platelet agents, E- and P-selectin blockade, and IL-8 inhibition—NET formation is often detectable. We speculate that NET formation may represent a key contributor to early proinflammatory and thrombodysregulatory events observed after xenotransplantation using multiply gene-edited organs. Ongoing efforts are focused on elucidating the underlying mechanisms of NET induction in this setting, particularly exploring the interactions of neutrophils with activated platelets, and identifying effective therapeutic strategies to prevent or mitigate NET-associated complications.

307.2 | Spatial Multimodal Characterization of Long-Term Immune Response After Pig-to-Human Kidney Xenotransplantation

Valentin Goutaudier[1], Erwan Morgand[1], Jeffrey Stern[2], Fariza Mezine[1], Karen Khalil[2], Claire Williams[3], Idris Boudhabhay[4], Lubka Roumenina[4], Aurélie Sannier[1], Aprajita Mattoo[2], Vasishta Tatapudi[2], David Ayares[5], Patrick Bruneval[1], Adam Griesemer[2], Massimo Mangiola[2], Robert A. Montgomery[2], Alexandre Loupy[1]

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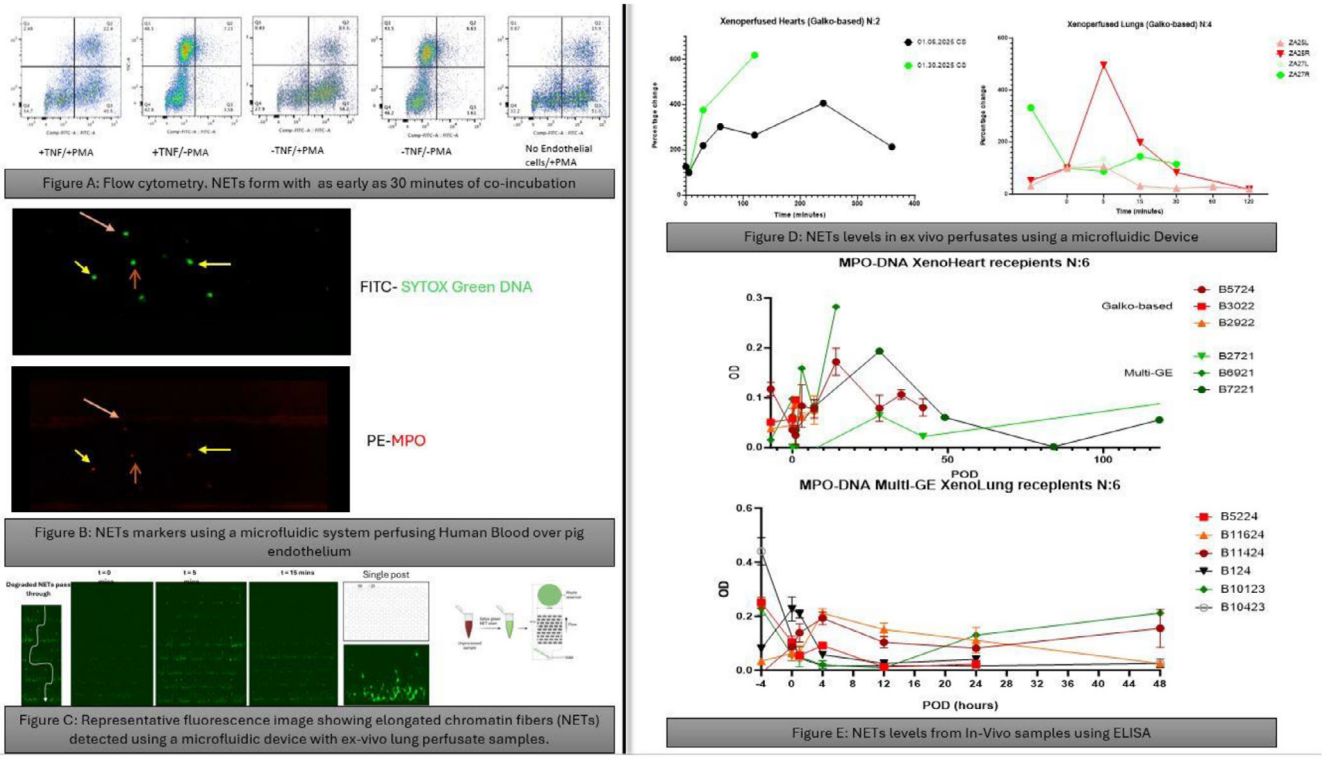


FIGURE 1 | 307.1

Introduction: We previously characterized the short-term immune response after pig-to-human kidney xenotransplantation, but little is known about its long-term evolution. Here, we aimed to decipher the long-term xenoimmune response in a human decedent model.

Methods: We analyzed a genetically modified pig kidney xenograft implanted for 61 days in a deceased human recipient with a pre-transplant negative xenoreactive crossmatch. We performed morphological evaluation, multi-immunophenotyping, 38-hyperplex complement cascade imaging, bulk transcriptomic profiling, and spatial transcriptomics at cellular and subcellular levels (NanoString CosMx) at sequential time points (POD10, POD33, POD45, POD49, POD61). Controls included pre-implantation xenografts, wild-type pig kidney autografts, and brain-dead pig kidneys.

Results: From POD10 to POD61, we observed a membranoproliferative glomerulonephritis (MPGN)-like pattern characterized by microvascular inflammation, predominantly in glomeruli, associated with intense linear capillary C4d deposition, glomerular deposition of IgM and IgA, endothelial activation, and early post-xenotransplant de novo positive xenoreactive crossmatch. This pattern was accompanied by glomerular double contours, interstitial edema, and endarteritis from POD45. Capillary inflammation primarily consisted of CD68+ and CD15+ innate immune cells throughout all follow-up time points. At POD45, we observed co-localized deposits of IgM, IgA, C1q, C3c, C4d, factor B, and complement factor H regulator along glomerular capillaries. The xenograft exhibited increased expression of genes related to humoral response, including monocyte and macrophage activation, NK cell burden, endothelial activation, interferon-gamma response, and T and B cell interaction, which improved over time after rejection treatment (Figure 1).

Conclusion: We described for the first time a new entity of xenoantibody-mediated injury characterized by an MPGN-like pattern, mainly driven by complement activation through glomerular xenoimmune complexes, which improved after rejection treatment. These findings support the development of personalized therapies to improve xenotransplantation outcomes.

307.3 | Exploring the Efficacy of Complement-Modulating Protective Coatings for Porcine Endothelial Cells Via Combination of Metabolic Glycoengineering and Click Chemistry

Ekaterina Umnyakova, Jannes Felsch, Simona Jacquemai, Shugirshan Suthagar, Haijie Zhao, Alexander J. Lander, Said Rabbani, Daniel Ricklin

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Molecular Pharmacy Group.

Introduction: During xenotransplantation, activation of host defense mechanisms, particularly the complement cascade, can induce inflammatory responses that result in xenograft dysfunction or rejection. Protective surface coatings capable of modulating complement activation confer a promising therapeutic approach to enhance xenograft viability, yet their evaluation demands specialized experimental models that enable biocompatible cell modification and mimic xenotransplant conditions.

Methods: We developed a coating approach for endothelial cell models by integrating metabolic glycoengineering with bioorthogonal click chemistry. Porcine iliac artery endothelial cells (PIECs), relevant to xenotransplantation applications, were cultured in the presence of azide-modified monosaccharides to

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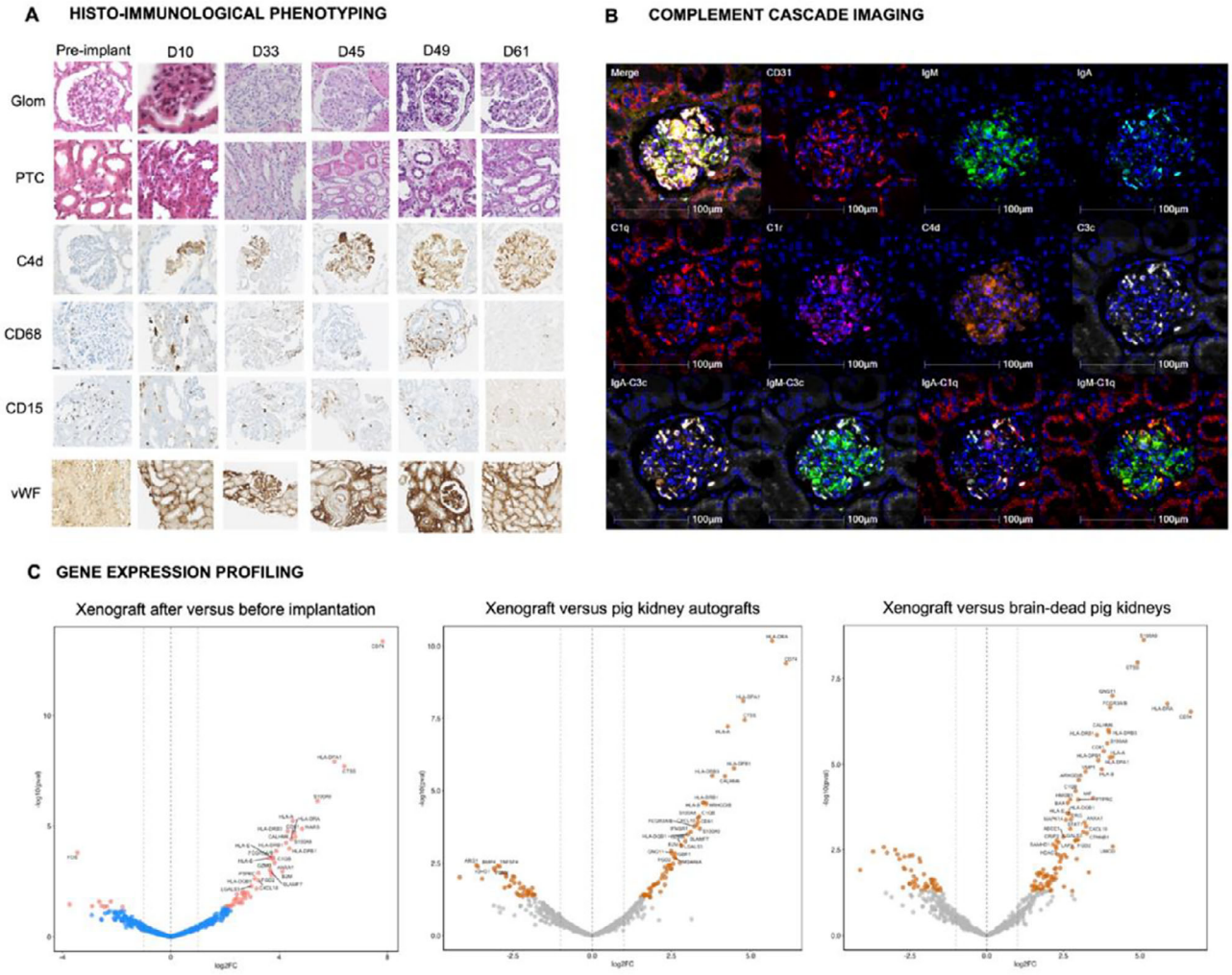


FIGURE 1 | 307.2

introduce reactive azide groups onto cell-surface glycans. This enabled the covalent attachment of an alkyne-functionalized peptide (5C6) that specifically recruits the physiological complement regulator factor H (FH; Figure 1A). The surface distribution of azides, coated peptides, and recruited FH was monitored by fluorescence microscopy and functional efficacy by measuring opsonization upon serum exposure.

Results: By optimizing conditions for glycan modification, we achieved peptide coating of PIEC with controllable density and even surface distribution while preserving endothelial cell viability. PIEC coated with 5C6, but not with a control peptide (scr5C6), efficiently recruited FH from purified sources and normal human serum onto 5C6. When exposed to Mg_2EGTA serum, 5C6-coated cells showed enhanced resistance to alternative pathway-mediated complement attack (Figure 1B). Beyond efficacy screening, this study provided critical insights into mechanisms underlying glycan modification, peptide coating stability, and complement activation dynamics specific to endothelial cells in xenotransplantation.

Conclusion: Our endothelial cell-based system offers a powerful platform for screening and optimizing complement-regulating coatings for xenotransplantation and may facilitate broader mechanistic studies and preclinical evaluation in xenotransplantation settings.

307.4 | Expression of Plexin-B2 on Swine Endothelial Cells Suppresses Neutrophil-Mediated Cytotoxicity in Xenotransplantation Models

Soichi Matsumura[1], Yoichi Kakuta[1], Shota Fukae[1], Masataka Kawamura[1], Shigeaki Nakazawa[1], Akira Maeda[2], Naoki Kan[2], Kengo Nakahata[2], Takehisa Ueno[2], Shuji Miyagawa[2], Norio Nonomura[1]

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Introduction: Human neutrophils are a major barrier to graft survival in xenotransplantation, particularly in the early stages of the innate immune response. In our previous studies, we reported that CD47 and CD31 suppress neutrophil activation. On the other hand, recent studies on anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV) have shown that Plexin-B2 acts as a ligand for neutrophil semaphorin 4D (SEMA4D)

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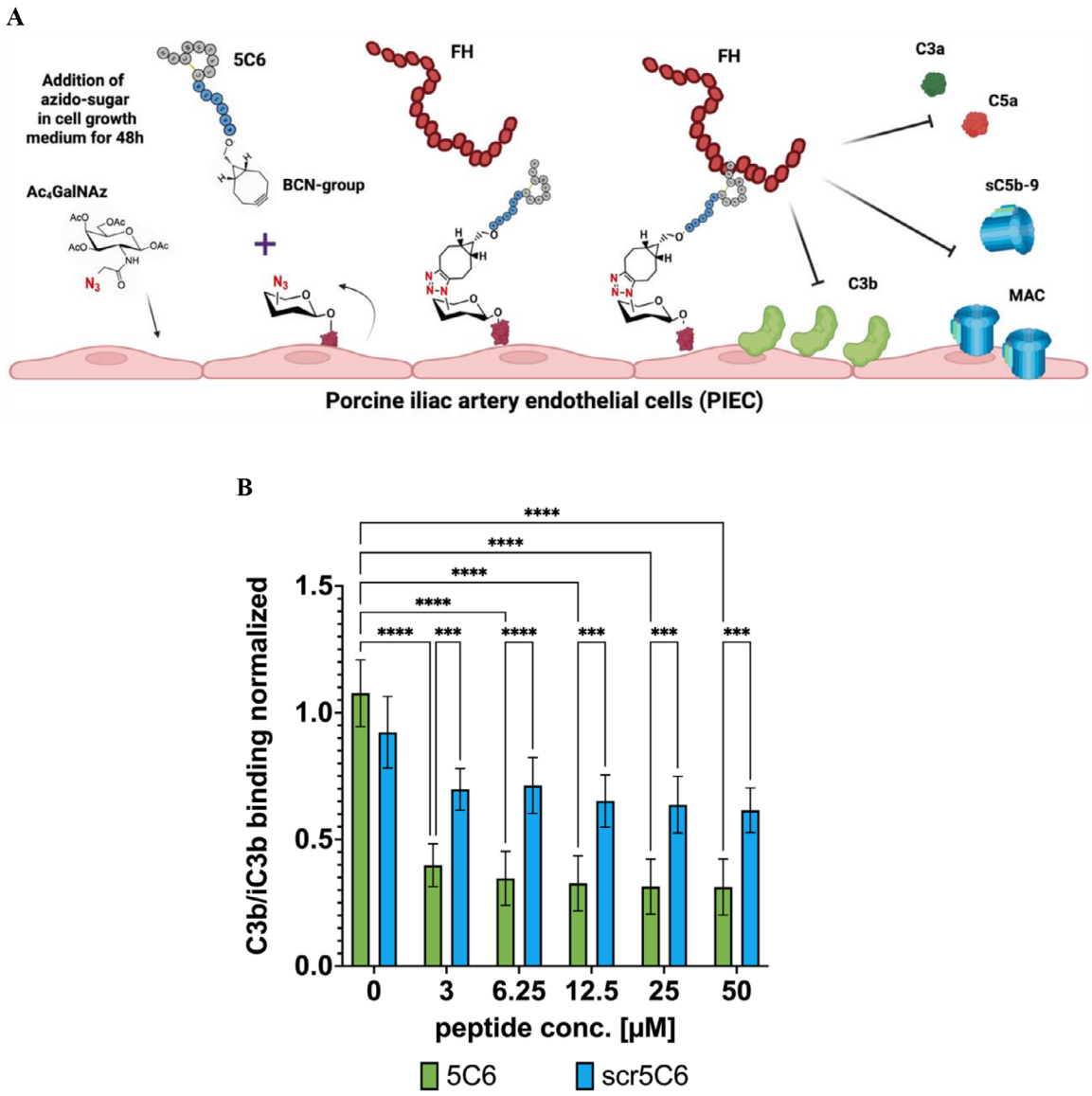


FIGURE 1 | 307.3 (A) Coating approach to provide complement control via regulator recruitment. Incubation of endothelial cells in $Ac_4GalNAz$-supplemented culture medium leads to the incorporation and presentation of azido-groups on the glycocalyx. This allows the covalent coupling of a clickable 5C6 derivative to azide-bearing glycans via a triazole bridge. 5C6-coating of cell surfaces enables the recruitment of endogenous FH, downregulating opsonization with C3b, MAC formation, and the release of anaphylatoxins (C3a, C5a). (B) Complement downregulation was evaluated in 50% Mg_2EGTA serum using different concentrations of peptides 5C6 and scr5C6 (scrambled negative control). Bars indicate the mean of the replicates; error bars indicate standard deviation, ****: significant ($p \leq 0.0001$).

and suppresses neutrophil activation. Semaphorins are a group of approximately 30 molecules with a wide range of functions discovered since 1992, including "immune semaphorins," which regulate the immune system from macrophages to T cells. However, the inhibitory effect of Plexin-B2 in xenotransplantation remains unclear. In this study, we compared the inhibitory effects of Plexin-B2 expression in swine endothelial cells (SECs) on neutrophil-mediated cytotoxicity, reactive oxygen species (ROS) production, and neutrophil extracellular trap (NET) formation with those of CD47 and CD31.

Methods: The cDNAs for Plexin-B2, CD47, and CD31 were cloned into the expression vector pCXN2 (chicken β-actin promoter + CMV enhancer) and transfected into SECs to generate SEC/Plexin-B2, SEC/CD47, and SEC/CD31 cell lines, respectively. Expression was confirmed by flow cytometry. Human neutrophils were isolated from healthy volunteers, and the inhibitory functions of these molecules were assessed by cytotoxicity assays using 10 or 50 nanomolar concentrations of phorbol 12-myristate 13-acetate (PMA). In addition, ROS production and NET formation in neutrophils were evaluated using flow cytometry and SYTOX Green staining, respectively. Statistical significance was determined using Student's t-test.

Results: First, the expression of each molecule on SECs was confirmed by flow cytometry. The cytotoxicity of SEC/Plexin-B2 was significantly reduced compared to wild-type cells with 10 nM PMA stimulation ($p = 0.0145$) and 50 nM PMA stimulation ($p = 0.00053$) for 5 h. In contrast, the suppression of cytotoxicity was not clearly observed in SEC/CD47, but it was significantly reduced in SEC/CD31 compared to wild-type cells ($p = 0.0213$ at 10 nM, $p = 0.0056$ at 50 nM).

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Furthermore, Plexin-B2 expression significantly attenuated ROS production ($p = 0.019$) and NET formation ($p = 0.0063$) compared to wild-type cells, confirming its inhibitory effect on neutrophil activation. For SEC/CD47, the ROS assay did not show clear suppression but indicated significant downregulation of NET formation compared to wild-type cells ($p = 0.0025$).

Conclusion: These findings suggest that endothelial expression of Plexin-B2 may provide a novel strategy to suppress neutrophil-induced xenograft injury and improve graft survival by targeting neutrophil–endothelial cell interactions in xenotransplantation.

Funding: This work was supported by the Japan Society for the Promotion of Science (JSPS) KAKENHI Grant Number:24K12507.

307.5 | Complement Inhibition in Xenotransplantation – Why, What, and When?

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Introduction: The complement system, an integral part of innate immunity, includes over 60 proteins and can be activated through three pathways: classical, lectin, and alternative. Uncontrolled complement activation can lead to complications in allo- and xenotransplantation, including graft rejection. Transgenic expression of human complement regulatory proteins in donor organs has been used to mitigate these issues, but challenges still remain. Here, we aim to summarize current literature supporting the role of complement inhibition in preserving graft function in xenotransplantation. We also examine clinical experiences with C3/C3b-targeted complement inhibition with pegcetacoplan in post allotransplantation complications such as antibody-mediated rejection (AMR), thrombotic microangiopathy (TMA) and C3 glomerulopathy and extrapolate these findings to xenotransplantation. Furthermore, we explore the mechanistic differences among clinically approved complement inhibitors and discuss their potential integration into immunosuppressive regimens for xenotransplantation.

Methods: A literature review from 2000 to 2025 was conducted, focusing on complement activation in donor organ injury and rejection processes.

Results: All complement pathways contribute to xenotransplantation-related injury. For example, ischemia reperfusion injury (IRI) can activate all pathways, while preformed or de novo anti-pig antibodies can trigger the classical pathway. Complement activation has been documented across various xenograft types and is a key driver of hyperacute rejection. Despite reducing hyperacute rejection with the introduction of human complement regulatory transgenes, residual complement activity still causes inflammation and ultimately lead to graft rejection. Emerging evidence underscores the critical role of complement activation in post-transplant complications such as graft rejection, mediated through mechanisms such as IRI, TMA, AMR as well as cell-mediated transplant rejection.

Conclusions: Clinical experience with complement-targeted therapies in allotransplantation showed promising efficacy. With the availability of multitude of approved inhibitors targeting various components of the complement cascade, there is an opportunity to tailor therapeutic approaches to block specific pathways relevant to xenograft rejection. Incorporating complement inhibitors into immunosuppressive protocols may provide a more comprehensive approach to immune modulation, thereby improving xenograft outcomes. As an example, pegcetacoplan has recently been used in animal xenotransplantation models, decedent models and living recipients with promising signs of efficacy. Future studies should focus on optimizing the timing, dosage, and combination of complement inhibitors to maximize their protective effects in the xenotransplantation setting.

310.1 | Retention of a Native Kidney With Ureter Ligation Compensates for Renin-Angiotensin System Function After Pig-to-Baboon Kidney Transplantation

Kohei Kinoshita[1], Maho Terashita[1], Akihiro Maenaka[1], Ryousuke Satou[2], Akemi Katsurada[2], Ivy A. Rosales[3], Gweneth E. Lavalla[1], David Ayares[4], Tatsuo Kawai[1], L. Gabriel Navar[2], David K. C. Cooper[1]

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Purpose: The ability of pig kidneys to fulfill physiological roles after transplantation (Tx) into baboons, including the renin-angiotensin-aldosterone system (RAAS) function, remains unclear. In prior studies where bilateral native nephrectomy was performed at pig kidney Tx (KTx), persistent dehydration required frequent saline infusions to prevent elevated serum creatinine (Cre). Additionally, recent pig-to-decedent human models with nephrectomy reported low or undetectable circulating renin. This differs from clinical KTx, where native kidneys are retained. We explored this topic by preserving one native kidney (after ureter ligation to observe life-supporting xenograft function) during pig KTx.

Methods: Baboons ($n = 6$) received kidneys from gene-edited pigs ($n = 4$, triple-knockout [TKO] with growth hormone-receptor-knockout and 6 human protective proteins: hCD46, hCD55, hTBM, hEPCR, hHO1, hCD47). Two baboons underwent bilateral nephrectomy (Group A) and four retained one native kidney with ureter ligation so that it could not contribute to salt and volume excretion (Group B). Induction therapy included anti-thymocyte globulin, anti-CD20mAb, and C1-esterase inhibitor. Maintenance therapy included anti-CD154mAb, rapamycin, methylprednisolone, and IL-6R blockade with tocilizumab. Monitoring included clinical parameters and endocrine assays for RAAS in collected blood samples.

Results: The immunosuppressive regimen supported graft survival >1 year ($n = 2$), >6 months ($n = 2$), and >3 months ($n = 2$). Group A exhibited features of dehydration: e.g., increasing Cre levels (requiring weekly, bi-, or tri-weekly normal saline administration) and higher potassium levels. Group B maintained normal Cre levels without fluid support. Naive pigs showed positive pig renin, but pig renin was undetectable at all post-transplant time-points in both groups (Figure 1A). Baboon renin concentration was measurable only in Group B (Figure 1B), indicating that the remaining kidney contributed to renin production. Group B showed higher baboon renin

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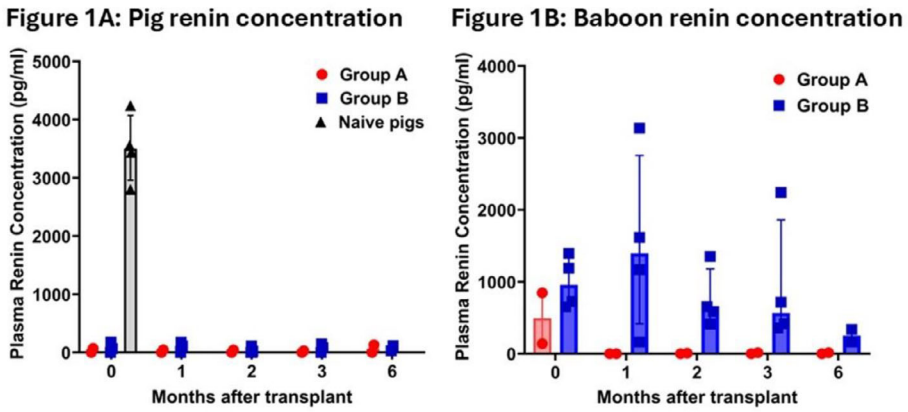


FIGURE 1 | 310.1

concentrations and activity than Group A, resulting in elevated angiotensin I, which indicated that angiotensinogen was cleaved by baboon renin. There were no differences in antiotensinogen or aldosterone levels between groups.

Conclusions: The absence of detectable pig renin highlights a potential physiological challenge in xenotransplantation. However, (1) retaining one native kidney with ureter ligation compensates for RAAS deficiencies observed after pig-to-baboon kidney transplantation (with bilateral native nephrectomy) and prevents dehydration. In clinical pig-to-human kidney xenotransplantation, preservation of native human kidneys may similarly maintain the RAAS with human renin. (2) Investigations need to be continued to determine whether the loss of RAAS function is related to an absence of (i) production or (ii) secretion of pig renin, or (iii) incompatibility of pig renin to interact with human angiotensinogen.

Funding: The work on xenotransplantation in our laboratory is supported in part by NIH NIAID U19 grant AI090959 and in part by a Kidney X Prize from the US DHHS and the American Society of Nephrology.

310.2 | Cardiac Xenograft as a Bridge to Allotransplantation in a Non-Human Primate Model: Lessons Learned

William Swicord[1], J. H. Simmons[2], Sarah J. Neal[2], Clementine Vo[3], Kanwarpal Bakshi[3], Julie Juliani[4], Julie Fenske[4], Kristen Getchell[5], Isabela Moreno[5], Vincent Yeung[5], David C. Cleveland[1], John D. Cleveland[1]

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Objective(s): Xenotransplantation of genetically engineered porcine hearts (GEPH) has been utilized as destination therapy in adult human subjects under FDA expanded access criteria. We envision this technology can save the lives of human infants with critical congenital heart disease who are currently dying on the cardiac allotransplant waiting list. A successful animal model is necessary to study this application and prove concept.

Methods: Three olive baboons aged 29, 15, and 29 months and weighing 6.9, 4.8, and 6.1 kg respectively underwent orthotopic cardiac xenotransplantation from size matched Yucatan miniature swine. Animals were maintained on immunosuppression (IS) centered on CD40 ligand blockade (Tegoprubart, Eledon Pharmaceuticals) for up to 8 months. Function of the xenograft was tracked on weekly echocardiogram and invasive hemodynamic monitoring. Immunologic responses were followed with flow cytometry. Xenografts were removed and surgically replaced with allografts from prospectively crossmatched, ABO compatible, size-matched donor baboons. Immunosuppression following this was transitioned to tacrolimus and mycophenolate mofetil. Functional and immunologic outcomes following allotransplantation were monitored in the aforementioned fashion.

Results: All animals survived >4 months with xenografts that exhibited normal systolic function up to the time of explant. There was no evidence of anti-pig antibody above pre-transplant levels nor was there evidence of same species sensitization (Figure 1). One out of three survived the allotransplant operation. The first animal was allowed to retain a small portion of porcine inferior vena cava (IVC) to facilitate repeat transplant. The second succumbed following a surgical error made in attempt to remove all porcine tissue. The third succumbed to volume overload during induction therapy prior to allotransplantation. Baboon #1 developed acute rejection 71 days following allotransplant and survived 105 days before elective euthanasia. Anti-pig antibodies increased in the setting of conventional immunosuppression and retained pig IVC.

Conclusion: Herein is proof of concept that bridge to cardiac allotransplantation with a cardiac xenograft is feasible. Prolonged exposure to GEPH does not cause same species sensitization, but conventional immunosuppression is not capable of preventing reactivity to xeno-antigens. All pig tissue must be removed prior to transition to conventional IS regimens and surgical strategies must be tailored to allow for this. Further study is warranted in the animal model.

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	Porcine Donor (Pig) - HECs				Allo-Donor (Baboon) - PBMCs			
NHP ID	Pre-Xeno Screen		Pre-Allo Screen		IgG	IgM	B Cells (CD20+)	T Cells (CD3+)
	IgG	IgM	IgG	IgM				
Baboon 1	Low IgG	Low IgM	Low IgG	Low IgM	No Increase	No Increase	No Increase	No Increase
Baboon 2	Low IgG	Low IgM	Low IgG	Low IgM	No Increase	No Increase	No Increase	No Increase
Baboon 3	Low IgG	Low IgM	Low IgG	Low IgM	No Increase	No Increase	No Increase	No Increase

Figure 1. Porcine heart endothelial cells (HECs) were treated with serum from the screened non-human primate (NHP) for the xeno-heart or allo-heart transplant. Low IgG or IgM levels is defined as anti-pig antibody levels lower compared to pooled baboon serum (pre-xeno screen) or pre-transplant levels (pre-allo screen). Peripheral Blood Mononuclear Cells (PBMCs) (pre-transplant and most recent timepoint) from the allo-donor were treated with serum from the xeno-heart NHP. No evidence of allo-sensitization against IgG, IgM, B cells (CD20+) and T cells (CD3+) from allo-donor PBMCs.

FIGURE 1 | 310.2

310.3 | GLP Preclinical Trial of Pig-to-Baboon Thymokidney Xenotransplantation Using Inbred GalT-KO Miniature Swine Donors

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Introduction: The International Xenotransplantation Association has recommended clinical xenotransplantation trials based on preclinical studies performed under Good Laboratory Practice (GLP), in which consistent life-supporting xenograft survival of >6 months has been achieved. Here, we present the first preclinical GLP study of pig-to-baboon thymokidney (TK) xenotransplantation using single knock-out (GalT-KO) swine as donors.

Methods: Recipient baboons ($n = 5$) with low natural non-Gal antibodies underwent total thymectomy and induction therapy with rATG and rituximab. On Day 0, they underwent bilateral nephrectomy and splenectomy and received transplants of TK grafts from inbred GalT-KO miniature swine. Immunosuppression (IS) included MMF, rapamycin, anti-CD40 mAb, and Belatacept. Kidney function was assessed through biochemical analyses and biopsies. Donor-specific antibodies (DSA) and absolute T cell, B cell, and recent thymic emigrant (RTE) counts were monitored. Mixed lymphocyte reaction (MLR) assays were performed monthly.

Results: Two animals were followed until POD 180, and 3 animals were continued through POD 365. Mean creatinine was 1.88 ± 0.53 and 2.8 ± 0.29 mg/dL on Days 180 and 370, respectively for the two groups. Kidney biopsies indicated mild transplant glomerulopathy and mild interstitial fibrosis without any signs of rejection or immunoglobulin deposition. After the post-transplant decrease, DSA remained stable. RTEs defined as CD45RA+CCR7+CD28+CD95-CD31+ cells, were detected in the circulation within two months after transplantation. In all animals except Animal 5, at least one MLR indicated pig-specific hyporesponsiveness.

Conclusion: In a GLP setting, we achieved 1 year life-supporting TK xenograft survival in baboons utilizing single GalT-KO miniature swine as donors. TK grafts maintained good function with no signs of rejection prior to termination. Post-transplant detection of RTE suggests that functioning pig thymus grafts may contribute to pig-specific hyporesponsiveness. Our results suggest that single GalT-KO inbred miniature swine donors may be appropriate for use in clinical trials. Future studies will focus on enhancing thymus-dependent tolerance to achieve IS-free survival.

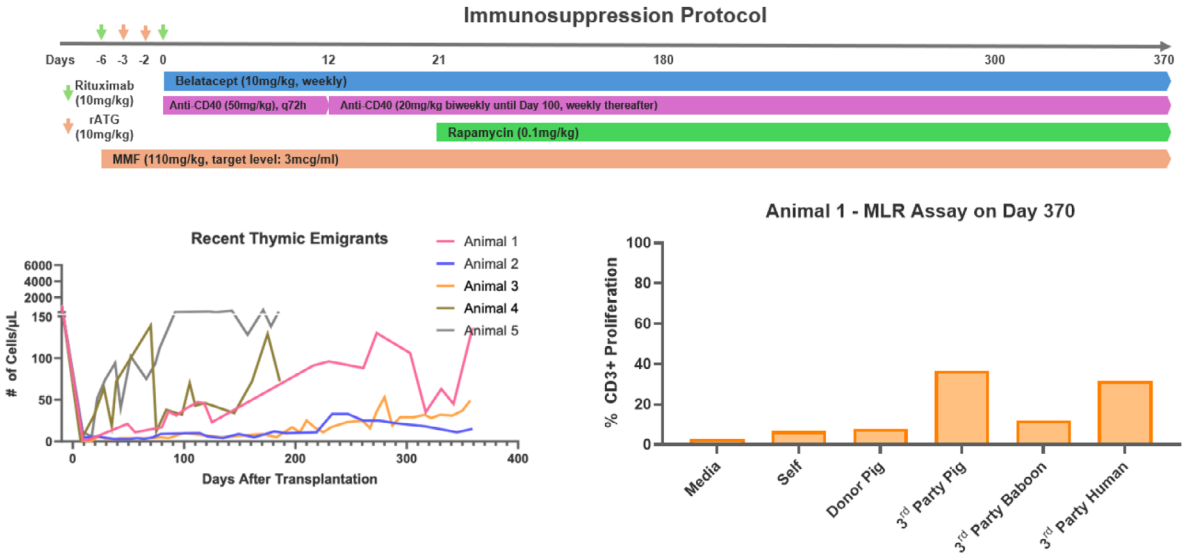
Funding: This project was funded by NefroHealth and ChoironeX. Research reported in this publication was performed in the CCTI Flow Cytometry Core, supported in part by the Office of the Director, National Institutes of Health, under awards S10OD030282. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

310.4 | Incomplete Cross-Species Regulation of Complement Associated With 10 Ge Xenokidney Failure in Life-Supporting Pig-to-Baboon Transplantation

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Animal ID	Creatinine (Endpoint)	Endpoint pathology		
		Transplant Glomerulopathy (cg)	Tubular Atrophy (ct)	Interstitial Fibrosis (ci)
Endpoint POD 370				
Animal 1	2.9	3	2	2
Animal 2	2.4	1	0	0
Animal 3	3.1	2	1	1
Endpoint POD 180				
Animal 4	1.71	1	1	2
Animal 5	1.33	0	1	1

FIGURE 1 | 310.3

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Introduction: Complement-mediated injury is a known barrier to successful xenotransplantation despite advances in source pig genetic engineering and human complement regulatory transgene expression. This study evaluated the contribution of complement activation to xenograft failure following life-supporting kidney transplantation from 10-gene-edited (10GE) pigs into nonhuman primates (NHPs), within the context of an Investigational New Drug (IND)-enabling nonclinical safety and efficacy study.

Methods: Twelve baboons received a single 10GE Xenokidney with either calcineurin inhibitor (CNI)-based (Group 1) or CD40/CD40L costimulation blockade-based (Group 2) immunosuppression. All xenokidneys expressed human complement regulators hCD46 and hCD55 but lacked hCD59. Xenokidney biopsies were assessed by immunofluorescence for C3c and C5b-9 deposition. A bioluminescent modified Ham (bmHam) assay was used to model complement activation and test efficacy of pharmacologic inhibitors in baboon serum including C1 esterase inhibitor, anti-C1s, anti-C5, and the alternative pathway inhibitor APL-2.

Results: Complement activation, characterized by early C5b-9 deposition without C3c binding, was observed in the majority of recipients and was associated with microangiopathic injury and xenokidney failure, independent of cellular- or antibody-mediated rejection. Eight of twelve animals demonstrated histologic evidence of thrombotic microangiopathy (TMA) on necropsy without evidence of cellular- or antibody-mediated rejection (no donor-specific antibody formation). Ischemia-reperfusion injury was associated with C5b-9 deposition on early biopsies; local and systemic infections including respiratory viruses were associated with accelerated complement activation and xenokidney failure.

Loss of complement regulatory transgene expression (particularly hCD55) was observed in failing xenokidneys. The bmHam assay demonstrated that standard complement inhibitors (eculizumab, sutimlimab, and APL-2) were ineffective in baboon serum, whereas tesidolumab (anti-C5) provided reliable inhibition.

Only one recipient received tesidolumab and maintained long-term xenokidney function with minimal microangiopathy.

Conclusion: Complement activation was the primary driver of 10GE Xenokidney failure in this pig-to-NHP kidney xenotransplantation study, occurring despite human complement

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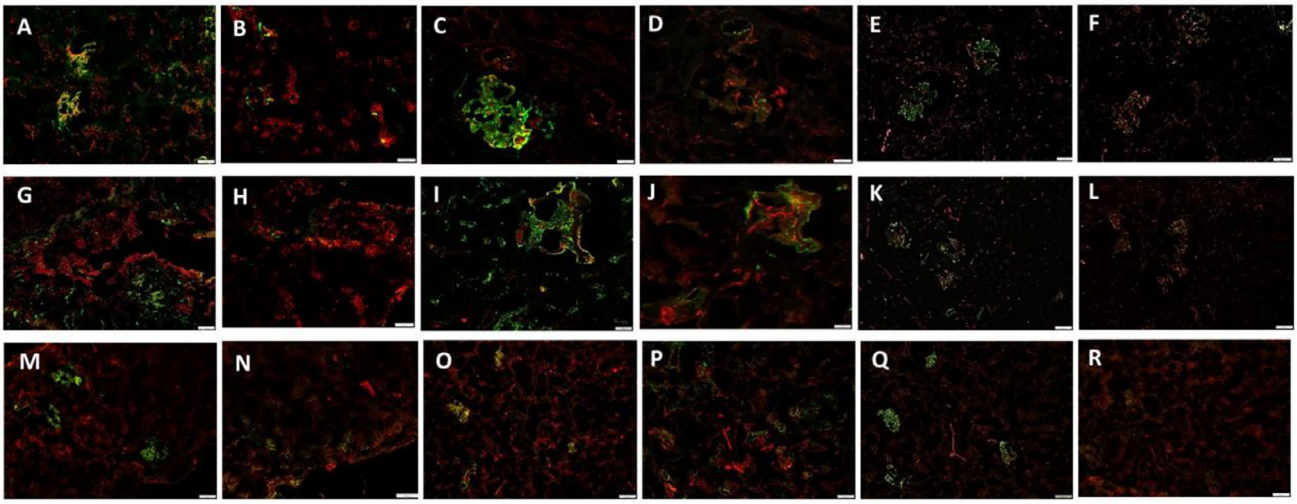


Figure 1: Xenokidney injury associated with complement activation in the absence of cellular or antibody-mediated rejection as demonstrated by immunofluorescence of B004519 (Group 2, panels A-F), B000820 (Group 1, panels G-L), and B000521 (Group 2, panels M-R). Membrane attack complex (MAC) is present in early biopsies (A, G, and M), suggesting ischemia reperfusion injury as initial trigger. Xenokidneys from recipients maintained with both immunosuppression regimens demonstrate C5b9 binding (C and I) in the absence of IgG binding (F and L). Weak and nonspecific IgM binding (B004519, panel E; and B000820, panel K) in presence of systemic infection (adenovirus in both B004519 and B000820) associated with increased C5b9 deposition and accelerated xenokidney failure. B000521 was initiated on maintenance C5 blockade with tesidolumab and MAC formation did not increase between POD 181 (O) and POD 366 (M) despite weak IgM binding (Q). Minimal C3 binding on early biopsies (panels B, H, and N) indicating no ongoing complement activation, while C3 binding on necropsy timepoint (B000820, panel I) suggests ongoing complement activation.

FIGURE 1 | 310.4

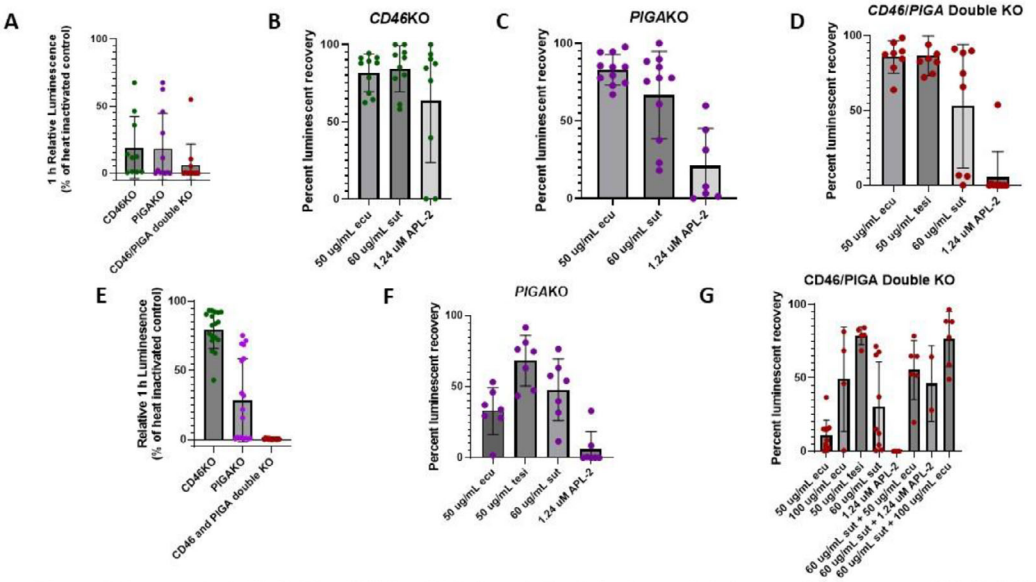


Figure 2. Human and Baboon Serum treated with C5, C1s, or C3 inhibitors. **A.** Bioluminescent mHam starting 1 hour relative luminescence with human serum on CD46KO (n=10), PIGAKO (n=11), CD46/PIGA Double KO (n=8). Individuals were selected specifically to have increased activity on each cell line. **B.** Human serum is spiked with complement inhibitors and luminescent recovery at t=1 h is indicated on the CD46KO cell line. Ecu (n=10), Sut (n=10), APL-2 (n=9). **C.** Human serum is spiked with complement inhibitors and luminescent recovery at t = 1 h is indicated on the PIGAKO cell line. Ecu (n=10), Sut (n=11), APL-2 (n=7). **D.** Human serum is spiked with complement inhibitors and luminescent recovery at t = 1 h is indicated on the CD46/PIGA Double KO cell line. Ecu (n=8), Tesi (n=7), Sut (n=8), APL-2 (n=10). **E.** Bioluminescent mHam starting 1 hour relative luminescence with baboon serum on CD46KO (n=18), PIGAKO (n=18), CD46/PIGA Double KO (n=15). **F.** Baboon serum is spiked with complement inhibitors and luminescent recovery at t = 1 h is indicated on the PIGAKO cell line. Ecu (n=7), Sut (n=7), APL-2 (n=7). **G.** Baboon serum is spiked with complement inhibitors and luminescent recovery at t = 1 h is indicated on the CD46/PIGA Double KO cell line. Ecu 50 ug/mL (n=12), Ecu 100 ug/mL (n=4), Tesi 50 ug/mL (n=5), Sut 60 ug/mL (n=9), APL-2 (n=6), Sut 60 ug/mL + Ecu 50 ug/mL (n=6), Sut + APL-2 (n=2), sut 60 ug/mL + ecu 100 ug/mL (n=6). Each dot represents a single replicate of a different individual. Ecu = eculizumab (C5 inhibitor), tesi = tesidolumab (C5 inhibitor) sut = sutimlimab (C1s inhibitor), APL-2 = pegcetacoplan (C3 inhibitor).

FIGURE 2 | 310.4

transgene expression and immunosuppression. Inflammation-associated complement activation in the absence of adaptive immune rejection highlights the need for effective and species-compatible complement blockade. These findings support the inclusion of maintenance anti-C5 therapy, and potentially dual-pathway inhibition, in clinical protocols for xenotransplantation.

Funding: This study was supported by a grant from United Therapeutics Corporation.

310.5 | A Survival Analysis of Pig-to-Non-Human Primate Orthotopic Heart Transplants

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Background: Progress in this xenotransplantation has centered around progressive genetic modifications in donor pigs and the use of novel immunosuppressive agents. Despite the significant progress that has been achieved, there has not yet been a rigorous analysis of the survival benefit of genetic modifications or specific immunosuppressive agents. We performed a retrospective survival analysis to determine the relative survival benefit of specific genetic modifications and immunosuppressive agents.

Methods: Fifty pig-to-non-human primate orthotopic heart transplants were performed between 2017 and 2023 at the University of Maryland, Baltimore. Porcine donors (Table 1) were obtained from Revivicor, Inc (Blacksburg, VA, USA). All subjects received induction immunosuppression with anti-thymocyte globulin, rituximab, glucocorticoids, and costimulation blockade (anti-CD40 mAb, anti-CD154 mAb, or Belatacept). Subjects with known or suspected perioperative cardiac xenograft dysfunction (PCXD, $n = 24$) were excluded from the analysis (see Figure 1). Retrospective multivariate Cox proportional hazard regression analyses were performed using Stata/BE 18.0.

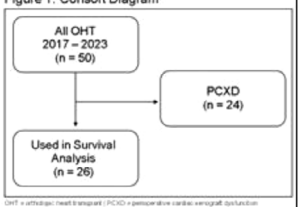
Results: A total of 50 pig-to-NHP OHTs were performed, of which 24 experienced PCXD and were excluded from further analysis. Among the remaining 26 subjects, survival ranged from 4 to 264 days with a median of 56 days (Figure 2A). Table 2 shows frequencies of variables for this cohort. Results of a multivariate Cox regression are shown in Table 3. β4GalNT2 KO and TBM HT showed a statistically significant survival benefit (HR 0.0064 and 0.0014, respectively). Anti-CD40 mAb and CTLA4-Ig showed statistically significant survival benefits (HR 0.0357 and 0.0782, respectively) as compared to use of anti-CD154 mAb. Curiously, inclusion of DAF HT showed a statistically significant survival penalty (HR 38.5051). This appears to be driven by two long-lived recipients of DAF HT negative xenografts (Figure 2D). Results for EPCR, CD47, and HO-1 could not be calculated due to collinearity.

Table 1: Description of Genetic Modifications in Porcine Donors

Abbreviation	Description	Phenotypic Attribute
GTKO	α-galactosyltransferase knock-out	Knock-out of enzyme responsible for Gal carbohydrate antigen
β4GalNT2 KO	β1,4-N-acetylgalactosyltransferase knock out	Knock out of enzyme responsible for Sda antigen
CMAH KO	complete metabolic profile (CMP)-N-acetylneuraminic acid hydroxylase knock out	Knock-out of enzyme responsible for Neu5GC antigen
DAF HT	Decay accelerating factor (CD55) human transgene	Knock-in of transgene for DAF, antagonizing formation of C3-convertase
CD46 HT	CD46 (membrane cofactor protein) human transgene	Knock- in of transgene for CD46, cofactor for degradation of C3b and C4b by factor I
TBM HT	Thrombomodulin human transgene	Knock-in of transgene for thrombomodulin, regulating thrombin activity promoting protein C activation
EPCR HT	Endothelial protein C receptor human transgene	Knock-in of transgene for EPCR, which promotes protein C activation
CD47 HT	CD47 (integrin associated protein) human transgene	Knock-in of CD47, interacts with SIRPα on macrophages to inhibit phagocytosis
HO-1 HT	Heme oxygenase 1 human transgene	Knock-in of transgene for HO-1, conferring anti-apoptotic properties
GHR KO	Growth Hormone Receptor knock-out	Knock-out of GHR, intended to mitigate pathological graft growth

KO = knock-out | HT = human transgene

Figure 1: Consort Diagram



OHT = orthotopic heart transplant | PCXD = perioperative cardiac xenograft dysfunction

Table 2: Cohort Variable Frequencies

Variable	Yes	No	Total
Gal KO	26	0	26
β4GalNT2 KO	23	3	26
CMAH KO	19	7	26
DAF HT	20	6	26
CD46 HT	25	1	26
TBM HT	24	2	26
EPCR HT	21	5	26
CD47 HT	21	5	26
HO-1 HT	19	7	26
GHR KO	18	8	26
Anti-CD40 mAb	20	6	26
Anti-CD154 mAb	3	23	26
CTLA4-Ig	3	23	26

KO = knock-out | HT = human transgene | mAb = monoclonal antibody
* Results could not be determined due to collinearity

Table 3: Multivariate Cox Proportional Hazard Regression

Variable	Hazard Ratio	Std. Err.	95% Confidence Interval	P-value
β4GalNT2 KO	0.0064	0.0119	0.0002 - 0.2482	0.007
CMAH KO	0.1593	0.2540	0.0070 - 3.6282	0.249
DAF HT	38.5051	72.7961	0.9468 - 1565.8970	0.053
CD46 HT	0.0860	0.2147	0.0006 - 11.4788	0.326
TBM HT	0.0014	0.0033	0.0000 - 0.1359	0.005
EPCR HT*	-	-	-	-
CD47 HT*	-	-	-	-
HO-1 HT*	-	-	-	-
GHR KO	5.2214	7.1729	0.3536 - 77.1126	0.229
Anti-CD40 mAb	0.0357	0.0386	0.0043 - 0.2971	0.002
CTLA4-Ig	0.0782	0.0900	0.0082 - 0.7455	0.027

KO = knock-out | HT = human transgene | mAb = monoclonal antibody
* Results could not be determined due to collinearity

FIGURE 1 | 310.5

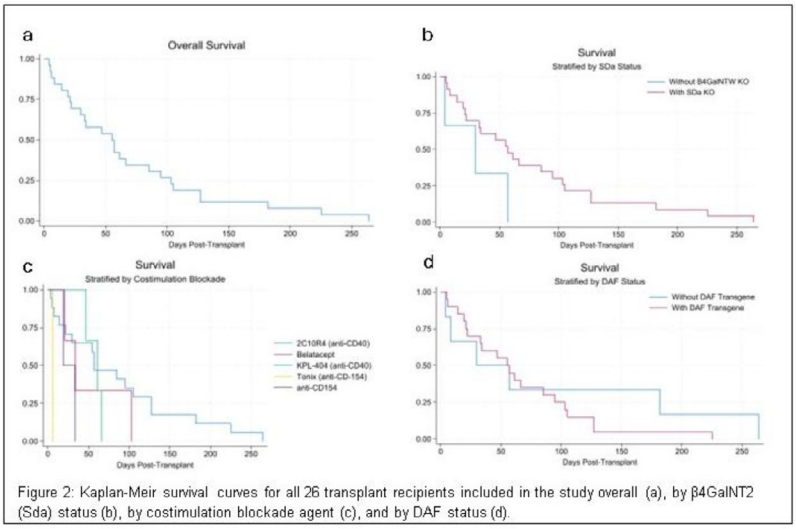


Figure 2: Kaplan-Meir survival curves for all 26 transplant recipients included in the study overall (a), by β4GalNT2 (Sda) status (b), by costimulation blockade agent (c), and by DAF status (d).

FIGURE 2 | 310.5

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Conclusion: This represents the first survival analysis informing the potential harm or benefit of specific genetic modifications or immunosuppressive agents in the xenotransplantation literature. While our results are limited by an ultimately small sample size, we are able to conclude that knockout of β4GalNT2 and inclusion of TBM HT conveyed a statistically significant survival benefit, while use of anti-CD154 mAb in the immunosuppressive regimen was associated with a statistically significant survival penalty. Inclusion of DAF HT appears to convey a large survival penalty although this effect may be driven by outliers and merits further investigation. Incorporation of data from additional prior studies (e.g., kidney transplants, heterotopic heart transplants) may add further statistical significance to enrich these results.

310.6 | Pre-Transplant Anti-Porcine Antibodies Do Not Predict Xenograft Survival in Pig-to-Non-Human Primate Orthotopic Heart Transplantation

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Background: There is an increasing need to accurately screen candidates who may benefit from xenotransplantation. To date, the field has relied on flow-cytometric techniques using porcine cells to screen for anti-porcine antibodies (APA) in the sera of non-human primate (NHP) recipients. We sought to evaluate whether pre-transplant APA are predictive of recipient survival in our experience with pig-to-non-human primate (NHP) orthotopic heart transplants (OHTs). To do this, we performed a retrospective survival analysis incorporating pre-operative APA levels.

Methods: Fifty pig-to-NHP OHTs were performed from 2017 to 2024 at the University of Maryland, Baltimore. Pre-transplant recipient serum samples were assayed for anti-porcine IgG and IgM. Briefly, recipient serum was inactivated and incubated with porcine aortic endothelial cells (PAECs) of either donor origin or from an immortalized GGTA1 KO PAEC line.Binding was assessed by flow cytometry (Cytek Aurora or BD Accuri C6 Plus, CA) and analyzed using FlowJo software (FlowJo LLC, Ashland, OR). Normalized geometric mean fluorescence intensity (MFI)

Table 1: Multivariate Cox Proportional Hazard Regression, IgG & IgM

Group	Pre-Transplant APA	Hazard Ratio	Std. Err.	95% Confidence Interval	P-value
All Subjects (n = 25)	IgG	1.0338	0.0537	0.93370 - 1.1446	0.523
	IgM	0.9415	0.0634	0.8251 - 1.0744	0.371
10 GE Only (n = 16)	IgG	0.9702	0.0683	0.8451 - 1.1138	0.668
	IgM	0.9031	0.078	0.7624 - 1.0698	0.238
10 GE & Anti-CD40 (n = 11)	IgG	1.0281	0.1388	0.7890 - 1.3395	0.838
	IgM	0.879	0.1046	0.6962 - 1.1099	0.278

FIGURE 1 | 310.6

Table 2: Multivariate Cox Proportional Hazard Regression, IgG, IgM, & CDC

Group	Pre-Transplant APA	Hazard Ratio	Std. Err.	95% Confidence Interval	P-value
Subjects with TKO CDC (n = 8)	IgG	1.0324	0.1022	0.8505 - 1.2535	0.747
	IgM	0.9367	0.1201	0.7286 - 1.204	0.61
	CDC (TKO)	0.9783	0.1271	0.7584 - 1.2623	0.867
Subjects with DS CDC (n = 5)	IgG	2.2108	1.5067	0.5813 - 8.4072	0.244
	IgM	1795.537	11127.78	0.0095 - 3.38 e+08	0.227
	CDC (DS)	1.362	2.1256	0.639 - 29.0134	0.843

FIGURE 2 | 310.6

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for the antibodies binding to cells was calculated and normalized to values obtained from serum from a baboon with confirmed rejection. Subjects with known or suspected perioperative cardiac xenograft dysfunction (PCXD, $n = 24$), were excluded from the analysis. Retrospective multivariate Cox proportional hazard regression analyses were performed using Stata/BE 18.0.

Results: Of 50 pig-to-NHP OHTs performed, 24 experienced PCXD and were excluded. Results of a multivariate Cox regression are shown in Tables 1 and 2. Table 1 reports hazard ratios using all subjects for whom pre-transplant APA was available ($n = 25$), for 10 gene-edited (10GE) xenograft recipients only ($n = 16$), and for 10GE recipients receiving anti-CD40 ($n = 11$). In all cases, hazard ratios for pre-transplant IgG and IgM are not statistically significant, with effects approaching 1 in all cases. A subgroup analysis was performed using subjects for whom pre-transplant complement-dependent cytotoxicity (CDC) was available using either triple-KO cells (GGTA1 KO, β4GalNT2 KO, CMAH KO, $n = 8$) or donor-specific (DS, $n = 5$) PAECs (Table 2). Again, all hazard ratios are not statistically significant.

Conclusion: Our experience with pig-to-NHP OHT shows that pre-transplant APA is not predictive of post-transplant survival. In a more limited set of subjects, we find that pre-transplant CDC is also not predictive of survival. These results are unexpected and run counter to established practice in xenotransplantation. Possible explanations include the inherent variability of this technique as well as the possibility that overall APA measures obscure measurement of specific immunodominant APA. This result highlights the need to establish robust, standardized approaches to screening potential xenograft recipients.

311.1 | Transgenic HLA-E on Porcine Endothelial Cells Attenuates Human NK Cytotoxicity and Degranulation Via CD94/NKG2A-MEDIATED Inhibition

Jonathan Schulz[1,2], Zahra Habibabady[1], Megan Dufault[1], Amir Sanatkar[1], Victoria Diaz[1], Kasinath Kuravi[3], Maria Kokkanaki[3], Steve Butler[3], Richard N. Pierson III[1]

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Introduction: Natural Killer cells are critical mediators of innate immunity and a major barrier to xenotransplantation. HLA-E, a non-classical MHC class I molecule, binds the inhibitory CD94/NKG2A receptor on human NK cells (hNK), suppressing their activation. This study evaluates the impact of HLA-E expression on genetically engineered (GE) porcine aortic endothelial cells (PAECs) in reducing human NK cell-mediated cytotoxicity and degranulation.

Methods: hNK were isolated from peripheral blood and cultured with IL-2. GE PAECs included Gal-knockout (GalKO/WT) cells, 10GE triple-knockout (GalKO, β4GalKO, CMAHKO) cells with human genes (hCD46, hCD55, hTBM, hEPCR, hHO1, hCD47, GHR knockout), and HLA-E-expressing 11GE cells (10GE+HLA-E = 11GE cells). Cytotoxicity was measured via an LDH release assay with hNK cocultured at an effector-to-target (E:T) ratio of 10:1 for 2.5 h. Degranulation was quantified by CD107a flow cytometry after a 2-h coculture. To probe the HLA-E pathway, an anti-CD94 blocking antibody was added to hNK before incubation with 11GE cells. Additionally, a static coculture fluorescent microscopy assay using DiOC6 (live cells) and PI (dead cells) was performed to visualize and quantify NK cell-mediated cytotoxicity.

Results: HLA-E expression significantly modulated hNK cell responses toward porcine endothelial cells. In the LDH cytotoxicity assay, 11GE PAECs exhibited a markedly reduced cytotoxicity level (52.18%, $n = 5$), which was statistically significant compared to both GalKO PAECs ($p = 0.0030$) and 10GE PAECs (86.5%, $n = 4$, $p = 0.0329$). No significant difference was observed between 10GE and GalKO groups ($p = 0.5004$). In the degranulation assay (CD107a), NK cells cocultured with 11GE targets showed reduced activation (13.46%, $n = 3$) relative to GalKO (21.68%, $n = 3$) and 10GE (16.8%, $n = 2$) targets. Furthermore, blockade of the CD94 receptor on NK cells reversed the inhibitory effect of HLA-E, restoring cytotoxicity and degranulation to levels comparable with HLA-E–negative targets. This effect was reproduced in two independent experiments, supporting the reliability.

The proportion of PI-positive cells was markedly lower in cocultures with HLA-E-expressing PAECs (36%, $n = 5$) versus GalKO (66%, $n = 5$) and 10GE cells (54%, $n = 4$), corroborating the LDH and CD107a findings.

Conclusion: These results provide direct mechanistic evidence that HLA-E expression inhibits hNK cell effector functions via the CD94/NKG2A inhibitory pathway. The observed reduction in cytotoxicity and degranulation underscores the relevance of this checkpoint in the xenogeneic context. The significant difference between 11GE and 10GE PAECs—differing only by HLA-E—highlights its specific immunomodulatory role and supports the inclusion of HLA-E in future genetically engineered pigs to enhance innate immune compatibility in pig-to-human xenotransplantation. Additional studies using baboon NK cells are ongoing to validate translational relevance in preclinical models.

311.2 | Modulating Human Neutrophil Rolling on Porcine Endothelium: Effects of Sialoadhesin and GPIb Blockade Combined With Genetic Engineering Under Microvascular Flow

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Purpose: We investigated the role of Gal 1-3αGal, aGPIb, and sialoadhesin carbohydrates (CHOs) in the interaction of human PMNs with wild-type (WT), GalTKO, and 10GE porcine aortic endothelial cells (EC) under physiologic microvascular shear stress and temperature, using human coronary endothelial cells (HCE) as a negative control.

Methods: Microfluidic channels were seeded with WT, GalTKO, 10GE, and human coronary ECs. ECs were stimulated with 10 n/μL rh-TNFα for 3 h (to induce IRI-mediated E-selectin upregulation) and perfused with fresh human whole blood stained with calcein to detect non-RBC cells. 1F1 was perfused over EC while a-GP1b was added to blood for 30 mins, both before the perfusion over 60 min under microvascular flow conditions

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(5 dynes/cm^2 shear stress) at 37°C. A total of 181 images were captured during each channel perfusion, and stacked images were then processed using ImageJ software. Applying signal threshold, size of the objects, and circularity, neutrophils were distinguished and analyzed for PMN rolling and movement (mean speed [pixels/frame] and track duration [Frames]). HCE was used as a negative control for PMN interactions. Results from multiple experimental conditions were analyzed using one-way ANOVA with post-hoc tests.

Results: Absence of Gal 1-3αGal significantly decreased PMN rolling and its duration compared to WT. Sialoadhesin blockade (mAb 1F1) and GPIb inhibition further reduced the duration of PMN tracking and increased the mean speed of migration on EC ($p < 0.005$ for all experimental groups except WT+GP1b vs Gal+GP1b). PMN rolling closely correlated with diffuse PLT adhesion, primarily occurring at EC-EC junctions among all cell lines.

Conclusion: PMN rolling on and migration over porcine EC under IRI conditions is reduced by GalTKO gene modification and further attenuated by sialoadhesin blockade and GPIb inhibition. These findings support that inhibition of carbohydrate-driven pro-inflammatory interactions mediated by PMNs and other blood elements reduces inflammation and improves xenograft outcomes. PMN localized rolling and migration in association with adherent PLTs implicates coagulation pathway activation in the residual sequestration observed with ECs treated with 1F1 and GPIb blockade. These results suggest that genetic and pharmacologic strategies targeting thrombodysregulation may further mitigate inflammation in xenografts. We will evaluate the effect of these agents on multi-GE ECs next

Funding: This work was supported by the sialoadhesin blocker came from the NHP Reagent Resource Center (NHPRRC, in Worcester, MA, USA).

311.3 | Expression of Human CD47 in a Novel Transgenic Pig Protects Porcine Endothelial Cells Partially From NK Cell Cytotoxicity

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Introduction: Natural killer (NK) cells pose a major barrier to successful xenotransplantation due to their capacity to recognize and, through the engagement an array of activating receptors, destroy porcine endothelial cells, the first point of contact between donor organ and the recipient immune cells. While the CD47–SIRPα receptor-ligand interaction is a well-established negative regulator of macrophage activity, its capacity in modulating NK cell effector functions in the context of xenograft rejection remains less well defined. Here, we investigated the immunomodulatory effects of human CD47 (hCD47) on porcine aortic endothelial cells (PAECs), derived from new hCD47 transgenic animals on NK cell responses.

Methods: Human NK cells were isolated from healthy donors and activated with interleukin-2 for a minimum of 5 days. PAEC derived from GGTA1-knockout/human CD47 transgenic pigs were used as target cells. NK cytotoxicity was assessed via live-cell imaging using CellTrace labeling and live/dead staining, under both static conditions (96-well plates) and dynamic flow conditions in 2D Ibidi microfluidic channels, applying a shear stress of 4 dyn/cm^2. Cocultures were performed at an effector-to-target ratio of 1:2 for 2 h. NK cell dynamics and interactions with PAEC monolayers were evaluated by tracking cell trajectories from time-lapse microscopy recordings.

Results: Expression of hCD47 on PAECs significantly reduced NK cell-mediated lysis, suggesting a protective role for CD47–SIRPα in counterbalancing the activation signals triggered during NK-PAECs interactions. Moreover, under shear stress conditions, NK cells displayed diminished adhesion and motility on hCD47-expressing PAECs compared to their active crawling on wild-type cells, indicating impaired adhesion and surveillance. Blocking of the CD47–SIRPα axis through peptide or antibody inhibition only partially restored NK cytotoxicity. Notably, in the presence of heat-inactivated human plasma, which contains preformed anti-pig antibodies, hCD47 did not protect PAECs from NK cell–mediated antibody-dependent cytotoxicity.

Conclusions: These findings highlight hCD47 as a promising molecular tool for modulating innate immune responses in xenotransplantation. hCD47 expression on donor vasculature may offer a dual strategy to suppress both macrophages and NK cell-mediated graft injury thereby enhancing xenograft survival.

311.4 | Complement Regulation in Genetically Modified Pig Endothelial Cells Under Xenogeneic and Inflammatory Conditions

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Introduction: Multigene modifications in donor pigs have significantly advanced xenotransplantation by overcoming interspecies innate immune barriers. A prominent model includes α1,3-galactosyltransferase-knockout pigs expressing human CD46 and thrombomodulin (3GM), which have demonstrated promising life-supporting graft function in preclinical settings. As the initial interface between graft and host, endothelial cells (ECs) are critical regulators of complement activation, coagulation, and inflammation. Here, we investigate how these genetic modifications modulate complement responses in pig ECs during xenogeneic and inflammatory conditions.

Methods: Primary ECs from 3GM pigs were cultured under physiological shear stress and perfused with normal human serum (NHS) or recalcified citrated human plasma to assess complement and coagulation activation, respectively. Inflammatory conditions were simulated using recombinant human TNF-α

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(rhTNF-α) perfusion. Antibody (IgG, IgM) and complement deposition (C1q, C3b/c, and C5b-9) and coagulation markers (clot formation time) were assessed, and bulk RNA-seq was performed to investigate transcriptomic changes.

Results: 3GM ECs showed significantly reduced antibody and complement deposition following NHS perfusion, as well as better anticoagulant properties upon citrated human plasma exposure, indicating effective regulation of xenogeneic complement and coagulation pathways. However, this protection was reduced under rhTNF-α-induced inflammation conditions. Interestingly, the increased complement deposition under inflammatory conditions was not attributed to increased deposition of IgG, IgM, or C1q, although differences in endothelial glycocalyx dynamics were observed. Transcriptomic analysis revealed that 3GM ECs exhibit reduced expression of key proinflammatory, coagulation, and complement-related genes (e.g., VCAM1, CCL2, C1QBP, SERPINE1) compared to wild-type (WT) ECs. In addition, upon xenogeneic activation, 3GM ECs displayed minimal transcriptomic changes and distinct responses to that of WT ECs. In contrast, rhTNF-α-induced inflammation led to more aligned gene expression profiles between 3GM and WT ECs, where upregulation of immune and defense response pathways was observed, potentially contributing to increased complement and coagulation dysregulation.

Conclusion: Our findings highlight that 3GM pig ECs were able to effectively prevent complement activation in xenogeneic settings. However, this protection is compromised under inflammatory conditions. These results suggest that incorporating additional anti-inflammatory transgenes may be necessary to maintain immune quiescence and improve graft survival in clinical xenotransplantation.

Funding: This work was supported by the Swiss National Science Foundation.

311.5 | Quantification of Islet Cell Cluster Size Heterogeneity in the Juvenile Porcine Pancreas

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Introduction: While whole-organ pancreas and islet allotransplantations offer effective alternatives for type 1 diabetes (T1D) treatment, their clinical use is limited by the shortage of human donors. Xenotransplantation is a potential solution to this challenge, with porcine pancreases emerging as an up-and-coming alternative source of islets. While neonatal or juvenile piglets are becoming increasingly favored for obtaining pancreatic islets, no optimal age for clinical application has been established. Debates continue regarding the islet size and function, which are mostly considered immature and are thus referred to as islet cell clusters (ICCs). Aim: This study investigated the ICC size heterogeneity in tissue samples from six juvenile porcine pancreases, all 14 days of age.

Methods: The abundance of ICCs of different sizes (defined as small, intermediate, and large) was assessed in large images of pancreas sections. Automated workflows using NIS Elements software were developed to measure and cluster areas of immunofluorescent-stained beta- and alpha-cells, and to quantify the proportions of insulin- and glucagon-positive regions within the different-sized clusters.

Results: Small, well-developed islets (~100 μm) were consistently identified across all juvenile pancreases, with a density of 6.2 ± 2.5 clusters/mm^2 (Mean ± S.D.). Homogeneous numbers of intermediate-sized (14.3 ± 4.6 clusters/mm^2) and small-sized (72.2 ± 12.6 clusters/mm^2) clusters were also observed. Insulin staining predominated in small and intermediate clusters (4-fold higher than glucagon), while large clusters exhibited significantly more glucagon staining, consistent with characteristics of mature islets.

Conclusion: Although further studies are needed to confirm the clinical suitability of juvenile porcine ICCs, our findings suggest that 14-day-old piglets already possess significant amounts of small, mature islets. Optimizing islet isolation techniques, such as gradient centrifugation used in human islet isolation, may enable efficient purification of small and more mature islets from juvenile porcine pancreases for transplantation.

311.6 | Macroencapsulated Transgenic InsGLP-1M3R Neonatal Porcine Islets Reverse Diabetes in Immunocompetent Rats

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Background and Aims: In the context of xenotransplantation, cellular encapsulation is a promising strategy to evade the host immune system whilst providing a nurturing environment for islet cell survival and function. We have previously shown that transgenic InsGLP-1M3R porcine islets have human-like insulin secretory response and that they can efficiently cure insulin-dependent diabetes in an immunosuppressed xenotransplantation model. Here, our aim was to use such functionally-improved islets to prepare macroencapsulated islet pouches that were then tested both in vitro and in vivo.

Materials and Methods: Pancreatic islets were isolated from transgenic neonatal piglets and kept in culture in a maturation medium for 8 days. Islet pouches were prepared by seeding 15 000 IEQ on a 1 cm^2 sheet of decellularized pig skin, covering the islets with a thin layer of an alginate-agarose gel solution and encasing the ensemble between two sheets of PET membranes (0.4 μm pore size) that were then heat-sealed. Obtained pouches were kept in culture over a period of 3–4 weeks, with glucose stimulation tests performed on a weekly basis. Streptozotocin-induced diabetic rats received a pouch each behind the seminal vesicle and were kept in an 80% oxygen atmosphere for 48 h following implantation. Blood glucose was monitored weekly, and blood samples were taken to evaluate the immunologic anti-pig response by FACS analysis of serum samples. Normoglycemic rats had their pouches removed 6 months post-implantation, and follow-up continued for hyperglycemia to occur.

Results: Weekly glucose stimulation of incubated islet pouches showed a robust 3.5-fold increase in insulin secretion in response to 15 mM glucose. Interestingly, the amount of both basal and stimulated insulin increased during the in vitro culture period.

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Out of 10 pouch recipients, 5 became normoglycemic (< 200 mg/dL) within 30 days of implantation. One hundred percent of these recipients remained so during a 6-month follow-up period. Upon graft removal, an irreversible rise in blood glucose was observed in 5 out of 5 rats, as shown in figure 1. FACS analysis of serum samples showed absence of anti-pig antibodies (IgM and IgG) in 9 out of 10 recipients, with one individual showing a 30% increase of IgG within 30 days post-implantation. Macroscopic observation of explanted pouches showed no signs of fibrosis or physical damage to the pouches. Moreover, a well-developed capillary network surrounding each pouch was observed.

Conclusion and Perspectives: Neonatal porcine islet macroencapsulation on an acellular collagen matrix sustains islet survival and function both in vitro and in vivo. The host immune reaction was successfully evaded using a combination of alginate-agarose gel and microporous PET membranes. The ensemble showed good biocompatibility and integration.

Funding: Financial support by "Service public de Wallonie, SPW Economie, Emploi, Recherche" Grant "8318 SPW".

312.1 | The Impact of Innate Immune Cell Activation in Genetically Engineered Pig-to-Nonhuman Primate Orthotopic Liver Xenotransplantation

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Objective: Liver xenotransplantation using genetically-engineered pigs offers a potential solution to the global organ shortage. However, non-human primate (NHP) recipients exhibit limited survival, necessitating an in-depth investigation of underlying mechanisms.

Methods: Nine genetically-engineered pig-to-NHP orthotopic liver xenotransplantations were performed. Recipients' clinical and immunological parameters were monitored longitudinally. Graft tissues were analyzed via immunohistochemistry (IHC) at various time points. Single-cell RNA sequencing characterized immune cell subsets and activation in peripheral blood mononuclear cells (PBMCs) and graft tissues from three recipients with extended survival.

Results: Recipients of Triple-knockout (GTKO/CMAHKO/β4GalN2KO/hCD55/hTBM) livers survived 5, 5, and 3 days, respectively, compared to $\leq$2 days for GTKO-only (GTKO or GTKO/hCD55/hCD59/hTBM) livers. In longer-surviving recipients, coagulation parameters and platelet counts remained stable for the first 3 days but declined thereafter. Shorter-surviving recipients exhibited severe coagulopathy and thrombocytopenia immediately post-transplant. Grafts from shorter-surviving recipients exhibited extensive necrosis and red blood cell destruction, whereas those from longer-surviving recipients showed focal necrosis, minimal T cell infiltration, and limited antibody and complement deposition. However, grafts and PBMCs from longer-surviving recipients showed increased macrophages (CD163+, CD5L+) and NK cells (CCL5+, GNLY+), and activated dendritic cells (PTPRC+, SKAP1+), accompanied by a significant rise in IL-6 levels in recipient peripheral blood. The IL-6 surge coincided with cardiac and renal damage, ultimately leading to recipient death.

Conclusions: Triple-knockout pig donors could mitigate early coagulopathy and thrombocytopenia in NHP recipients, extending survival. However, innate immune cell activation and the associated IL-6-mediated pro-inflammatory response contribute to recipient mortality. Targeting these pathways may improve outcomes in liver orthotopic xenotransplantation.

Funding: This work was supported by the Excellent Young Scientists Fund of Hunan Province (No.2022JJ20087) and Hunan Key R&D Program (No.2024DK2005).

312.2 | Application of a Novel Spatial Transcriptomic 6000-Plex Panel in Pig-to-Human Xenotransplantation

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Introduction: Kidney xenotransplantation is a promising approach that has the potential to open new treatment avenues for patients currently on transplant waiting lists. Here we applied for the first time a 6000-plex gene panel to decipher the immune response after transplantation of a genetically edited porcine thymokidney into a human recipient.

Methods: We performed in-depth multimodal phenotyping of tissue biopsies taken at 7 sequential follow-up times from Day 0 through Day 61. Initial bulk transcriptomic studies showed a molecular signature evocative of antibody-mediated rejection developing as early as Day 10. In addition to a pre-transplant pig kidney control sample, we selected 4 post-transplantation time points to further explore and used the CosMx Spatial Molecular Imager to localize and profile the human immune cells infiltrating the xenograft in their spatial context. We designed a novel bioinformatic approach to accurately distinguish the approximately 3000 human immune cells from the approximately 70 000 pig structural cells.

Results: Across all time points, macrophages were the most prevalent human cell type, in agreement with previous analyses. The human immune rejection response peaked at the D33 time point after which anti-rejection treatment was provided, leading to a successful decrease in the immune-molecular signs of xenoantibody-mediated rejection. We observed human myeloid cells present in every one of the post-transplant glomeruli in the sampled biopsies and characterized differential expression of pro- and anti-inflammatory myeloid genes over the course of treatment (**Figure**). We identified spatially-enriched human

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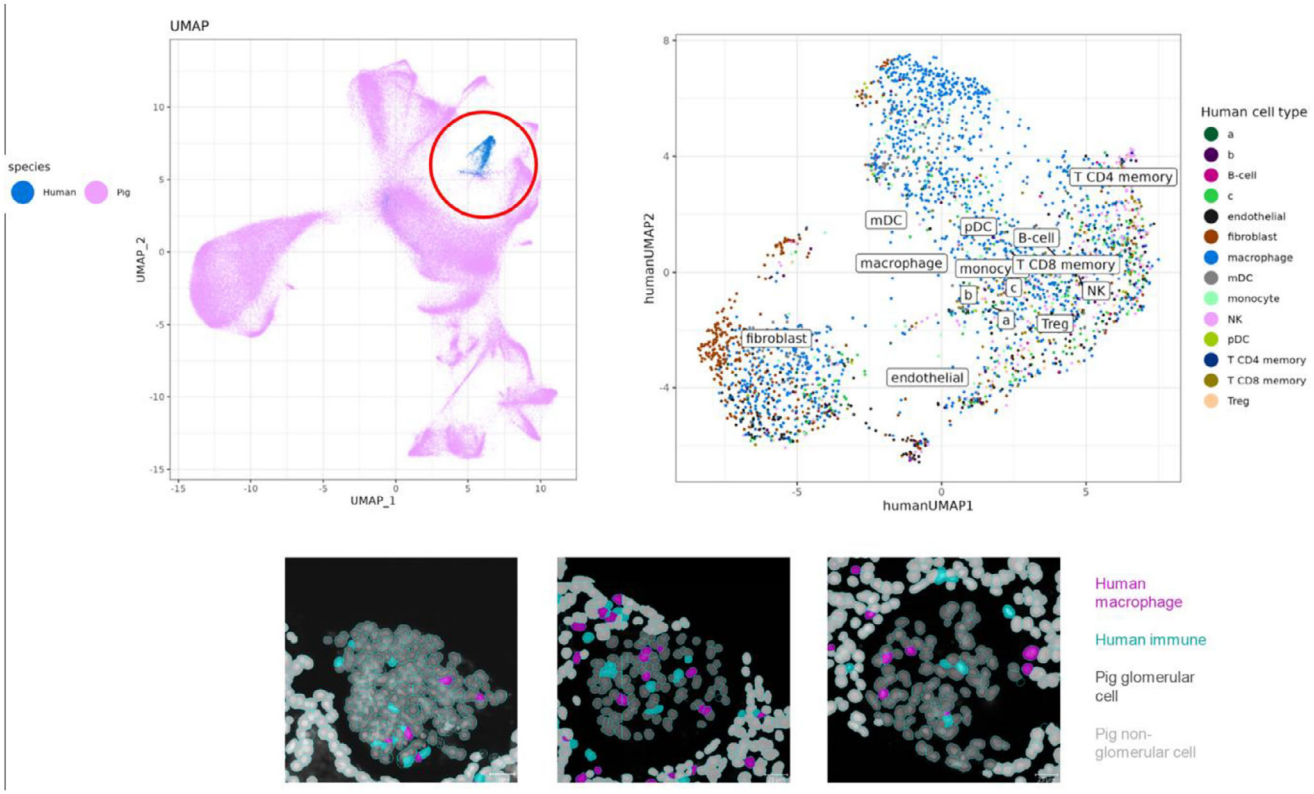


FIGURE 1 | 312.2

macrophage – pig structural ligand-receptor interactions present at early and late timepoints and catalogued spatially correlated modules of co-expressed genes.

Conclusion: Overall, our data suggest xenoantibody-mediated rejection, characterized by human innate immune cells infiltration in the porcine xenograft, localized within and outside of glomeruli. Discovery of these molecular mechanisms and the successful attenuation of the xenograft organ rejection with targeted treatment paves the way for potential new anti-rejection therapies to further enable human clinical trials.

312.3 | Histopathologic Evaluation of Cardiac Xenotransplantation: A Review of 13 Autopsy Cases

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Introduction: It is important to observe histopathologic findings for the diagnosis and treatment of rejection in cardiac xenotransplantation. However, there is still a limited literature describing the histopathologic findings in cardiac xenotransplantation.

Methods: We performed 13 porcine cardiac xenotransplantations to cynomolgus monkeys. Autopsy of transplanted porcine heart was carried out. Tissue samples were analyzed using H&E, histochemical staining for PAS, Elastic fiber, Masson-Trichrome, and immunohistochemical staining for CD3, CD4, CD8, CD20, CD56, CD68, C4d.

Results: 4 died or were euthanized within hours. 9 survived for 9, 13, 15, 18, 21, 22, 46, 60, and 216 days. Two of the short-term surviving transplants at 0 day showed intravascular macrophages and strong C4d staining. Myocardial infarction and intravascular fibrin thrombi were present at 9, 22, 46, and 60 days. Mild C4d endothelial staining was present at 22, 46 days. Long-term survival transplant at 216 days showed confluent myocardial infarction with interstitial hemorrhage and fibrosis. Severe arteriosclerosis with intimal thickening and medial sclerosis were present. In our case, there were no findings of acute cellular rejection, such as lymphocytic infiltrates with myocyte damage.

Conclusion: In our study, no clear histopathological findings could be diagnosed as acute cellular rejection. It is necessary to develop diagnostic pathology criteria or grading schemes to properly treat rejection and evaluate prognosis.

Funding: This research was supported by a grant of the Korea Health Technology R&D Project through the Korea Health Industry Development Institute (KHIDI), funded by the Ministry of Health & Welfare, Republic of Korea (grant number HI20C1234).

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312.4 | The Histologic Identification of IgA Deposits in Three Cases of Porcine to Human Xenokidney Transplantation

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Introduction: Nonhuman primate (NHP) xenotransplants demonstrate the deposition of natural, pre-formed IgA antibodies on biopsies of rejected alpha 1,3-galactosyltransferase gene knockout (*GGTA1* KO) pig kidneys. Serum IgA antibodies have been shown to rise in tandem with natural IgG and IgM antibodies during rejection in NHP recipients. Whether these IgA antibodies, like IgG and IgM, play a role in immune-mediated kidney injury remains unknown. Here we describe three cases of pig to human xenokidney transplants and the pattern of IgA deposition on biopsy during their clinical care.

Methods: Three humans underwent xenokidney transplants with two different genetically modified pig kidneys. Case A, a decedent with a *GGTA1* KO Thymokidney maintained in the ICU for 61 days, was successfully treated for antibody-mediated rejection (AMR) on POD33 and subsequently maintained excellent graft function until planned explant on POD61. Case B, a living recipient with a *GGTA1* KO Thymokidney after LVAD placement, underwent explant on POD47 due to progressive graft failure in the setting of hemodynamic instability and was later identified to have an antibody-mediated process on molecular transcriptomics. Case C, a living recipient with a 10GE Xenokidney, was successfully treated for AMR on POD17 and subsequently maintained excellent graft function until development of a second severe rejection on POD128 and underwent explant on POD130. All three cases underwent protocol and for-cause biopsies with immunoglobulin and complement staining. In Case C, serum IgA titers were correlated with IgA staining on biopsy at the time of AMR diagnosis and after treatment.

Results: All biopsies demonstrated evidence of glomerular mesangial proliferation. Case A became IgA positive starting on POD10. IgA diminished in positivity after treatment of AMR. This deposition was similar to IgG, IgM, C3 and C4d staining. Case B was negative for IgA on POD13 but developed IgA positivity by POD32 along with IgM and C3 positivity. IgA positivity increased by POD42, as did IgG. Case C became IgA positive starting on POD9. IgA diminished in positivity after AMR treatment. This deposition was similar to IgG, IgM, C3 and C4d staining (Figure 1). Serum IgA titers in Case C on POD17 (AMR onset) and POD85 (AMR resolution) were found to correlate with IgA positivity on biopsy (Figure 2).

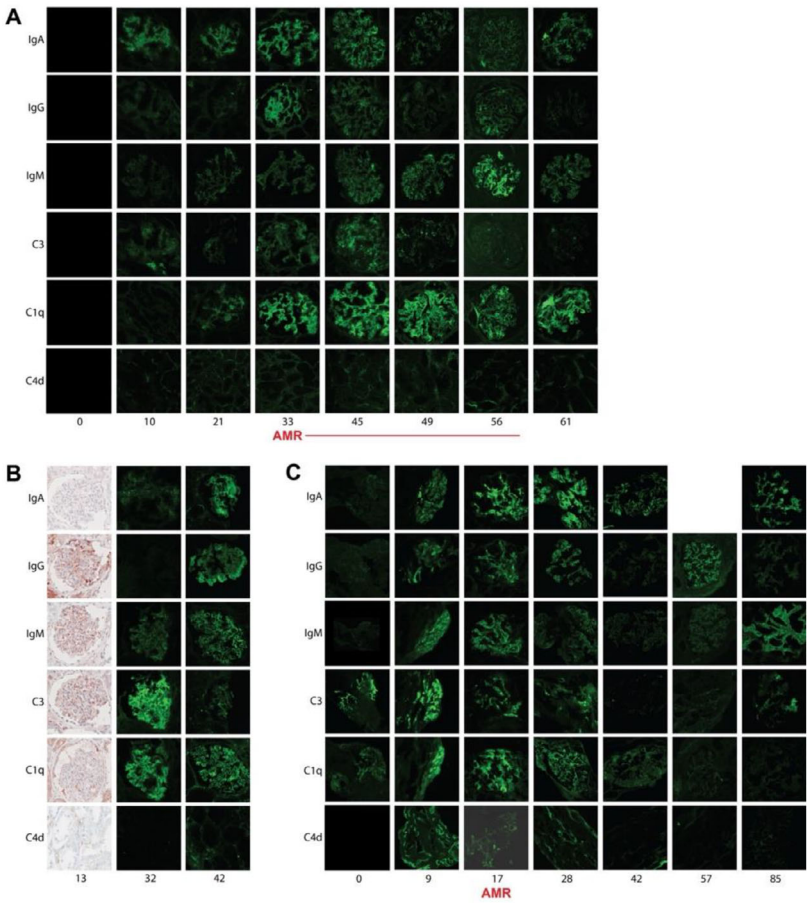


FIGURE 1 | 312.4

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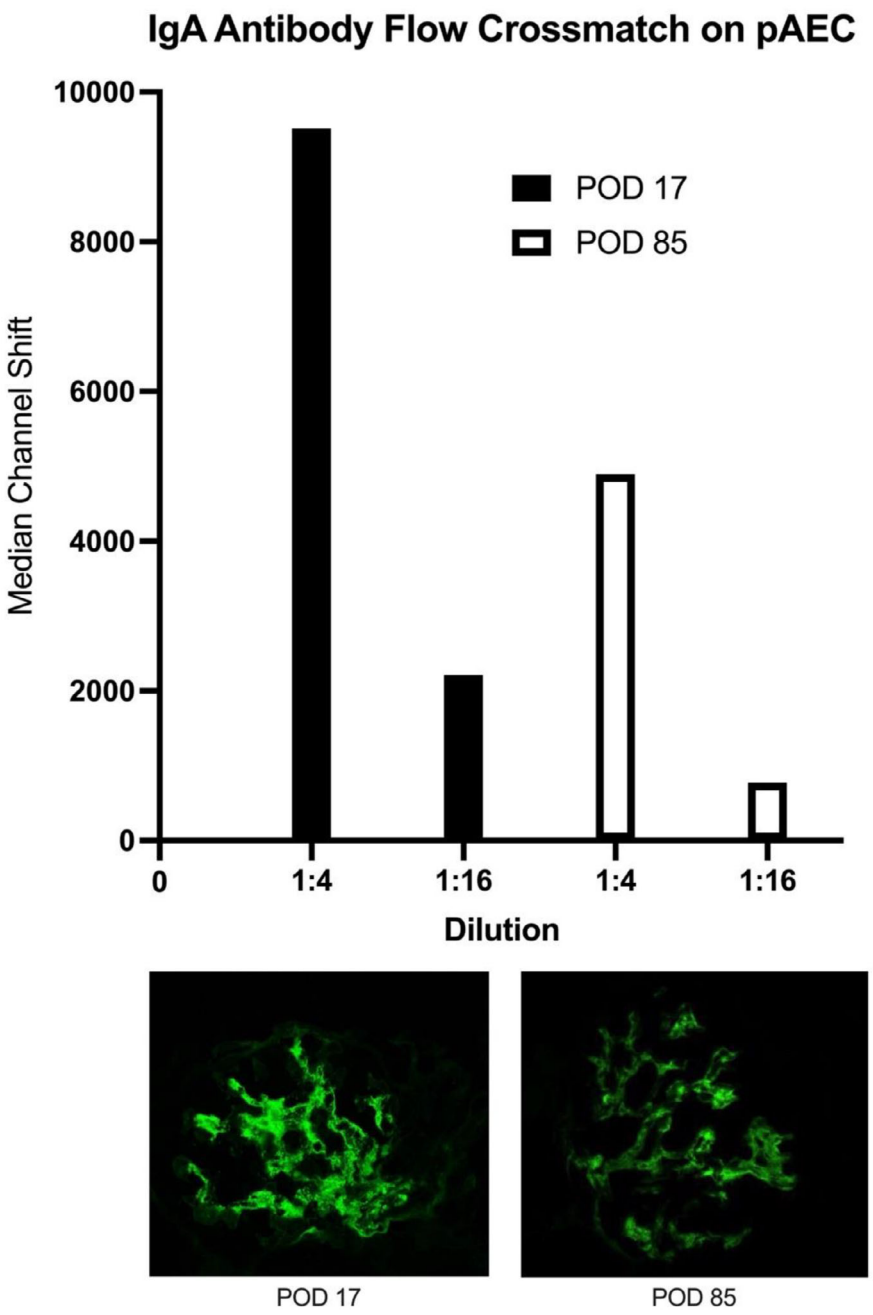


FIGURE 2 | 312.4

Conclusion: The relevance of IgA antibodies in immune-mediated kidney injury in pig to human xenotransplants is unknown. In two of three pig to human xenotransplant cases at our institution, IgA staining preceded AMR diagnosis and diminished after treatment, following a pattern similar to other immunoglobulin and complement staining consistent with AMR diagnosis. Serum IgA titers also appeared to diminish after AMR treatment and correlated with decreased IgA staining on biopsy.

Funding: This data is supported by funding from United Therapeutics Corporation, PBC. Tal Eitan was supported by the National Cancer Institute, National Institutes of Health, through Grant Award Number T32 CA193111.

312.5 | A Novel IgG and IgM Cleaving Endopeptidase, IceMG, for Xenotransplantation

Alessandro Martinino, Zishen Li, Timothy Smith, Davide Schillrio, Zachary Elore, Janghoon Yoon, Emma Fatzaun, Rafaela Beloni, Victoria Z. Ferrara, Matthew Tunbridge, Nagham Bazzi, Kwan wai Fung, Meghan Hu, Shengli Song, Annette Jackson, Stuart Knechtle, Aravind Asokan, Jean Kwun
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Introduction: Xenotransplantation is a promising approach to tackle the severe organ shortage. However, antibody-mediated rejection (AMR) remains a significant obstacle, with preformed

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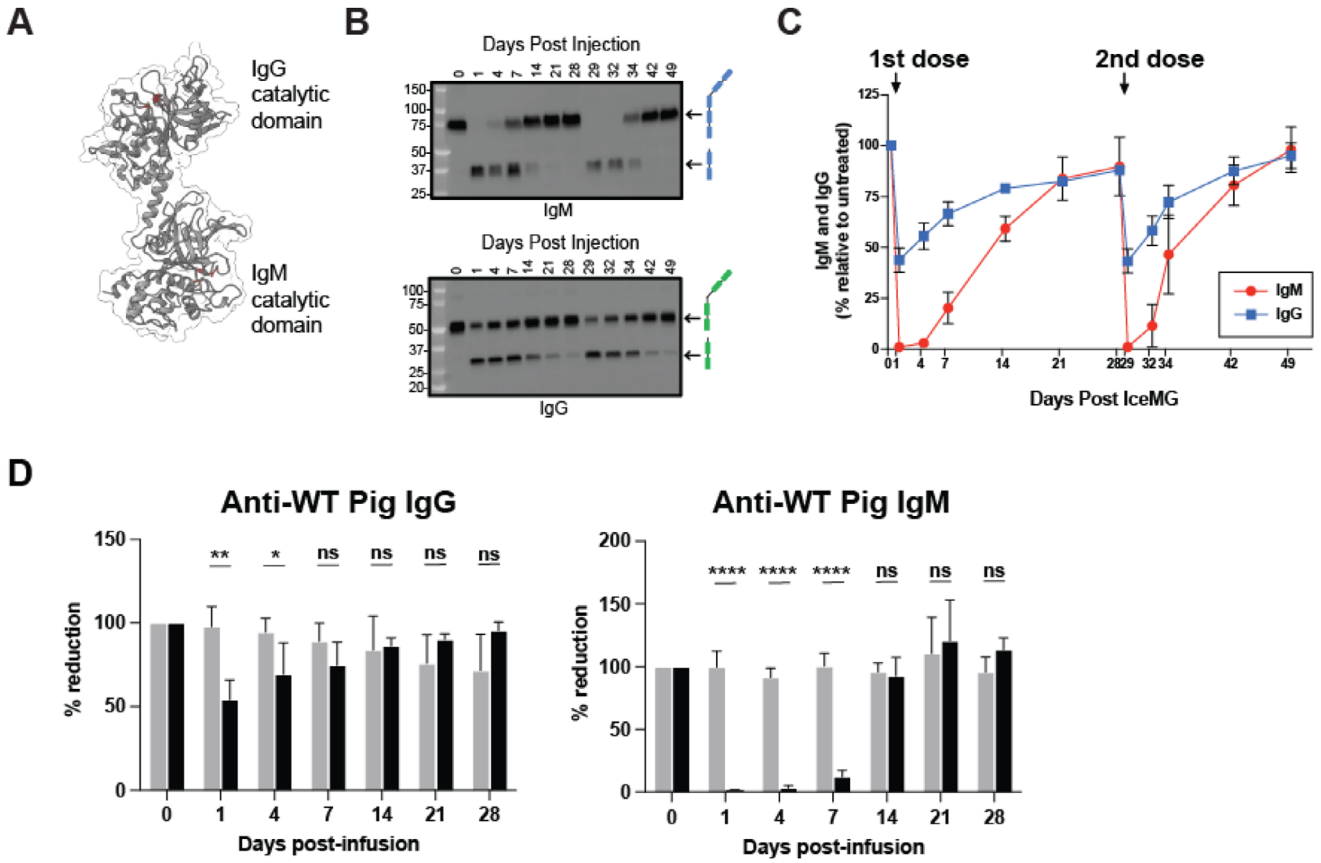


FIGURE 1 | 312.5

xenoreactive-antibodies (IgG and IgM) playing key roles. We aim to investigate the effectiveness of IceMG, a newly developed recombinant endopeptidase that simultaneously cleaves IgG and IgM, offering a potential therapeutic strategy to prevent or mitigate AMR in xenotransplantation.

Methods: Eight rhesus macaques were sensitized with two sequential skin allografts, with four receiving two intravenous doses of IceMG (2 mg/kg, Figure 1A) spaced 28 days apart. Circulating total IgG and IgM levels with their split products were assessed with western blot. We also evaluated allo-specific, xenoreactive and vaccine-specific antibodies using flow-crossmatch. We also monitored B cell subpopulations after IceMG treatment.

Results: IceMG administration resulted in a statistically significant but temporary reduction in total IgG and IgM levels (Figure 1B,C). Likewise, allo- and xenoreactive IgG and IgM, as measured by T cell and B cell flow crossmatch, showed a significant decline, returning to baseline within 2 weeks (Figure 1D). Using a pathogen-specific multiplexed reporter cell panel, COVID-19–vaccinated NHPs showed a reduction in IgG and IgM responses against SARS-CoV-2 antigens, including the spike protein, RBD-SD1, and S2 domain antigens. Repeated IceMG treatments produced similar antibody depletion/repletion kinetics. IceMG significantly but transiently reduced the frequency of IgM+ B cells ($p = 0.04$) without affecting IgG+ or IgD+ B cells, suggesting a temporary loss of membrane IgM.

Conclusion: IceMG effectively cleaves both IgG and IgM simultaneously, showing potential as a new therapeutic option for AMR in organ transplantation, including xenotransplantation. Further studies are needed to refine dosing strategies and assess long-term immune effects.

312.6 | Human Xenoreactive Antibodies Against Porcine Aortic Endothelial Cells

Imad Aljabban[1], Jacqueline Kim[1], Ian Jaffe[1], Karen Khalil[1], Tal Eitan[1], Amy Dandro[2], Ana Fazio-Kroll[2], David Ayares[2], Simon Williams[1], Brendan Keating[1], Ming Wu[3], Aprajita Mattoo[1], Vasishta Tatapudi[1], Edward Skolnik[1], Jeffrey M Stern[1], Adam Griesemer[1], Robert A Montgomery[1], Massimo Mangiola[1]

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Introduction: A barrier to successful xenotransplantation is the presence of donor-specific antibodies that could initiate an antibody-mediated response. Pre-operative crossmatch testing is a valuable technique for detecting the presence of such xenoreactive antibodies, and can inform strategies to mitigate the risk of rejection. Here, we evaluate human anti-pig reactivity against porcine aortic endothelial cells (pAECs) in a pig-to-human decedent xenotransplant model.

Methods: A brain-dead decedent underwent bilateral native nephrectomies followed by transplantation of a GGTA1 KO Thymokidney, with clinically approved immunosuppression administered for a planned 61-day period. Undiluted, heat-inactivated serum samples from the decedent at various time-

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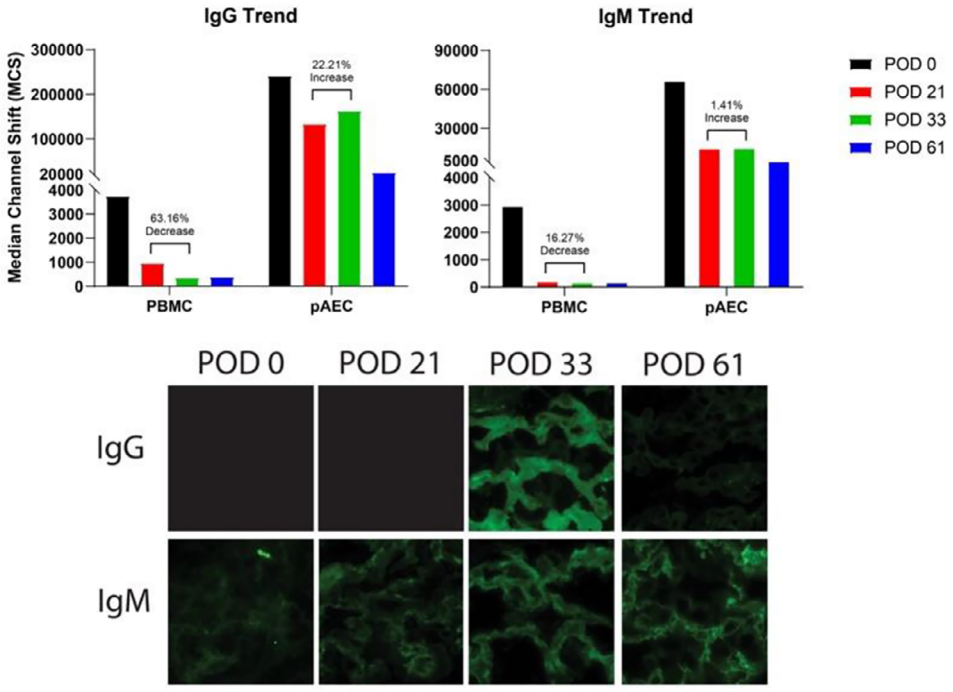


Figure 1. Detection of Anti-Pig IgG and IgM on Pig PBMCs and pAEC Following Pig-to-Human Xenotransplantation in a Brain Dead Decedent. FCXM was conducted on days 0, 21, 33, and 61 after kidney xenograft reperfusion and correlated with IF.

FIGURE 1 | 312.6

points were incubated with either porcine peripheral blood mononuclear cells (PBMCs) or pAECs. Human anti-pig antibody reactivity was assessed via flow cytometric crossmatch (FCXM) and quantified by measuring the median channel shift (MCS) in fluorescence intensity. Serial xenograft biopsies were collected and analyzed by immunofluorescence (IF) to detect glomerular deposition of human IgG and IgM.

Results: Mean IgG and IgM reactivity against pAECs was approximately 100-fold and 28-fold higher, respectively, compared to PBMCs. By post-operative day (POD) 33, early antibody-mediated rejection was identified, coinciding with a rise in serum creatinine (data not shown). Flow cytometric crossmatch (FXCM) using PBMCs demonstrated a 61.16% decrease in IgG and a 16.27% decrease in IgM reactivity. In contrast, FXCM with pAECs showed a 22.1% increase in IgG and a 1.41% increase in IgM reactivity (Figure 1A). These changes in anti-pig IgG and IgM responses correlated with increased glomerular deposition of IgG and IgM on immunofluorescence at POD 33 (Figure 1B). Following initiation of anti-rejection therapy, the IgG immunofluorescence signal decreased.

Conclusions: FCXM using pAECs revealed significantly higher anti-pig IgG and IgM reactivity compared to PBMCs, suggesting the presence of antigenic targets on pAECs that are either absent or expressed at lower density on PBMCs. Crossmatch on pAECs may provide a more reliable means of detecting xenoantibody mediated responses compared to PBMCs.

Funding: Work funded in part by Lung Biotechnology, a wholly owned subsidiary of United Therapeutics Corporation.

315.2 | Targeting Innate Immune Barriers to Boost Xenogeneic Chimerism: The Role of Macrophage-Mediated Xenophagocytosis

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Recent advances in transplanting hypoimmunogenic pig organs have marked a major step in transplantation medicine. However, despite the heroic number of genomic edits made and intense immunosuppression, these porcine organs still failed within months of transplant into human patients, likely due to the vast number of other interspecies differences. Therefore, interspecies blastocyst complementation is a promising approach to generate human organs from pluripotent stem cells, but xenogeneic donor cell contribution remains low due to poorly understood xenogeneic barriers. In addition to the previously proposed xenogeneic barrier driven by developmental incompatibilities, here we identify an innate immune barrier, termed xenogeneic phagocytosis (xenophagocytosis), mediated by host macrophages. After injecting rat PSCs into mouse blastocysts, we found that donor chimerism sharply declines between embryonic days 9.5 and 11.5, coinciding with the emergence of mouse primitive macrophages. Genetic depletion of host macrophages significantly improved

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rat donor chimerism in the mouse embryo—achieving up to 77% chimerism in lung and 57% in heart. We identify the mechanism by which host macrophages selectively phagocytose xenogeneic donor cells: xenogeneic cells display elevated phosphatidylserine, an "eat-me" signal recognized by host macrophages through phagocytic receptor Axl. Axl positive macrophages actively recognize and eliminate donor cells through xenophagocytosis. Genetic knockout of Axl from host embryos or overexpression of the "don't eat-me" signal CD47 on donor cells markedly enhanced donor rat chimerism. Xenophagocytosis blockade also enhances chimerism in human to mouse chimeras, demonstrating the generalizability of this strategy. These findings offer mechanistic insights and practical strategies not only for overcoming the xenogeneic barrier to generating human organs in livestock, but also for improving rejection outcomes in xenotransplantation.

Funding: This work was supported by Leducq Foundation. Dr. Ralph & Marian Falk Medical Research Trust. Japan Agency for Medical Research and Development (AMED).

Poster Abstracts

P1.01 | Improving Pig Red Blood Cell Transfusion Safety With Anti-CD154 Co-Administration in Nonhuman Primates

Hee Jung Kang[1], Juhye Roh[1], Eun Mi Park[1], Haneulnari Lee[1], Hye Won Lee[1], Ju Young Lee[2], Jeong Ho Hwang[2], Joohyun Shim[3], Hyunil Kim[3]

[1]Laboratory Medicine, Hallym University College of Medicine, Anyang, Korea; [2]Animal Model Research Group, Korea Institute of Toxicology, Jeongeup, Korea; [3]Transgenic Animal Research, Optipharm Inc., Cheongju, Korea

Background: Declining blood donation rates and a narrow supply-demand margin pose challenges in transfusion medicine. Genetically engineered pig red blood cells (pRBCs) are a potential alternative to human RBCs. In nonhuman primates (NHPs), pRBC transfusions provide short-term hematological benefit without severe adverse reactions but induce anti-pRBC antibody formation, increasing risks upon repeated transfusions. Anti-CD154 antibodies block the CD40-CD154 co-stimulation pathway and suppress B cell activation. This study investigated whether co-administering anti-CD154 with pRBCs could reduce antibody induction and transfusion-related risks in NHPs.

Methods: Triple knockout (TKO) pigs (Optipharm, Korea) provided RBCs for transfusion. After 25% blood volume withdrawal, NHPs received TKO pRBCs with PG-405 (anti-CD154(Fab)-NTIG-IL-10, ProGen; 30 mg/kg IV), repeated 3 days later (TKO XTf_aCD154, $N = 4$). Eight weeks later, 2 of these NHPs underwent repeat transfusion with the same anti-CD154 protocol (ReXTf_aCD154, $N = 2$). Blood samples were collected at multiple time points to assess hematologic, biochemical, cytokine, complement, and anti-pRBC antibody profiles. Data were compared with prior cohorts without anti-CD154: TKO XTf ($N = 8$) and ReXTf ($N = 2$).

Results: Anti-CD154 did not affect lymphocyte subsets or general blood and cytokine profiles. However, anti-pig antibody induction was significantly reduced in TKO XTf_aCD154 compared to TKO XTf. On Day 21, agglutination titers were lower in TKO XTf_aCD154 (median 2 vs. 128, $p = 0.0057$). In repeat transfusions, ReXTf_aCD154 showed lower pre-transfusion titers (median 5.6 vs. 45) and preserved the hematologic benefit up to 24 h without signs of adverse reactions, unlike ReXTf.

Conclusions: Anti-pRBC antibody induction remains a barrier to clinical xenotransfusion. Co-administration of anti-CD154 significantly suppressed antibody responses and mitigated adverse effects during repeated transfusions. pRBCs combined with anti-CD154 may offer a feasible alternative to human RBCs for transfusion. (Grant No. 22-CM-EC-18)

P1.02 | Systemic Inflammatory Biomarkers Versus Graft Specific HTBM in Monitoring Xenograft Status

Prachi Sardana[1], Megan Dufault[2], Farzana Rahman[1], Amy Dandro[1], Kasinath Kuravi [1], Willard Eyestone[1], David Ayares[1], Richard Pierson [2]

[1]Transgene Analytics, Revivicor (Subsidiary of United Therapeutics), Blacksburg, Virginia, USA; [2]Center for Transplantation Sciences and Department of Surgery, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts, USA

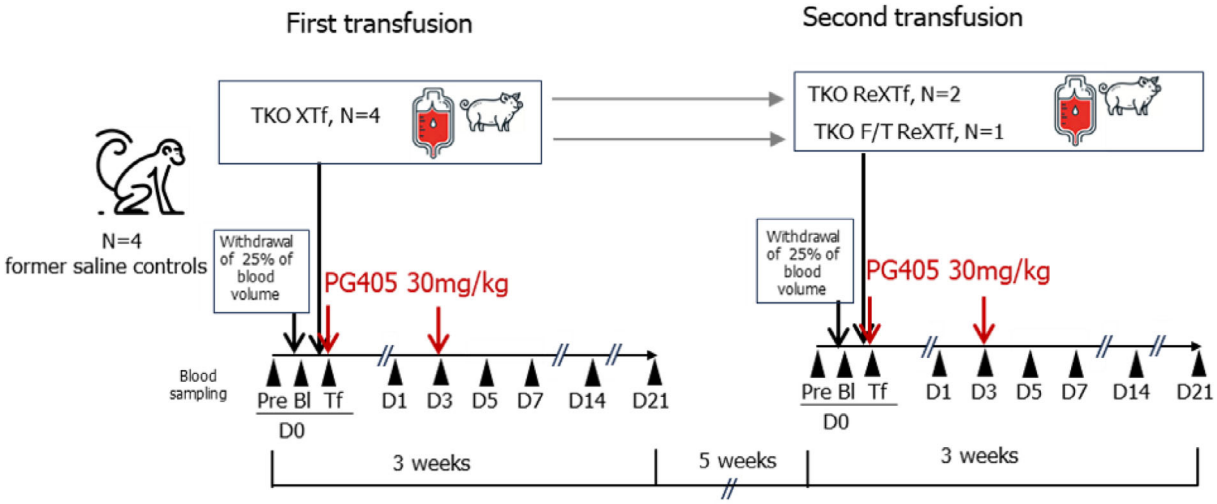


FIGURE 1 | P1.01

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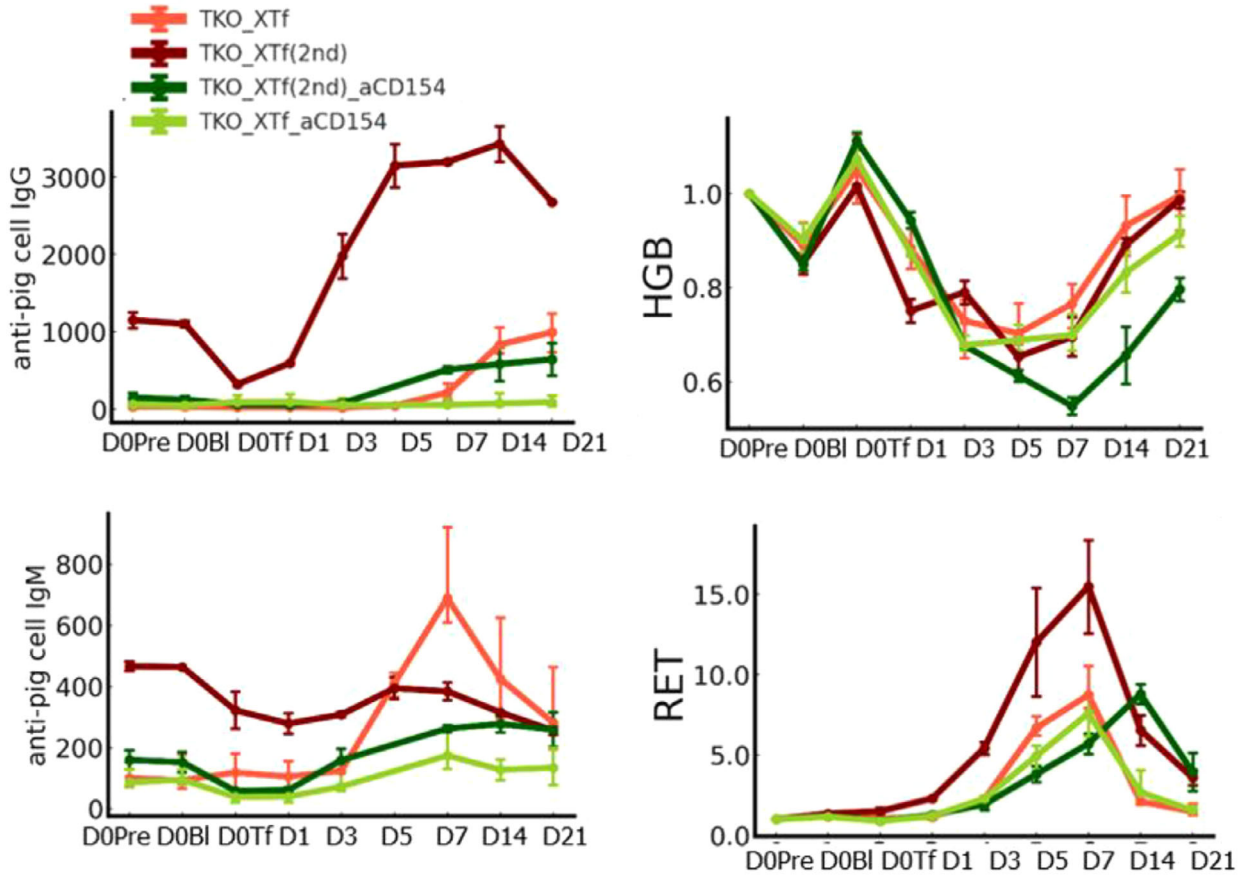


FIGURE 2 | P1.01

Introduction: Xenograft transplantation is a promising treatment for end-stage organ failure. However, widespread application remains complicated by biomolecular interspecies incompatibilities, leading to graft rejection. Currently, genetic and therapeutic methods are being developed in pigs to non-human primate (NHP) transplant models to prolong graft survival. To monitor graft condition, there is a need to measure several biomarkers in transplant recipients. Systemic inflammatory markers like C-Reactive Protein (CRP), Interleukin-6 (IL-6), and Monocyte Chemoattractant Protein 1 (MCP-1), are well-studied and relatively easy to measure. While CRP/IL-6/MCP-1 provide an understanding of the recipients' pro-inflammatory status, it may be more useful to study graft-specific markers. Humanized thrombomodulin (hTBM) is present in some genetically modified porcine xenografts to prevent pro-coagulatory effects stemming from interactions between porcine TBM and NHP/human thrombin. The following study retrospectively measured CRP/IL-6/MCP-1/hTBM in serum and plasma samples from kidney and heart xenograft baboon recipients, aiming to determine which marker is the best predictor of early graft failure.

Methods: Biological parameters were retrospectively evaluated in plasma and serum samples using ELISA. CRP (cat# CRP-3, Life Diagnostic, Inc., West Chester, Pennsylvania, USA), IL-6 (cat# BMS641-2, Thermo Fisher, Vienna, Austria), and MCP-1 (cat# EP16RB, Thermo Fisher, Vienna, Austria) were measured on a SpectraMax iD3 ELISA plate reader, following the manufacturers' instructions. Transgenic human-specific TBM (CD141) was measured using an ELISA kit (ab46508, Abcam, Boston, MA) on a Biotek ELISA plate reader, following the manufacturer's instructions.

Results: Kidney recipients presented with an initial spike in CRP, while heart recipients presented with an initial spike in IL-6. Differences in treatment regimen, including the administration of an IL-6 receptor antagonist for heart recipients, may affect the reliance of CRP and IL-6 as inflammatory markers. Kidney and heart recipients presented with similar average MCP-1 trends. While both exhibited stabilized levels following an initial increase within 24 h of transplant, kidney recipients yielded significantly higher levels of hTBM relative to heart recipients. Unlike CRP/IL-6, hTBM levels remained stable in xenokidney recipients presenting with acute gastric dilatation (AGD) but no early graft failure.

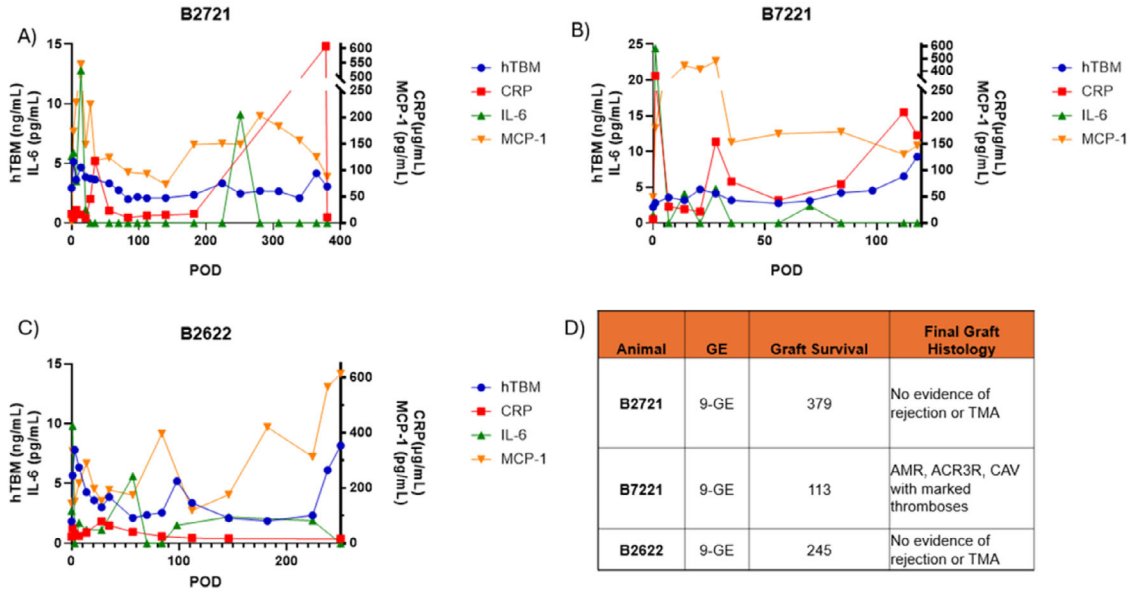
Conclusions: Due to graft-specificity and a significant role in anti-inflammation and anti-coagulation, hTBM may serve as a biomarker to monitor organ status and should be studied further.

P1.03 | Xenotransplantation Hope: Flying Pigs and the People Who Make It Happen

Sofie á Rogvi, Anja Jensen
Department of Public Health, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark

Introduction: After years of non-human primate research and a moratorium on xenotransplantation, recent advancements have

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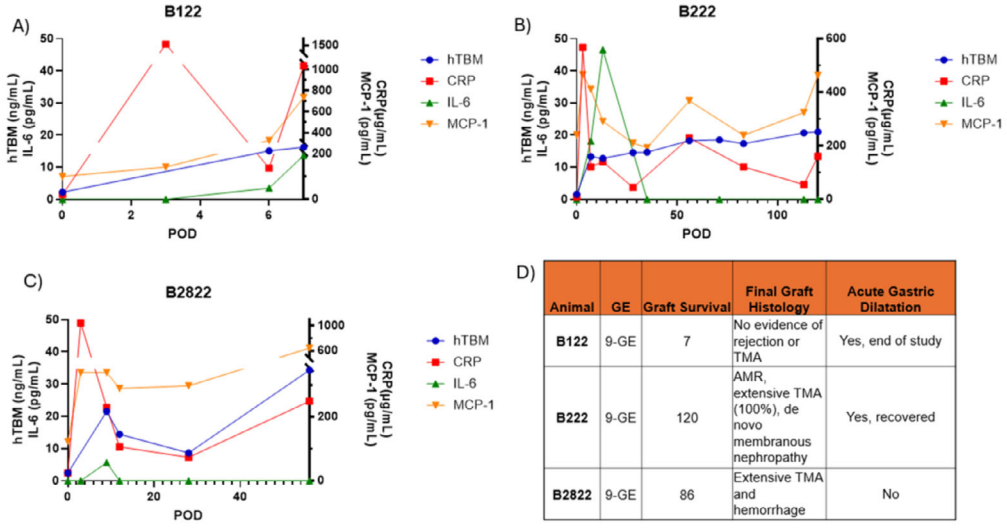


D)

Animal	GE	Graft Survival	Final Graft Histology
B2721	9-GE	379	No evidence of rejection or TMA
B7221	9-GE	113	AMR, ACR3R, CAV with marked thromboses
B2622	9-GE	245	No evidence of rejection or TMA

Figure 1: Soluble hTBM, CRP, IL-6, and MCP-1 trends in baboons receiving heart xenografts. Recipients presented with a spike in IL-6 around POD 1-3. A) B2721 exhibited stable levels of hTBM below 5ng/mL throughout the course of the study, with a spike in CRP correlating with pneumonia around POD364; good graft function until end of study (EOS); B) B7221 presented with progressive graft hypertrophy and antibody mediated rejection (AMR), accompanied by transiently elevated MCP-1 levels and a rise in hTBM and CRP at EOS; C) B2622 presented with elevated hTBM and MCP-1 towards EOS, correlating with gradual xenograft hypertrophy, subacute abdominal compartment syndrome, progressive anemia, weight loss, and decreasing graft function. D) Summary of genetic edits (GE), graft survival, and final graft histology. Post operative date (POD); thrombotic microangiopathy (TMA); acute cellular rejection grade 3 (ACR3R), cardiac allograft vasculopathy (CAV)

FIGURE 1 | P1.02



D)

Animal	GE	Graft Survival	Final Graft Histology	Acute Gastric Dilatation
B122	9-GE	7	No evidence of rejection or TMA	Yes, end of study
B222	9-GE	120	AMR, extensive TMA (100%), de novo membranous nephropathy	Yes, recovered
B2822	9-GE	86	Extensive TMA and hemorrhage	No

Figure 2: Soluble hTBM, CRP, IL-6, and MCP-1 trends in baboons receiving kidney xenografts. Recipients presented with a spike in CRP around POD3. B122 and B222 presented with acute gastric dilatation (AGD) following transplant. A) B122 was euthanized on POD7 due to severe acute gastric dilatation, accompanied by elevated CRP, IL-6, and MCP-1 from POD6, where hTBM remained stable. Final pathology revealed mild acute tubular injury but no evidence of rejection. B) B222 exhibited AGD, correlating with a spike in IL-6, but recovered gradually with a normal appetite and stools by POD21. Transiently elevated hTBM > 15ng/mL after POD35 correlated with deteriorating graft function, with a biopsy at POD42 showing TMA in approximately one-third of glomeruli. C) Increasing CRP, MCP-1, and hTBM correlated with weight loss and a decreased appetite in B2822 beginning around POD35. By EOS, the xenograft was extensively hemorrhagic with diffuse edema, and the animal presented with a subacute ureter obstruction. D) Summary of genetic edits (GE), graft survival, final graft histology, and presence of AGD. Post operative date (POD); thrombotic microangiopathy (TMA)

FIGURE 2 | P1.02

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reignited interest in the field. Previously, phrases like "xenotransplantation is the future and always will be" or "xenotransplantation will happen when pigs fly" were common. However, with developments in gene-editing technologies like CRISPR-Cas9, decedent experiments, several pig-to-human transplants in recent years, and forthcoming clinical trials, the landscape is changing. This study focuses on the people making all of this happen. It explores the future scenarios and potential envisioned by those working in xenotransplantation, and investigates the work, the network, and the dedication driving xenotransplantation pioneers.

Methods: This anthropological study is based on semi-structured interviews with 19 US surgeons, researchers, and nurses involved in xenotransplantation experiments and focuses on their experiences and how they imagine xenotransplantation futures. Additionally, we included media material covering xenotransplantation in 2021–2024. The data were transcribed and thematically analyzed. Using the concepts of "imaginaries" and "potentiality", we analyze the perceived unlimited potential of pig organs, the articulated need for this technology, and the hopes and experiences of medical professionals.

Results: The findings provide insights into the personal hopes, dilemmas, and professional aspirations of xenotransplantation surgeons, researchers, and nurses. They envision a future where xenotransplantation treatments are available for patients with no options. Some imaginaries extend to fantasizing about tailored pigs bred for individual patients, eliminating the need for immunosuppression, and surpassing allotransplantation. Scenarios include pig organs delivered by drones to patients in need. However, questions arise about the practicalities and the accessibility of this technology: Will it be available to all, or only to a privileged few? As such, based on insights from the actual people dedicated to developing xenotransplantation, our research shed light on the ethical, organizational and practical implications of these highly innovative procedures.

Conclusion: This study provides valuable anthropological insights into the personal experiences, practical expertise, and medical dedication that drives and justify xenotransplantation experiments. It highlights the importance of considering the emotions and motives of clinical practitioners. As xenotransplantation progresses, it is crucial to balance the potential benefits of xenotransplantation with the ethical and practical challenges experienced in the clinic, ensuring that the technology serves humanity equitably. Most importantly, we argue for social science attention to the medical professionals involved as they represent a valuable source of knowledge for developing and improving the social, organizational, and practical aspects of xenotransplantation.

Funding: This study is part of the project "TechnEmotion: The Interaction between Technology and Emotion in Transplant Medicine" supported by a Sapere Aude grant from the Independent Research Fund Denmark (Grant no. 1055-00059B).

P1.04 | Brain-Dead Donors in Xenotransplantation Research: Global Trends and Legal-Ethical Challenges in Korea

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This study reviews international practices and experiences with brain-dead donor research in xenotransplantation and critically examines Korea's current legal and ethical framework. Preclinical xenotransplantation studies using non-human primates face critical limitations due to interspecies physiological differences, making direct translation to human clinical outcomes uncertain. To bridge this gap, research involving brain-dead donors has emerged internationally as an intermediate model, allowing evaluation of graft viability and immune compatibility under near-human physiological conditions. Brain-dead individuals, sustained by life-support measures despite irreversible loss of brain function, offer unique opportunities for biomedical research beyond the capabilities of traditional models. Several xenotransplantation studies have successfully utilized this model to assess post-transplant outcomes and refine preclinical evaluation. In Korea, however, brain-dead individuals are regarded solely as organ donors, and research involving them is prohibited. This reflects not only the legal and cultural view that brain death is inseparable from organ donation, but also a broader societal reluctance to fully accept brain death as true death.

Funding: This work was supported by a grant of the Xenotransplantation Research and Development Project through the Korea Health Industry Development Institute (KHIDI), funded by the Ministry of Health & Welfare, Republic of Korea (No. RS-2023-KH136898).

P1.05 | Production and Characterization of Transgenic Pigs for Xenotransfusion by Knock-In of Human CD46 and TBM or CD59 and CD47 Genes at the GGTA1 Exon 4 Locus

Jun-Hyeong Kim, Nayoung Ko, Hyoung-Joo Kim, Yongjin Lee, Jae-Kyung Park, Kyungmin Kwak, Sung-Hun Park, Byung Hyun Kim, Hyunil Kim, Joohyun Shim
Transgenic Animal Research, Optipharm Inc., CheongJu, Korea

Introduction: The continuous decline in blood donations has become a critical global issue, emphasizing the urgent need for alternative solutions to meet the growing global demand. One promising approach is the development of transgenic pigs capable of overcoming immune rejection, enabling their blood to be used in therapeutic applications. To this end, we aim to generate transgenic pigs by eliminating genes responsible for xenoantigen production and inserting human genes that regulate immune rejection, thereby enabling the development of xenogeneic porcine red blood cell (pRBC) products.

Methods: Two types of transgenic cells were generated by inserting *hCD46-2A-hTBM* (TG1) or *hCD59-2A-hCD47* (TG2) vectors into exon 4 of the *GGTA1* gene, which encodes alpha-1,3-galactosyltransferase, via homologous recombination. Subsequently, cloned transgenic pigs were generated via somatic cell nuclear transfer based on these cells. Blood samples were collected from the resulting pigs, and FACS analysis was performed to verify the proper insertion and expression of the inserted human genes.

Results: Cloned transgenic pigs were produced using transgenic cell lines carrying the TG1 and TG2 constructs. In peripheral blood mononuclear cells (PBMCs), all transgenes were successfully expressed. However, in pRBCs, hCD46 and hTBM were not detected, whereas the other inserted genes, hCD59 and hCD47, showed clear expression. Notably, hCD46 and hTBM

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were expressed in PBMCs and other tissues, indicating that their lack of expression is specific to red blood cells. Further analysis at the mRNA level revealed that *hCD46* and *hTBM* transcripts were present in pRBCs.

Conclusion: In summary, to develop xenogeneic RBC products, we successfully produced transgenic pigs with the human immune regulatory genes *hCD46* and *hTBM* or *hCD59* and *hCD47* inserted into the *GGTA1* exon 4 locus. While the inserted genes were properly expressed in PBMCs and other tissues, hCD46 and hTBM were not expressed on the membrane of erythrocytes, in contrast to hCD59 and hCD47. Although mRNA transcription occurs in erythrocyte precursor cells, challenges seem to arise during post-transcriptional or subsequent processes. Further research is needed to clarify the precise mechanisms involved.

Funding: This study was supported by the Institute of Civil Military Technology Cooperation, funded by the Defense Acquisition Program Administration and the Ministry of Trade, Industry, and Energy of the Korean Government (grant no. 22-CM-EC-18).

P1.06 | Blood-Derived Endothelial Progenitor Cells Enable Non-Terminal Verification of Endothelial-Specific Transgene Expression in GTKO/MCP/TBM Pigs

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GTKO/MCP/TBM transgenic pigs were developed to mitigate coagulation-mediated vascular rejection in xenotransplantation by expressing human thrombomodulin (hTBM) under the control of the endothelial-specific pICAM2 promoter. In this study, blood-derived endothelial progenitor cells (EPCs) were isolated from the pig, characterized for their endothelial properties, and then used to verify the endothelial-specific expression of the transgene. The EPCs exhibited a typical cobblestone morphology. At passage 4, increased expression of ICAM2 was confirmed via real-time PCR. Flow cytometry analysis revealed expression of endothelial progenitor markers including CD34, CD133, CD31, vWF, VEGFR2, and VE-cadherin. The cells also demonstrated angiogenic potential in an in vitro tube formation assay and retained their morphology and marker expression up to passage 13. At this stage, 6–8% of the cells expressed the activated endothelial marker CD62E/CD62P, along with elevated levels of VEGFR2 and VE-cadherin. Notably, expression of hTBM was detected, indicating that transgene activation driven by the pICAM2 promoter is preferentially induced in activated ECs. These findings demonstrate that blood-derived EPCs from transgenic pigs can be used to evaluate endothelial-specific transgene expression without the need for vascular tissue biopsy. This approach may offer a reliable method for molecular verification and pre-transplant evaluation in future xenotransplantation research.

Funding: This study was supported by the 2025 RDA Fellowship Program of the National Institute of Animal Science, Rural Development Administration, Republic of Korea. This research was also supported by a grant of the Korea Health Technology R&D Project through the Korea Health Industry Development Institute (KHIDI), funded by the Ministry of Health & Welfare, Republic of Korea (grant number: HI23C0417).

P1.07 | Generation of 10 Gene Pigs in One Step With Combination of CRISPR/Cas9 and 2A Self-Cleaving Multigene Expression System

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Introduction: Xenotransplantation has been considerably advanced by identifying the genetic modifications needed for the donor pig. These include (1) triple knockout (KO) (TKO) of xenoantigen encoding genes:- GGTA1, B4GaLNT2 and CMAH, (2) KO of GHR, to limit organ growth, and (3) addition of human genes: (i) complement inhibitors (CIs), CD46/CD55/CD59; (ii) thromboregulatorsy hTBM/hEPCR; (iii) apoptosis inhibitors hHO-1(iv) a macrophage inhibitor hCD47. However, challenges remain in generating multi-transgenic pigs for production in a time- and cost-efficient manner. The aim of this study is to generate multi-transgenic donor pigs with ten genetic edits (10GE), introducing six essential human transgenes as a single multi-cistronic vector (MCV) in one step.

Methods: We constructed five MCVs, each containing six human transgenes organized into three bicistronic units, utilizing the 2A self-cleaving peptide system. Each transgene pair was linked by a 2A peptide, to allowing the expression of both transgenes from a single promoter. Homology arms were incorporated to facilitate Homology Directed Repair (HDR) at the CMAH or GGTA1/Neo locus landing pads. Each MCV was transfected as a linear fragment (~28kb), flanked by HDR arms, into GGTA1 knockout fibroblasts, along with the appropriate CRISPR guides and Cas9. Vector targeting was confirmed by PCR, ddPCR, and Southern blot. Transgene expression was evaluated in heart, lung, and kidney tissue samples using an automated capillary western blot assay system and immunohistochemistry (IHC). Transgene function in aortic endothelial cells was confirmed in vitro by Complement–Dependent Cytotoxicity (CDC) assay (hCD46, hDAF), Activated Protein C (APC) assay (hTBM, hEPCR) and SIRP-areceptor activation assay (hCD47)

Results: No significant differences in transgene expression were observed between the two landing pads in the 10GE pigs. Physiological levels of hTBM and hEPCR were low when co-expressed in a bi-cistron controlled by pTBMp, while expression of hCD55, hCD46, and hCD47 was robust at physiologically acceptable levels under CAGp. hHO-1 levels remained low regardless of the upstream gene. hCD47 expression was strongest when placed immediately downstream of the CAG promoter, but was barely detectable when positioned second, downstream of the 2A sequence. Nevertheless, in vitro assays showed that hCD47 protein expressed in endothelial cells from different MCVs retained the ability to bind and activate SIRP-α, albeit to varying degrees. MCVs expressing two (hCD46 & hCD55) or three (hCD46, hCD55, and hCD59) CIs performed equally in CDC assays. In all, 10GE genotypes had similar APC levels that were comparable to a human endothelial cell line.

Conclusion: To date, live pigs have been generated with four of these MCVs, and organs from three 10GE genotypes have been used in xenotransplantation experiments in baboons and human recipients.

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P1.08 | Interspecies Chimerism With Human Expanded Potential Stem Cells in Porcine Embryos

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Introduction: Exogenesis is a promising strategy for generating humanized organs by replacing specific cell lineages with human cells in genetically engineered pig embryos that harbor organogenesis-disabled niches. Embryo complementation with patient-specific stem cells has the potential to produce transplantable xeno-organs while further reducing immunological and physiological barriers. However, achieving high integration of human cells into developing organs in evolutionarily distant species remains a major challenge. Insufficient chimerism is often due to a developmental mismatch, causing stem cells to be competitively eliminated from the blastocyst or tissue niche. In order to improve interspecies chimerism, we employed human expanded pluripotent stem cells (EPSCs) alongside refined injection and in vitro culture protocols for porcine embryos.

Methods: Here, we applied four modified expanded potential culture conditions (EPSCM, EPSCM-1, EPSCM-2, and EPSCM-8C) that enable conversion of human primed induced pluripotent stem cells (hiPSCs) to earlier developmental states, resembling the morula and 8-cell embryo stages. We assessed the expression of pluripotency- and totipotency-associated genes in these lines and compared their chimeric competency in parthenogenetic blastocysts. The development of interspecies chimeric embryos was monitored over 2 days using the Primo Vision system. Stem cell survival, proliferation, and integration in porcine embryos were confirmed through immunofluorescence imaging.

Results: Human primed iPSCs converted into stable EPSC lines exhibited a consistent dome-shaped morphology over more than 20 passages and showed upregulation of pluripotency markers (*SOX2, OCT4, NANOG, KLF17, DPPA3* and *DPPA5*) and totipotency-associated genes (*TPRX1, MAEL, DUXA and DUXB*). Normal porcine blastocyst formation was observed after the injection of ten GFP-labeled human PSCs derived from four modified EPSC conditions, resulting in a 94% recovery rate after 2 days. Quantification of GFP-positive human cells per blastocyst revealed that cells cultured in EPSCM-2 conditions displayed the highest chimeric competency, contributing 15–40 cells per embryo after 2 days. Expression of the cell-adhesion molecule E-cadherin confirmed that the injected hPSCs formed functional adherens junctions with cells of the host blastocyst. The viability test of the injected cells within chimeric blastocysts, after PARP and CASPASE staining revealed that only a few human PSCs underwent apoptosis.

Conclusion: We demonstrated that hEPSCs, owing to their enhanced pluripotent properties and differentiation potential, represent promising donor cells for the generation of interspecies chimeras. Our long-term goal is to produce functional, rejection-free humanized pig hearts using genetically modified Auckland xeno-embryos for preclinical studies and, ultimately, clinical transplantation trials.

Funding: This work was supported by Leducq Foundation (23CVD01 "Xenogenic and exogenic pig organs for transplantation into humans"). German Centre for Cardiovascular Research (FKZ 81Z0600601 "Platform Reprograming"). European Research Council (788381 "Deep biomodeling of human cardiogenesis").

P1.09 | Site-Specific Integration of HO1 and CD47 At the CMAH Locus in GTKO Pigs for Immunomodulation in Xenotransplantation

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Xenotransplantation from pigs to humans holds great promise for addressing organ shortages. However, immunological barriers, including hyperacute rejection mediated by α-Gal epitopes and macrophage phagocytosis, must be overcome. To this end, we generated genetically modified pigs with targeted knock-in of human heme oxygenase-1 (HO1) and CD47 expression cassettes into the exon 4 of the cytidine monophosphate-N-acetylneuraminic acid hydroxylase (CMAH) gene in a GGTA1-knockout (GTKO) background. Initial analysis of GT, CMAH, and B4GAL expression across various porcine tissues revealed unexpectedly low endogenous CMAH expression levels, prompting us to utilize alternative promoters for transgene expression. We employed the human HO1 promoter to drive regulated HO1 expression, considering its potent biological activity and potential for adverse effects upon overexpression. Constitutive, high-level expression of human CD47, a "don't eat me" signal, was achieved using the previously established pEF1α promoter. A bicistronic vector encoding HO1 and CD47 was constructed and demonstrated inducible expression upon PMA and human serum treatment in porcine cells, confirming its regulatory capacity. The knock-in vector was precisely integrated into the CMAH locus of GTKO porcine cells using CRISPR/Cas9 technology. Somatic cell nuclear transfer resulted in the generation of two cloned piglets, with one surviving for 1 week. Post-mortem analysis of the deceased piglet's heart, kidney, liver, lung, and spleen by Western blotting confirmed the presence of both human HO1 and CD47 proteins in all examined tissues. Immunohistochemistry (IHC) analysis showed HO1 expression predominantly in the liver and lung. Flow cytometry analysis of peripheral blood mononuclear cells (PBMCs) from the surviving piglet demonstrated the expression of human CD47 on the cell surface. These results demonstrate the successful targeted knock-in of human HO1 and CD47 into the CMAH locus of GTKO pigs, achieving transgene expression in vital organs. The use of specific promoters allows for regulated HO1 expression and high-level CD47 expression, representing a significant step towards generating donor pigs with enhanced resistance to immune rejection for xenotransplantation. Further studies on the surviving pig and subsequent generations will be crucial to evaluate the long-term efficacy and safety of this genetic modification strategy.

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Funding: This work was supported by the Cooperative Research Program for Agriculture Science and Technology Development (project number PJ01422802) of the Rural Development Administration, and by a grant from the Korea Health Technology R&D Project through the Korea Health Industry Development Institute (KHIDI), funded by the Ministry of Health and Welfare, Republic of Korea (grant numbers: HI23C041702).

P1.10 | Expression of a Retroviral Immunosuppressive Protein on the Xenotransplant – A Way to Reduce Pharmacological Immunosuppression?

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Although the porcine organs used for xenotransplantation have been genetically modified to prevent hyperacute rejection and reduce cell- and antibody-mediated rejection, intensive pharmacological immunosuppression is still required to prevent rejection. The expression of an immunosuppressive protein on the surface of the pig transplant could help to prevent rejection. Retroviral transmembrane envelope proteins containing a highly conserved domain, the so-called immunosuppressive (isu) domain, are known to be immunosuppressive. These proteins, including the transmembrane envelope protein p15E of the porcine endogenous retrovirus (PERV), have been shown to inhibit lymphocyte activation in vitro and modulate cytokine release and gene expression as shown by cytokine arrays and microarrays. In vivo studies have shown that expression of various retroviral transmembrane envelope proteins on murine tumor cells, which are otherwise eliminated by the immune system, can enable tumor formation in immunocompetent mice by suppressing host immune responses. In a model experiment, a construct encoding a part of p15E of PERV, including the isu domain, was transfected into human 293 cells, and the expression was analyzed by immunofluorescence and flow cytometry. These cells were subsequently cocultured with peripheral blood mononuclear cells (PBMCs) from healthy human donors. Although p15E expression on 293 cells was low, coculture induced human PBMCs to secrete higher levels of IL-10 and to upregulate the expression of IL-6, IFN-α, TNF-α, MMP1, and SEPP1. Additionally, p15E expression led to a downregulation of MHC class I molecules and preliminary data indicate that p15E expression could have a protective effect against cellular cytotoxicity. If the expression of p15E can be significantly increased, the expression of p15E on the surface of porcine xenotransplants could substantially reduce the requirement for systemic immunosuppression. Notably, expression of a transfected p15E construct represents an optimal strategy for such an approach, as p15E is encoded endogenously in all pigs, and pigs are immunologically tolerant to it, not producing antibodies against the protein. The incorporation of the immunosuppressive molecule p15E into pig xenotransplants could represent a significant advancement in the field of transplantation, potentially providing an additional advantage over allotransplantation.

Funding: This work was supported by Deutsche Forschungsgemeinschaft TRR127.

P1.11 | LEA29Y Expression Affects Myocardial Scar Formation After Ischemia/Reperfusion in Pigs

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Background: The processes of scar formation during myocardial remodeling after ischemia/reperfusion (I/R) are intricate and tightly regulated by the immune system and its effector cells. The fusion protein LEA29Y is a widely used immunosuppressive medication that inhibits T-cell co-stimulation, thereby limiting immune cell activation. A Pig model with systemic LEA29Y-Expression has been established as a model for general immunosuppression, enabling investigation of I/R injury and remodeling under immunocompromised conditions. Investigations include an emphasis on collagen content as a representation of scar formation, as well as the quantification of immune cells.

Methods: Using a catheter-based Intervention, 60 min of Ischemia in the left anterior descending artery (LAD) was induced, followed by a permanent reperfusion, leading to an infarction in the Left Ventricle (LV). Animals were sampled after 3, 9, 14, and 26 days and compared to controls. Ejection fraction was calculated, ECG was recorded, and complemented by histological staining with HE, Sirius Red, IBA1, CD3-T-Cells, and neutrophils. Histological Analysis was performed using "QuPath", an open source bioimage analysis program that allows for the analysis of wholly scanned slides, thus enabling a high-resolution global measurement of stained tissue in percent and micrometers square.

Results: LEA29Y and WT animals displayed similar infarct sizes. LV angiography showed a significantly higher loss of ejection fraction in the transgenic pigs after 3 days (-15.48 ± 1.069 vs. -7.554 ± 1.873, $p = 0.011$), but not after 9 days or later. QuPath analysis of stained collagen areas detects scar formation over the course of the investigation period. It showed a peak of collagen at d14, with less height and slower decline at d26 in LEA29Y Animals compared to WT (Day 3: 13,33% of LEA29Y LV vs. 12,6% of WT LV; Day 9: 18,36% of LEA29Y LV vs. 16,57% of WT LV; Day 14: 18,4% of LEA29Y LV vs. 20,75% of WT LV; Day 26: 16,49% of LEA29Y LV vs. 15,04% of WT LV).

Conclusion: Blocking of T-cell co-stimulation initially leads to a higher loss of pumping function of the LV and a delayed scar contraction. To further investigate the loss of T-cells and the connections between them and other immune cells, such as macrophages and neutrophils, immunohistochemistry stainings are conducted.

P1.12 | Bispecific Anti-CD40LXCD28 Antibody Prevents Mouse Xenogeneic GVHD

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Introduction: Graft-versus-host disease (GVHD) is a systemic complication of transplantation, primarily arising from the activation of donor CD4+ T cells that recognize recipient cells as foreign. The secondary signals required for CD4+ T cell activation mainly rely on two co-stimulatory molecules, CD40L and CD28. Blocking either of these two signals can induce immunological tolerance to suppress the occurrence of GVHD. In this study, we developed an anti-CD40LXCD28 bispecific antibody (bsAb) and tested its efficacy in the prevention of GVHD.
Methods: In an in vitro setting, we studied the affinity of anti-CD40LXCD28 bsAb to CD40L and CD28 and its efficacy in blocking CD40L-CD40 and CD28-CD80 interactions, as well as in inhibiting the activation of human T cells. Xenogeneic GVHD was induced in NSG mice ($n = 5$/group) by injecting human PBMCs (1.0×10^7cells/injection) three times a week for 3 weeks. Anti-CD40LXCD28 bsAb or a combination of control anti-CD40L and anti-CD28 bsAbs (400μg/ea) were injected following the same schedule. The body weight and GVHD scores of recipient mice were monitored for 8 weeks, and their changes were analyzed by two-way ANOVA with Tukey's multiple-comparison test.
Results: When left untreated, three of five PBMC-injected mice died during the monitoring period. In the mouse group treated with the combination of control bsAbs, significant body weight loss and elevated GVHD scores were observed compared to control PBMC-uninjected mice ($p < 0.05$ and $p < 0.0001$, respectively). In contrast, mice treated with the anti-CD40LXCD28 bsAb had no statistically significant changes in both body weight and GVHD scores compared to PBMC-uninjected controls, and demonstrated significantly reduced body weight loss and GVHD scores compared to the control bsAbs combination-treated mouse group ($p = 0.0001$ for both).
Conclusion: Anti-CD40LXCD28 bsAb effectively inhibited GVHD and was more potent compared to the combined treatment of anti-CD40L and CD28 antibodies.
Funding: This study was supported by the National Research Foundation of Korea (NRF-2016M3A9B594173823) and Korea Health Industry Development Institute (HI23C041703).

P1.13 | Dosing of Pegcetacoplan With Plasmapheresis for Treatment of Xenoantibody Mediated Rejection

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Introduction: Antibody-mediated rejection (AMR) is a concern in the implementation of clinical xenotransplantion. Plasmapheresis (PP) is an integral part of AMR management; however, limited data exist on the effect of PP on many novel complement inhibitors, including pegcetacoplan (PEG), an inhibitor of complement components C3 and C3b.
Methods: An expanded access single patient investigational new drug (IND) application was approved for a 10 gene-edit (10GE) porcine kidney transplanted into a 53-year-old ESRD patient, previous kidney donor on dialysis, with a cPRA of 99.98%. A clinically approved immunosuppression regimen consisting of lymphocyte depletion, corticosteroids, belatacept, tacrolimus, mycophenolate and a C5 inhibitor was utilized.
Results: There was immediate xenograft function and no evidence of clinically significant proteinuria until POD 16 (Figure 1A). A for cause biopsy was concerning for AMR (Figure 1B), prompting a course of PP and initiation of PEG. After initiation of treatment and 5 sessions of PP, sCr and proteinuria significantly improved (Figure 1A). The PEG dosing strategy, 1080 mg intravenous (IV) bolus plus 463 mg/day continuous infusion with 1080 mg IV boluses after PP, was determined by our previous successful treatment of AMR in a previous xenokidney (GGTA1 KO thymokidney) in a 2-month decedent. The patient was converted to subcutaneous administration after completion of the initial 5 PP sessions. A second course of 5 PP sessions was initiated after a POD 28 biopsy was concerning for ongoing AMR (Figure 1B). PEG 1080 mg SC three times weekly plus 1080 mg IV boluses post-PP was continued, and a biopsy on POD 42 revealed resolving AMR with negative C4d and C3 staining on immunofluorescence. There was no evidence of related adverse events with PEG using the off-label dosing strategy noted above. The patient is currently 86 days post-transplant and clinically stable.
Conclusion: PEG SC plus an IV bolus dosing strategy in the setting of PP was effective for treatment of AMR in a xenokidney transplant recipient. Future studies of the pharmacokinetics of PEG and PP will be prudent to validate these clinical findings.
Funding: This data is supported by funding from United Therapeutics Corporation, PBC. Pegcetacoplan was obtained through Apellis Pharmaceuticals' Compassionate Use Program.

P1.14 | Immunomodulatory Ability of Porcine Peripheral Blood-Derived Mesenchymal Stem Cells in Xenotransplantation

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Porcine mesenchymal stem cells (MSCs) have been reported as an effective method for overcoming immune rejection of allografts. Specifically, bone marrow-derived mesenchymal stem cells (BM-MSCs) have demonstrated significant immunomodulatory capabilities. Although obtaining BM-MSCs is challenging, peripheral blood-derived mesenchymal stem cells (PB-MSCs) can be easily harvested at any time through a significantly less invasive method. However, the immunomodulatory effects of PB-MSCs are poorly understood. In this study, the characterization of PB-MSCs isolated from peripheral blood mononuclear cells (PBMCs) was confirmed by MSC surface markers CD44, CD73, CD90,

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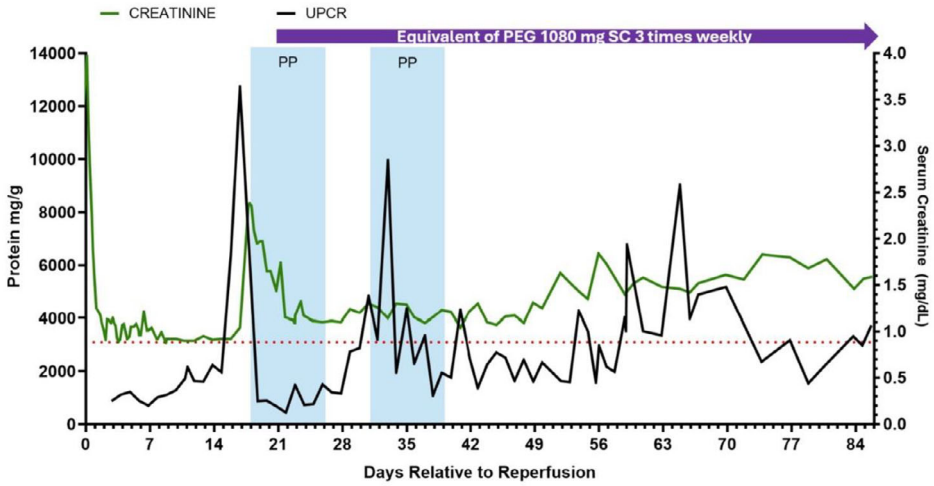


Figure 1a. There was immediate xenograft function with sCr nadir of 0.91 mg/dL by post-operative day (POD) 2 and no evidence of clinically significant proteinuria until POD 16. A rise in proteinuria prompted a for-cause biopsy on POD 17 and subsequent initiation of PP and PEG 1080 mg IV bolus followed by 463 mg/day continuous infusion (daily dosing based on equivalent of 1080 mg SC 3 times weekly regimen) and 1080 mg IV bolus after each PP session. The sCr and proteinuria returned to baseline values by POD 26 after completion of the first round of 5 PP sessions. A second course of PP was initiated after a POD 28 biopsy was concerning for ongoing AMR. PEG 1080 mg SC three times weekly plus 1080 mg IV boluses post-PP was continued and a biopsy on POD 42 revealed resolving AMR with negative C4d and C3 staining.

FIGURE 1 | P1.13

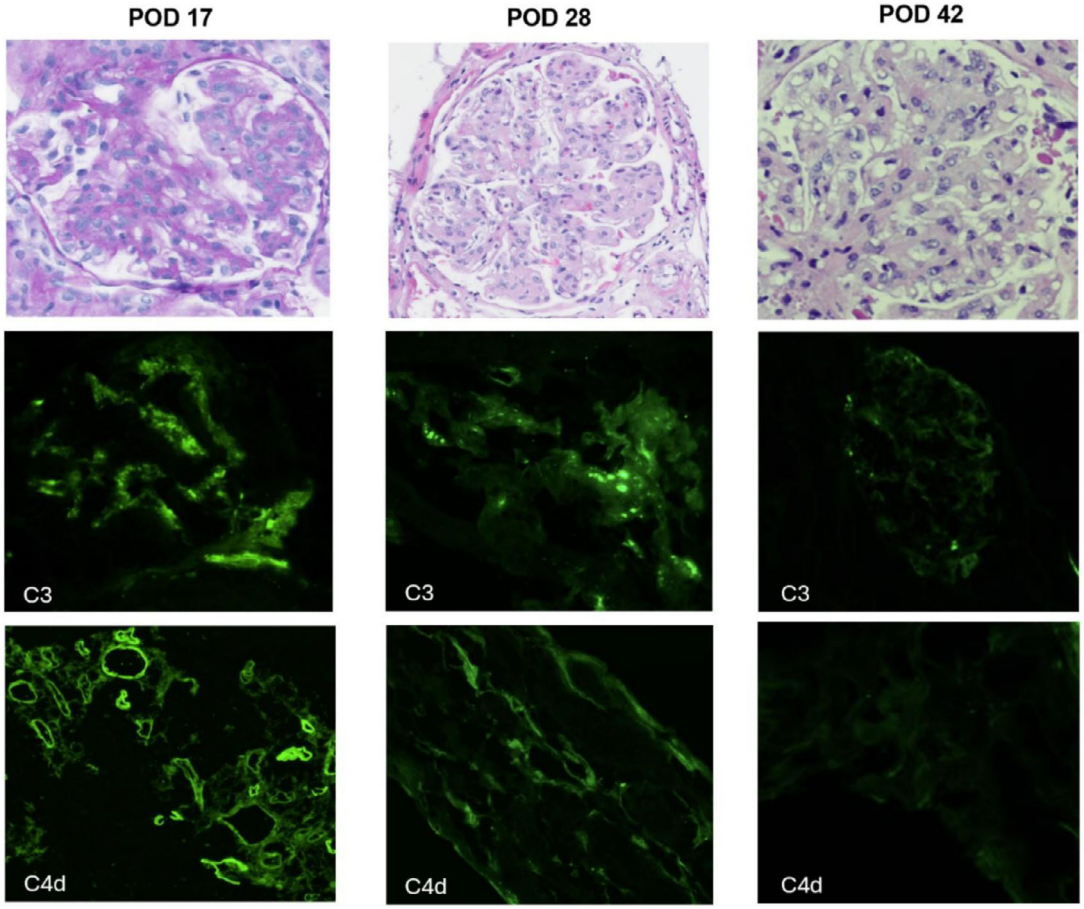


Figure 1b. Biopsy results from POD 17 shows evidence of glomerulitis and mesangiolysis concerning for AMR, with evidence of diffuse C4d and C3 staining. POD 28 biopsy was concerning for ongoing AMR. By POD 42, there was evidence of resolving AMR with negative C4d and C3 staining on immunofluorescence, observed 3 weeks after initiation of PEG.

FIGURE 2 | P1.13

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and CD105, and they were negative for CD11b and CD45. The results showed that PB-MSCs demonstrated similar potential for cell proliferation and differentiation into various cell types when compared to BM-MSCs. To assess the immunomodulatory effects of BM-MSCs and PB-MSCs, we examined inflammation-related cytokine expression in human monocyte (THP-1) cells treated with phorbol-12-myristate-13-acetate (PMA) and lipopolysaccharide (LPS), and then indirectly cocultured with MSCs. As a result, M1 macrophage surface markers CD86 and CCR7 were decreased, and the M2 surface marker CD163 was increased, while pro-inflammatory markers TNF-α and IL-6 were decreased and the anti-inflammatory marker IL-10 was increased. Furthermore, xenogenic cytotoxicity assessment results revealed that when porcine cells were directly cocultured with PMA and LPS-differentiated THP-1 cells educated with MSCs, PB-MSCs demonstrated a greater reduction in xenogenic cytotoxicity compared to BM-MSCs, suggesting the need for further research. Therefore, the more effective immunomodulatory function of PB-MSCs compared to BM-MSCs has been confirmed. In addition, future research will elucidate the molecular mechanisms underlying the immunomodulatory functions of PB-MSCs and evaluate their potential clinical applications in xenotransplantation and cell-based therapies.

Funding: This research was supported by the Korea Institute of Toxicology (Grant No. BB-2504, RS-2023-KH135254), Republic of Korea.

P1.15 | Impact of the High Baseline Anti-A/B Antibody Titer on the Clinical Outcomes in ABO-Incompatible Living Donor Liver Transplantation

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Background: Recently, advances in desensitization protocols have made ABO-incompatible (ABOi) living donor liver transplantation (LDLT) feasible option in the era of organ shortage. Although multiple sessions of plasmapheresis can successfully reduce preformed anti-A/B titer prior to transplantation, the clinical significance of baseline anti-A/B antibody titers remains uncertain. The aim of this study is to investigate the clinical outcomes of ABOi LDLT in patients with a high baseline anti-A/B antibody titer.

Methods: A total of 50 patients who received ABOi LDLT from 2010 to 2020 at two tertiary hospitals were evaluated retrospectively. Two centers used a protocol composed of rituximab, plasmapheresis, and/or splenectomy. The patients were classified according to baseline anti-A/B titer (<1:256, $n = 88$ or ≥1:256, $n = 62$) and compared the clinical outcomes among these groups. Graft survival rates were calculated using the Kaplan–Meier method according to the groups.

Results: In the high baseline titer group, the number of plasmapheresis required to reach the target titer (1:16) was significantly higher (4.4 ± 2.2 sessions) than in the low baseline titer group (1.9 ± 1.2 sessions, $p < 0.001$). 14 (16.4%) patients in high baseline titer group and 7 (9.2%) patients in low baseline titer group experienced postoperative titer rebound to ≥1:32, ($p = 0.014$). The occurrence of both cellular rejection and antibody-mediated rejection did not show a significant difference ($p = 0.251$ and $p = 0.147$, respectively). The 1-,3-, and 5-year graft survival was not different among groups (high titer vs. low titer; 94.2%, 83.3%, and 59.0% vs. 92.1%, 86.3%, and 79.5%, $p = 0.326$). Multivariate analysis showed that high baseline anti-A/B titer and postoperative rebound titer did not adversely affect clinical outcomes after ABOi LDLT.

Conclusions: Although the patients with high baseline anti-A/B titer showed a higher tendency of postoperative antibody rebound, the baseline and rebound anti-A/B titer may not be as important factors for clinical outcomes of ABOi LDLT if appropriate desensitization is performed.

P1.16 | Suppressive Effect of Mycophenolic Acid on the Cytotoxicity of Human Macrophages Against Swine Endothelial Cells

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Objective: Macrophages have been suggested to be involved in xenograft rejection, but the effects of immunosuppressants at different stages of macrophage differentiation have not been fully elucidated. In this study, we investigated the suppressive effect of mycophenolic acid (MPA), which mainly targets lymphocytes, on the cytotoxic activity of human macrophages against swine endothelial cells (SECs).

Method: CD14-positive monocytes were isolated from peripheral blood mononuclear cells of healthy donors using mag-

Figure 2. Antibody rebound rise

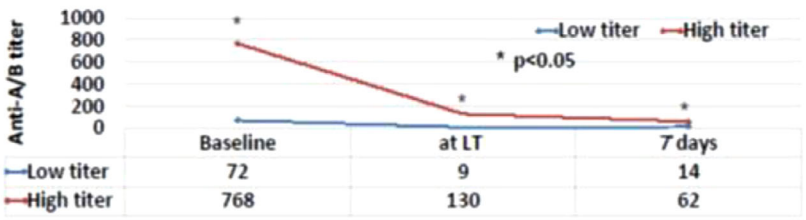


FIGURE 1 | P1.15

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netic activated cell sorting (MACS). The isolated monocytes were cultured with 100 ng/mL granulocyte-macrophage colony-stimulating factor (GM-CSF) for 7 days to induce differentiation into macrophages. Macrophages were then cocultured with SECs in the presence of MPA at concentrations of 2.5 or 5.0 μg/mL to evaluate its effect on cytotoxicity against SECs.

To assess the effect of MPA on the differentiation of monocytes into macrophages, 2.5 μg/mL MPA was added simultaneously with GM-CSF, and its effect on cytotoxic activity was evaluated. Cytotoxicity was measured using a water-soluble tetrazolium salt-8 (WST-8) assay. In addition, flow cytometry was used to assess changes in the expression of immunoglobulin-like transcript 4 (ILT4) and CD14, as well as cell viability.

Results: Addition of MPA after macrophage differentiation significantly reduced cytotoxicity compared to the control group, although a dose-dependent reduction was not observed (control 82.9%, MPA 2.5 μg/mL group 60.3%, MPA 5.0 μg/mL group 55.7%; control group vs. MPA 2.5 μg/mL, $p = 0.02$). Next, we examined the effect of MPA on macrophage differentiation. When MPA was added concurrently with GM-CSF and cultured for 7 days, cytotoxicity was markedly suppressed (control 94.2%, MPA 2.5 μg/mL 0.2%; $p < 0.001$). Flow cytometry analysis revealed that the expression of ILT4 and CD14 in the MPA-treated group gradually decreased over time compared to the control group, and that these ILT4/CD14-negative cells were not detectable, suggesting loss of cell viability during the differentiation process.

Conclusion: While MPA exhibited a mild inhibitory effect on cytotoxicity in fully differentiated macrophages with GM-CSF, it demonstrated a strong suppressive effect on cytotoxicity by inducing cell death during the macrophage differentiation stage.

P1.17 | Attitudes Toward Pig Islet Xenotransplantation for Type 1 Diabetes: A Scoping Review

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Background: Over 8 million people globally have type 1 diabetes. Islet allotransplantation offers an alternative to insulin therapy but is constrained by donor availability. Genetically modified pig islet transplantation presents a potential solution, yet understanding stakeholder attitudes is crucial before clinical adoption.

Methods: This scoping review followed Joanna Briggs Institute methodology and PRISMA guidelines. CINAHL, EMBASE, PubMed, PsychINFO, and SCOPUS were searched for studies examining attitudes toward pig islet xenotransplantation among patients, healthcare workers, and other stakeholders.

Results: From 199 sources, 16 met eligibility criteria, spanning ten countries from 2003 - 2023. The 23 780 participants included 1535 (6.5%) patients/family members, 216 (0.9%) healthcare workers, and 22 029 (92.6%) students. Attitudes were generally positive but declined sharply in some studies when risks, such as viral transmission, were disclosed.

Conclusion: Despite overall positive attitudes, risk perception significantly influences acceptance of islet xenotransplantation. The predominance of quantitative research highlights a need for qualitative studies and validated survey instruments to enhance understanding and comparability of stakeholder perspectives.

P1.18 | Optimizing aCD154-Free Immunosuppression and Monitoring in QKO Pig-to-Monkey Islet Xenotransplantation

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Background: Despite the promise of pancreatic islet allotransplantation for diabetes treatment, its clinical application is limited by the shortage of suitable human donors. Genetically modified porcine islets offer a viable alternative. While immunosuppressive therapies that block the CD40/CD154 pathway have shown efficacy in preclinical xenotransplantation studies, their translation to clinical practice remains challenging due to safety concerns, particularly with anti-CD154 agents. In this study, we explore the use of clinically applicable immunomodulatory regimens that exclude anti-CD154, alongside immune monitoring, in a pig-to-nonhuman primate islet xenotransplantation model.

Methods: Cynomolgus monkeys were rendered diabetic with streptozotocin and received an intraportal infusion of 50 000–100 000 IEQ/kg of islets from GGTA/CMAH/4GalNT2/iGb3s gene knockout (QKO) pigs. All recipients received a base immunosuppressive regimen of anti-thymocyte globulin (ATG) induction and tacrolimus, sirolimus and mycophenolate mofetil (MMF) maintenance. Cohort 1 received the base regimen only; Cohort 2 also received abatacept; Cohort 3 additionally received crovalimab and rituximab.

Results: Islet graft survival was 35 and 25 days in Cohort 1, 140 days in Cohort 2, and 217 days in Cohort 3. All recipients exhibited a significant decrease in peripheral blood T lymphocytes following transplantation. T cells began to recover early by post-transplantation day (POD) 14, with effector memory $CD8^+$ T cells increasing more prominently than other T cell subsets. In addition, no significant elevations in plasma IL-6 or TNF levels were observed. Recipients in Cohort 1 and Cohort 2 showed significantly elevated levels of membrane attack complex (MAC) and C5a, along with substantial infiltration of $CD3^+$ T cells and $CD68^+$ macrophages in the xenografts. In contrast, Cohort 3 recipients showed no significant changes in MAC or C5a levels and exhibited reduced infiltration of $CD3^+$ and $CD68^+$ cells. The proportion of xenograft-derived cell-free DNA (xdcfDNA) was markedly elevated at POD 7 and declined to low, by Day 28 in Cohorts 1 and 2. In Cohort 3, xdcfDNA levels remained consistent at baseline and stable.

Conclusions: Our experience demonstrates that the effector memory CD8+ T cells and activated macrophages may be detrimental to long-term graft survival, and the need for

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sustained immunosuppression to prevent immune responses in islet xenografts. These findings suggest the clinical applicability of a CD154-free immunomodulatory strategy in islet xenotransplantation using QKO porcine islets.

Funding: This research was supported by a grant of the Korea Health Technology R&D Project through the Korea Health Industry Development Institute (KHIDI), funded by the Ministry of Health & Welfare, Republic of Korea (grant number: RS-2023-KH136749).

P1.19 | Islet Recovery and Functionality Retained After 24-h Static Cold Storage of Neonatal Porcine Pancreas in University of Wisconsin Solution

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Introduction: Islet transplantation can restore glycemic homeostasis in a subset of patients with type 1 diabetes but is limited by a shortage of human donors and the need for multiple donors per recipient. Neonatal porcine islets (NPIs) are a promising alternative, providing a scalable, reproducible source of islets from genetically modified pigs. Porcine pancreases could be transported to Good Manufacturing Practice (GMP) facilities specialized for NPI isolation. Static cold storage with University of Wisconsin (UW) solution is the standard for pancreas preservation, supporting viability for up to 12 h. Extending this duration to 24 h could expand xenotransplantation applications by allowing longer transport times. This study examines the impact of 24-h cold storage of neonatal porcine pancreases (2–5 days old) in UW solution on islet recovery and NPI function.

Methods: Pancreases from both male and female neonatal piglets ($n = 16$/treatment) were either digested immediately post-surgery (fresh) or stored in UW at 4°C for 24 h before islet isolation. After a week of culture, islets were assessed for glucose-stimulated insulin secretion (GSIS), DNA content, insulin content, and morphology. Functionality was further tested by transplanting 3000 NPIs under the kidney capsule of male and female streptozotocin-induced diabetic B6.129S7-Rag^{1tm1Mom}/J mice (Fresh: $n = 24$; UW: $n = 25$) post-culture. Data expressed as mean ± SEM.

Results: Cell recovery based on total DNA content per pancreas was similar in both groups (Fresh: 90.50 ± 10.28 μg vs. UW: 82.45 ± 6.02 μg; p = ns). In contrast, the total cellular insulin content per pancreas was significantly higher in the fresh group (8.82 ± 1.29 μg vs. UW: 3.84 ± 0.49 μg; $p < 0.01$). The proportion of β-cells (Fresh: 22.08% ± 1.76% vs. UW: 16.41% ± 1.26%; $p < 0.05$), and calculated β-cell recovery was significantly lower in UW (Fresh: $2.60 \times 10^6 \pm 0.23$ vs. UW: $1.85 \times 10^6 \pm 0.15$; $p < 0.05$). The in vitro glucose-stimulated insulin secretion remained consistent, with similar percent insulin release at low (Fresh: 0.049% ± 0.006% vs. UW: 0.053% ± 0.007%; p = ns) and high glucose concentrations (Fresh: 0.098% ± 0.014% vs. UW: 0.111% ± 0.015%; p = ns). Stimulation indices were comparable, at 2.00 ± 0.18 and 2.12 ± 0.08 (p = ns) in the fresh and UW groups, respectively. The reversal rates of diabetes in the transplanted mice were comparable between fresh and UW, suggesting that UW treatment does not impair in vivo function.

Conclusions: 24-h hypothermic storage of neonatal pig pancreases in UW solution did not impair NPI recovery. In vitro and in vivo function was not impaired despite significantly lower insulin content, β-cell composition, and number. This supports the feasibility of transporting porcine pancreases to expand xenogeneic donor islet availability.

P1.20 | Nanodelivery of IL33 Locally Amplifies the ILC2 and Treg to Protect the Graft in Islet Transplantation

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Introduction: Islet transplantation is an effective treatment for severe diabetes mellitus. However, immune rejection has always been an inevitable problem in transplantation, which can lead to islet graft failure. Therefore, finding effective enhanced anti-immune rejection strategies has become an urgent concern in the field of islet transplantation. Our previous study showed that IL33 can protect islet grafts as an immunomodulator by expanding ILC2 and Treg. Here, we designed chitosan nanoparticles loaded with IL33, which can locally sustain the release of IL33, and co-transplanted with islets to regulate the immune microenvironment and improve islets' survival and function.

Methods: Chitosan nanoparticles loaded with IL33 (named as CS @ IL33) were synthesized by ion polymerization and characterized by an electron microscope. The encapsulation rate of IL33 was detected by the Elisa kit. Since IL33 can directly bind to the ST2 protein, which is located on the membrane of Treg and ILC2 cells, the CS @ IL33 labeling with cy5.5 was used to show the binding of nanoparticles and cells, which was visualized by confocal microscopy. The CS @ IL33 and islets were co-transplanted under the kidney capsule of streptozotocin-induced diabetic mouse. The number and proportion of ILC2 and Treg cells in the transplanted area were measured by flow cytometry and immunofluorescence. The blood glucose and body weight of mice were detected every other day. The islet function was evaluated by oral glucose tolerance experiment and the immunohistochemistry of insulin.

Results: TEM images showed that the diameter of spherical particles was around 100 nm, and the results of Elisa revealed that the packing rate of CS @ IL33 was 99.14%. The fluorescent images from Confocal microscopy demonstrated that the CS @ IL33 nanoparticles were bound uniformly to the Treg cell membrane. The flow cytometry and immunofluorescence showed that both CS @ IL33 and intraperitoneal injection of IL33 effectively amplified the ILC2 and Treg in the transplantation location. The results from the in vivo transplantation model showed the CS @ IL33 nanoparticles could better protect the islets without causing any other systemic inflammation.

Conclusion: We synthesized CS @ IL33, which can specifically amplify Treg and ILC2 in the graft location and reduce the immune rejection. The co-transplantation of CS @ IL33 and islets may regulate the local immune microenvironment and help to enhance the graft function, which may suggest a more effective way to improve the results of islet transplantation.

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Funding: This work was supported by the National Natural Science Foundation of China (Grant No.: 82272102).

P1.21 | Supply Chain Flexibility for a Porcine Beta Cell Replacement Therapy to Treat Type 1 Diabetes

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The porcine pancreas is a promising source for a scalable and standardized beta cell replacement therapy. A flexible supply chain will be key to optimizing time and cost efficiency and to make islet transplantation available to the global diabetes patient population. Pancreas source tissue cold ischemia time (CIT) and porcine islet cryopreservation were explored. Animal studies comply with Directive 2010/63/EU. Piglets were obtained at gestational day 109 to 115 or neonatal day 1. Pancreas tissue was stored at 4–8°C with variable intervals of CIT and 1 h as reference. Standard isolation medium, Institut Georges Lopez-1 Organ Preservation Solution (IGL-1), HypoThermosol (HTS), University of Wisconsin solution (UW), and Histidine-tryptophan-ketoglutarate solution (HTK) were used as pancreas preservation media. Endocrine cell isolation was performed via collagenase digestion and counterflow elutriation. On Day 1 and after 1 week of culture, samples were taken to determine cell numbers, viability, and composition. Cryopreservation was performed on fresh pancreas tissue, and cells were frozen in Cryostor 10 on Days 2 and 7 after isolation. In vivo maturation and diabetes reversal were assessed in nude and NSG mice under the kidney capsule. A clear negative correlation exists between CIT and beta cell yield ($p < 0.0001$). Beta cell yield was stable after 3 h of CIT, but 12 h or more resulted in a 40%–50% drop. No impact was seen on cell viability or in vivo maturation in nude mice, with only a minimal negative impact on beta cell purity ($p = 0.03$). Cell yields and quality were compared for CIT ≥ 12h in HTK, HTS, IGL-1, and UW and compared with the reference control. An average beta cell yield of 7.7 × 106 beta cells per g pancreas was obtained for the 1 h CIT control, 5.0 × 106 for HTS, 5.9 × 106 for IGL-1, and 3.2 × 106 for UW, with only 2.0 × 106 beta cells per g pancreas for HTK. Beta cell purity was 36.8% for the control, 42.0% for the HTS, 37.5% for IGL-1, 31.7% for IGL-1, and 30.7% for HTK. Cryopreservation on Day 2 allows higher beta cell recoveries compared to cryopreservation after 1 week of culture with a viable beta cell recovery of 114% ± 36% for fresh cells cultured for 1 week, 84% ± 28% for cells frozen on Day 2 after culture and 41% ± 15% for cells frozen after 1 week of culture. Purity with respect to beta cell composition and in vivo graft maturation and diabetes reversal in NSG mice was comparable for fresh and cryopreserved cells, irrespective of freezing day. Nevertheless, cryopreservation still results in reduced cell yields with a drop in beta cell recovery from 11 ± 5 × 106 to 6 ± 2 × 106 per gram pancreas for fresh and Day 2 cryopreserved cells, respectively. Pancreas CIT limits porcine beta cell replacement therapy flexibility, while cryopreservation does allow supply chain flexibility. Despite reduced yields, it enables cells to be produced at a centralized facility and preserved for future patient treatment in clinics worldwide.

P1.23 | Targeting the Activity of Beta Cells by Membrane Surface Redox Regulation for Islet Transplantation

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Introduction: Islet transplantation offers hope for type 1 diabetes treatment. While hypoxia-induced damage is one of the most important issues contributing to the graft loss in the early stage of islet transplantation. Given the weaker expression of the antioxidant system in beta cells compared to other cells, they are more susceptible to hypoxia-induced damage. Therefore, enhancing the resistance of beta cells to hypoxia damage has become a top priority. In this study, we designed fusogenic liposomes, termed Fulips, to regulate the survival and function of islet cells. The Fulips, equipped with 2,2,6,6-tetramethylpiperidine (TEMP) groups, decorated the membrane surface of islet cells after fusion, shielding them from oxidation-induced loss of activity by acting as ROS 'decoys' in the extracellular milieu. Additionally, ROS scavenging induced changes in molecular magnetism, quantitatively measurable through MRI relaxation time alterations.

Method: Islet cell activity was evaluated by flow cytometry, redox status by DCFH-DA fluorescence, and insulin levels post-glucose stimulation by ELISA. Neonatal porcine islets were extracted and subsequently transplanted under the renal capsule of NCG mice, serving as our standard transplantation model. Islet cells viability in grafts was assessed via TUNEL experiments on fixed pathology slices, while ROS content was evaluated using DHE pathological staining. All phantoms and animal MRI studies were performed on a Bruke 7.0 T small animal MRI scanner.

Results: In vitro, Fulips labeled cyanine 5.5 uniformly bound to islet cells membranes as observed by confocal microscopy. Under hypoxic conditions, the viability of islet cells was significant higher when cocultured with Fulips than cultured alone (89.50 ± 2.23% vs. 67.60 ± 2.79%). Flow cytometry results showed a reduction in intracellular ROS levels of hypoxia islets cells after liposome treatment (79.20 ± 2.78% vs. 55.70 ± 2.47%). Hypoxia reduced insulin secretion by beta cells, while Fulips treatment reversed this trend. The islet cells cocultured with Fulips were transplanted to the renal capsule of NCG mice, 3 days post-transplantation, magnetic resonance imaging indicated a reduction in T2 signal value (13419 ± 1120 vs. 5499 ± 408) and shortened T2 relaxation time (119.0 ± 19.91 vs. 43.80 ± 3.09) in the grafts, which suggested reduced ROS levels and increased islet survival. TUNEL staining confirmed the results, that there was a reduction of apoptotic cells in liposomes cocultured grafts compared to the control ones. DHE staining further demonstrated decreased ROS levels in liposome-treated grafts.

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Conclusion: This work underscored the role of the redox status in bridging the activity of islet cells in situ and the treatment response for type 1 diabetes therapy. Ultimately, targeting the redox metabolism of translated cells by chemical approaches could realize modulation and molecular imaging of treatment response in transplantation therapy.

Funding: This work was supported by the Natural Science Foundation of China (Grant No. 82272102), the Natural Science Foundation of Hunan Province, China (Grant No. 2025JJ50714), and the Natural Science Foundation of Changsha, China (Grant No. kq2403066).

P1.24 | Establishing a Xenotransplantation Program in Armenia to Enhance Surgical Training and Explore the Role of Polyamines in Liver Graft Rejection

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Introduction: Liver transplantation continues to face challenges of acute and chronic rejection, especially in xenotransplantation where interspecies immune barriers are profound. Polyamines—putrescine, spermidine, and spermine—are emerging as key regulators of immune responses, oxidative stress, and tissue regeneration. While their cytoprotective and immunomodulatory roles have been explored in oncology and aging, their application in transplant immunology remains largely untapped.

Methods: We propose a translational xenotransplantation model using porcine liver grafts to investigate polyamine dynamics during immune activation. The study will quantify tissue-specific and systemic polyamine levels during acute rejection and tolerance phases. Targeted interventions-such as dietary spermidine supplementation or modulation of polyamine-metabolizing enzymes-will be evaluated as adjuncts to standard immunosuppression. Molecular assays, including cytokine profiling, regulatory T-cell quantification, and hepatocyte autophagy markers, will be used to determine immunologic impact.

Results: Preclinical data indicate that elevated spermidine levels are associated with enhanced graft tolerance and decreased oxidative injury during liver ischemia-reperfusion. Initial findings from cardiac and renal xenografts also support the hypothesis that polyamines modulate cross-species immune responses. We anticipate that polyamine supplementation may reduce pro-inflammatory cytokine release, increase Treg populations, and promote hepatocyte regeneration, resulting in prolonged xenograft survival.

Conclusion: By integrating polyamine profiling with surgical and molecular innovation, this project positions polyamines as both biomarkers and therapeutic agents in transplantation. Their selective immunomodulatory action offers a potential paradigm shift from broad immunosuppression to metabolic immunoregulation. This approach could improve xenograft acceptance, reduce drug toxicity, and enhance long-term transplant outcomes, particularly in low-resource settings aiming to expand xenotransplantation programs.

P1.25 | Unravelling the Dynamic Process of NPI Maturation At the Single Cell Transcriptome Level

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Introduction: The in vitro culture period is essential for the maturation of neonatal porcine islets (NPIs) to achieve the properties required for successful transplantation. The composition of the media has a significant impact on the properties of the NPI product. Knowledge of the molecular signature of NPIs during the in vitro maturation period is essential for targeted improvement.

Methods: scRNA-seq analysis of NPIs at different culture days was used to study the molecular profiles of NPIs at single-cell resolution. In addition, beta-cell enriched samples of porcine pancreas from late embryonic, pre-weaning, and post-weaning animals were included in the analysis.

Results: A porcine beta-cell transcriptome atlas of the early postnatal period was established. The single-cell transcript profiles of NPIs during culture revealed that the in vitro maturation process is a highly dynamic process. Not only was a shift in the cell type composition during the culture period observed, but also changes in the transcriptome signature within the cell type cluster.

Conclusion: The in vitro maturation of NPIs revealed a high degree of plasticity, which was reflected in their transcriptome profile.

P1.26 | Genetic Diversity and Population Structure of GTKO-Engineered Xeno Pigs Across Generations Based on SNP Analysis

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Xeno pigs, crossbreeds of Chicago miniature pigs and Landrace pigs genetically engineered to lack the α1,3-galactosyltransferase (GTKO) gene, have been established as effective models for xenotransplantation by mitigating hyperacute immune rejection. In this study, the genetic diversity of Xeno pigs (generations 7–11) was assessed in comparison to control groups, including Massachusetts General Hospital (MGH) pigs (positive control for hematopoietic cell and solid organ transplantation research), Landrace (LR; negative control), and Yorkshire × Landrace (Y/L) pigs, using 67 283 single nucleotide polymorphisms (SNPs). Principal component analysis (PCA) revealed that Xeno pigs formed a distinct genetic cluster, clearly separated from MGH, LR, and Y/L breeds. Linkage disequilibrium (LD) analysis demonstrated a progressive increase in LD within the Xeno pig population across generations, while the LD size difference between Xeno pigs and conventional breeds remained substantial, indicating limited recombination and introgression from external genetic lines. Estimates of effective population size (Ne) further corroborated this trend. Genetic distance metrics and phylogenetic analyses confirmed significant divergence and low genetic relatedness between Xeno pigs and the other breeds. Furthermore, admixture

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analysis, with an optimal K value of 3 determined by the lowest cross-validation (CV) error, demonstrated that Xeno pigs maintained a distinct ancestral genome with minimal admixture from conventional breeds. These findings underscore the necessity of continuous genomic surveillance to ensure the maintenance of xenotransplantation-relevant traits and genetic homogeneity in the Xeno pig population through selective breeding.

Funding: This study was supported by the RDA Fellowship Program of the National Institute of Animal Science, Rural Development Administration, Republic of Korea. This study was conducted with the support of the Korea Health Industry Development Institute (KHIDI) Xenograft Research and Development Project (Project number HI23C0417) funded by the Ministry of Health and Welfare.

P1.27 | Preliminary Results of Obtaining Designated Pathogen-Free Pigs in a Pig Facility for Xenotransplantation in Brazil

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Introduction: In 2024, the first Brazilian pig facility (PF) was inaugurated with the aim of producing genetically modified pigs for xenotransplantation. This study aims to report the preliminary results of the first standardization procedure to produce DPF pigs at the Xeno Brazil PF, located in São Paulo-SP, Brazil.

Methodology: Embryos produced by in vitro fertilization were transferred to previously synchronized non-SPF recipient sows via laparotomy. The piglets were delivered by hysterectomy in the surgical center in the clean area of the PF. After removal, the uterus was immersed in a waterlock bath containing an antiseptic solution (Virkon at 10%) and opened in an adjacent neonatology room for the birth of three piglets. Artificial lactation was carried out without colostrum (Denkapig – 450g/L for the first 5 days and 250g/L until 30 days). To determine the DPF status of the piglets and the sow, samples of whole blood, serum, feces, nasal and oral swabs, and tissue (lung, diaphragm, and heart) were collected. The piglets and sow samples were pooled and tested for 71 pathogens using a molecular panel (qPCR) based on studies in the literature on xenotransplantation. Surface swab samples were collected from the operating room, waterlock, neonatology room, piglet incubators, milk preparation room, materials storage, and dressing areas after sanitization (neutral detergent, 0.2% sodium hypochlorite, and 1% virkon) and before stocking the animals, and tested for four environmental pathogens. The choice of pathogens tested was based on the scientific literature, as well as considering the reality of Brazilian pig health, since Brazil does not yet have its specific legislation for xenotransplantation.

Results: The sow was positive for seven pathogens (Campylobacter spp., Mycoplasma spp., Mycoplasma hyorhinis, Pasteurella multocida, Salmonella enterica, Streptococcus spp., and PCV2). The piglets were negative for all the evaluated pathogens (Table 1). None of the pathogens tested in the PF environment were detected (Table 2).

Table 1. Results of the pathogens analyzed in collected samples of whole blood, blood serum, swabs (nasal, oral, rectal) and tissues (lung, diaphragm and heart) of the sow and the piglets born in the pig facility.

PATHOGENS ANALYZED	SOW #24021	PIGLETS
Pathogens with double negative results*	Negative	Negative
Campylobacter spp.	Positive	Negative
Mycoplasma spp.	Positive	Negative
Mycoplasma hyorhinis	Positive	Negative
Pasteurella multocida	Positive	Negative
Salmonella enterica	Positive	Negative
Streptococcus spp.	Positive	Negative
Porcine Circovirus Type 2 (PCV2)	Positive	Negative
Total of analyzed pathogens	**71**	**71**
Total of positive pathogens	**7**	**0**

*** Pathogens with double negative results:** *Anaplasm spp. / Ehrlich spp., Aspergillus fumigatus, Bartonella spp., Betacoronavirus spp. , Bordetella bronchiseptica, Brucella spp., Chlamydophila spp., Clostridium difficile, Clostridium perfringens, Cryptosporidium spp., Eimeria spp., Giardia spp., Lawsonia intracellularis, Leptospira spp., Mycobacterium spp., Neospora caninum, Parainfluenza virus type 1, Rhodococcus equi,* Rotavirus A, *Salmonella spp., Sporothrix spp., Trypanosoma cruzi,* Saint Louis Encephalitis Virus, Eastern Equine Encephalomyelitis Virus, Venezuelan Equine Encephalomyelitis Virus, West Nile Virus, Influenza Virus, *Actinobacillus pleuropneumoniae, Actinobacillus suis, Bacillus anthracis, Brachyspira hyodysenteriae, Campylobacter coli, Campylobacter jejuni, Clostridium botulinum, Clostridium chauvoei, Clostridium novyi,* Coronavirus porcine, Encephalomyocarditis virus, *Erysipelothrix rhusiopathiae, Escherichia coli (O157:H7),* Hepatitis E, *Listeria monocytogenes, Mycoplasma hyopneumoniae, Mycoplasma hyosynoviae, Mycoplasma suis,* Porcine adenovirus, Porcine cytomegalovirus, Porcine parvovirus 1, Porcine parvovirus 2, Porcine parvovirus 3, Porcine parvovirus 4, Porcine parvovirus 5-7, Porcine rotavirus B, Porcine rotavirus C, SARS coronavirus, *Staphylococcus aureus* - Methicillin-Resistant, *Staphylococcus hyicus, Streptococcus porcinus, Streptococcus suis,* Transmissible gastroenteritis, *Trichinella spiralis, Yersinia enterocolitica, Yersinia spp.*, Porcine Cytomegalovirus (PCMV).

FIGURE 1 | P1.27

Conclusion: The method to obtain DPF piglets and the sanitization of the PF have proved to be efficient so far. Although the sow tested positive for seven pathogens, none of the 3 piglets born were positive. New piglet samples will be taken at 2 months of age to confirm their DPF status. Prior standardization of the techniques for obtaining DPF pigs facilitates the training of facility staff, as well as optimizing techniques and the use of resources, reducing costs, and minimizing errors when obtaining genetically modified pig clones for xenotransplantation.

Funding: This work was supported by the São Paulo Research Foundation (FAPESP) – grant number 21/11872-5, the National Council for Scientific and Technological Development (CNPq) – grant number 444075/2023-2, and the Água Branca Pig Farm, Itu, São Paulo, Brazil. Biotec Group, Manaus, Brazil.

P1.28 | Comparison of Morphological Quality of Fetal Fibroblasts FSED in Nuclear Transfer for Swine Embryo Production for Xenotransplantation

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Table 2. Results of environmental pathogens tested in the facility's clean area.

Clean Area Locations Tested	Facultative Aerobic Bacteria	Yeast and Mold	Enterobacteriaceae	Coagulase positive *Staphylococcus aureus*
Locations Tested *	<1 CFU/cm²	<1 CFU/cm²	<1 CFU/cm²	<1 CFU/cm²

* **Locations tested**: Ceiling of the milk preparation room, wall of the milk preparation room, milk preparation room floor, milk preparation room bench, material storage room ceiling, material storage room wall, floor of the material storage room, ceiling of the neonatology room, wall of the neonatology room, floor of the neonatology room, incubator 1 in the neonatology room, incubator 2 in the neonatology room, incubator 3 in the neonatology room, waterlock bath, dressing area ceiling (Male), dressing area wall (Male), dressing area floor (Male), ceiling of the dressing area (Female), dressing area wall (Female), dressing area floor (Female), ceiling of the sterilized materials exit area, wall of the sterilized materials exit area, floor of the sterilized materials exit area, surgical room ceiling, surgical room wall, surgical room floor.

**CFU/cm2: Colony Forming Unit per Square Centimeter, Limit of quantification for each microorganisms analyzed = 1.

FIGURE 2 | P1.27

Centro de Pesquisa sobre o Genoma Humano e Células Tronco - USP. Faculdade de Medicina - USP.

The production of cloned pigs via somatic cell nuclear transfer (SCNT) is a promising approach to generate organs suitable for xenotransplantation. The quality of donor cells, particularly oocytes and fetal fibroblasts (FF), plays a crucial role in the success of cloning procedures. This study aims to compare the morphological quality of FF used in SCNT and how it influences embryo development. FF were cultured until 80%–95% confluence and evaluated based on morphological criteria: cell shape (rounded), size (<15 micrometers), and population homogeneity. Each parameter was scored on a scale from 0–1 (poor), 2–3 (medium), to 4–5 (good), and a mean score was adopted. Only medium- and good- scored cells were used for cloning. Additionally, cumulus cells (CC) were employed as a control group to assess the impact of cell type on cloning efficiency, and parthenogenetic (PTN) embryos were used as second control to compare oocyte quality. Oocytes were also evaluated using the same morphological scoring criteria. Post-reconstruction and chemical activation, embryos were cultured in vitro, and developmental competence was assessed by cleavage and blastocyst formation rates on Day 7. Medium- and high-scores were compared, and data were analyzed by chi-square, considering $p < 0.05$ as significant. Notably, the PTN group exhibited difference in embryo development based on oocyte quality, with high-scored oocytes resulting in higher cleavage (111/116; 95.7%) and blastocyst rates (78/116; 67.2%), compared to medium-scored oocytes (20/25; 80.0% and 9/25; 36.0%), respectively ($p < 0.005$). No difference was observed between high- and medium-quality FF: cleavage rates were 120/193 (62.2%) versus 60/106 (56.6%), and blastocyst rates were 27/193 (14.0%) versus 18/106 (17.0%). Similarly, no significant differences were found when comparing CC groups: cleavage rates of 65/106 (61.3%) versus 26/46 (56.5%), and blastocyst rates of 18/106 (17.0%) versus 6/46 (13.0%). Likewise, no significant differences were observed between FF and CC. These findings suggest that FF morphology did not markedly impact developmental success beyond the influence of oocyte quality and consequently does not appear to influence swine SCNT success. Nevertheless, cells with optimal appearance, appropriate size, and homogeneous populations are preferred because they facilitate the micromanipulation procedures. Maintaining cells at 80%–95% confluence is indicative of cell cycle arrest in the G1/G0 phase, which likely contributes to consistent results across experiments. These findings highlight that, within the parameters tested, selecting high-quality donor cells based on morphology may be less critical than ensuring proper cell cycle status. Still, choosing healthy, well-characterized donor cells remains important for optimizing cloning efficiency in xenotransplantation applications.

P1.29 | A Rational Design Method for Tissue Dissociation Enzyme Optimization (RDMTDE Method)

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Enzyme-mediated cell isolation methods require tissue dissociation enzymes (TDEs) that contain optimal amounts of C. histolyticum collagenase and neutral protease activity. Salt-precipitated C. histolyticum culture supernatants were first sold as lyophilized products in the 1960s. These traditional collagenase products required researchers to pre-qualify new lots in an application before purchase. In the 1990s, reverse engineering of "good lots" of collagenase for specific applications led to the use of purified enzymes that improved product consistency but did not address TDE optimization. A mechanistic model for collagenase-protease mediated cell isolation reported in 2011 proposed that collagenase only degrades collagen, which leads to thinning of the collagen jungle found in the extracellular matrix (ECM), that in turn leads to exposure of protease-sensitive sites on ECM cell-anchoring proteins. Once proteases cut these anchoring proteins, cells are released from the tissue. The RDMTDE method is derived from the above model. It simplifies optimization of TDEs to one variable: neutral protease activity. The method requires that excess collagenase activity must be present in an enzyme-mediated cell isolation method that consistently provides sufficient yields of functional, viable cells, and the maximal amount of neutral protease activity required for cell recovery is ≤ the activity in Worthington's Type 1 Collagenase. The method has two requirements. First, you must determine the collagenase used in your current method (Reference Method). Second, it is necessary to use purified and characterized collagenase and neutral proteases to define the enzyme activities required for

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cell isolation. The three steps of the process are: (1) Estimate the mass of collagenase in the enzyme solution used in the Reference Method. (2) Prepare solutions of collagenase-protease mixtures where the mass of collagenase is fixed and the neutral protease activity is titrated to assess its effectiveness to release cells from tissue. A TDE Excel Calculator simplifies preparation of these solutions by entering the mass of collagenase (same for all solutions), volume of enzyme solution, and the amount of neutral protease activity expressed as the percentage found in Worthington Collagenase Type 1. The output from the calculator defines the amount of PD Collagenase 800 and Collagenase Gold to prepare each solution. (3) Isolate cells using the above solutions and compare the results to those using your current collagenase product. The results from these isolations should be comparable to the Reference Method. If not, purified clostripain can be added to improve cell yields. The method's primary benefit is defining the TDE composition required for cell isolation. This method can be used by any user of traditional or purified collagenase products. Once the enzyme composition is defined, further improvement can be made to determine the effect of these modifications on functional cell yields.

P1.31 | An Ancillary Material Supplier's Perspective on ISO 20399:2022

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Ancillary material (AM) users are responsible for assessing the risks of AMs by qualifying the AMs used in these processes. Complex AMs, such as tissue dissociation enzymes, can profoundly impact cell-gene therapy (CGT) products. USP <1043> laid out a framework for a risk-based approach to qualify AMs. More recently, the ISO 20399:2022 AM Standard recommended that AM suppliers collaborate with AM users to support the qualification of their products for use in CGT manufacturing processes. The poster highlights the importance of the supplier-user relationship in managing the risks of AM used in CGT products. The USP chapter and ISO Standard emphasize the importance of AM qualification in understanding how the AMs impact the CGT product's critical quality attributes. Qualification includes understanding how inherent AM variability impacts the CGT final product. However, AM specifications are often set by the supplier with limited input from AM users. Furthermore, AM variability is typically not part of suppliers' product documentation. AM users must request this information and rely on the cooperation of the AM supplier to understand the variables that may impact the CGT product. The solution to this gap is for AM suppliers and users to develop a strong working relationship that enables users to apply the Quality by Design (QbD) framework to their manufacturing process. AM suppliers can work to mitigate risks identified by AM users to identify the AM critical material attributes. An example of potential responses to major risk categories is shown in the table below.

Risk Categories	AM User Concern	AM Supplier Response
Safety	Animal origin materials	Develop a robust raw material qualification program based on the verifiable traceability of all components.
Consistency	Potency variability	Develop a deep understanding of the AM's manufacturing critical process parameters. Identify and control critical material attributes for robust CGT manufacturing. Utilize all appropriate validated characterization methods.
Sustainability	Reliability of supply	Provide resources to plan for anticipated demand expectations. Invest in a robust and reliable supply chain for all process inputs

The mechanism by which the AM supplier responds to the user concerns is through transparent, detailed documentation that supports the AM user qualification plan. This process starts with proactive efforts by both parties to build a trusted working relationship. While the AM user is ultimately responsible for the qualification of process inputs and manufacturing control, AM suppliers can better facilitate this endeavor by partnering with users to ensure adequate quality system controls are in place and appropriate supporting documentation is provided to users. AM suppliers should be willing to build relationships with AM users that facilitate qualification as trusted suppliers to meet the regulatory scrutiny of CGT manufacturing processes.

P1.32 | Quality Systems Driving Innovation: Implementing QMS, QA, and QC for Genetically Modified Pigs in Xenotransplantation

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Xenotransplantation is emerging as a transformative solution to the global organ shortage. However, the path from laboratory innovation to clinical application requires not only robust genetic engineering but also rigorous implementation of quality systems. This study presents the experience of XenoBr, a Brazilian biotech start-up, in establishing and operationalizing a comprehensive quality management system (QMS) to ensure the safety, reliability, and regulatory compliance of genetically modified porcine donors for xenotransplantation. A dual framework integrating quality assurance (QA) and quality control (QC) was implemented under GMP-aligned workflows. The QA strategy focused on structuring internal processes through standardized documentation, training, and an online document

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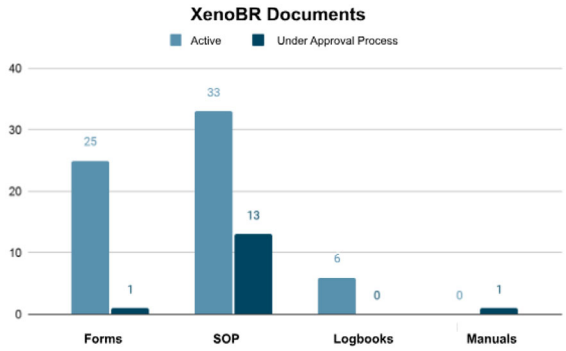


Figure 1. Bar graph from the Qualitfy platform showing document implementation in XenoBR.

FIGURE 1 | P1.32

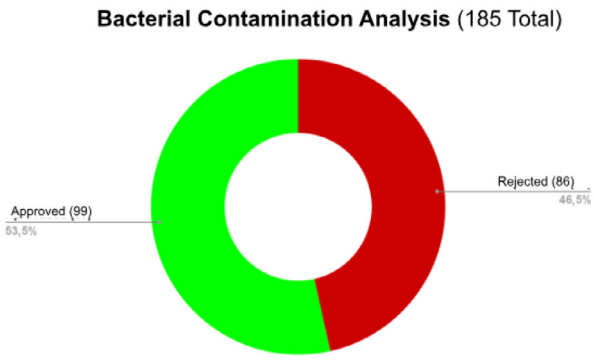


Figure 2. Pie chart of MicroSnap® test outcomes (approved vs. rejected samples).

FIGURE 2 | P1.32

and event management platform (Qualitfy) in line with ISO 9001 standards. A total of 64 documents - including SOPs, logbooks and traceability forms - were approved and implemented. QA also led the development of a Good Laboratory Practices (GLP) manual, which is now central to team training and audit readiness. In parallel, QC activities focused on biosafety, genetic stability, and microbial contamination. Microbiological monitoring used validated ATP bioluminescence assays (MicroSnap) and mycoplasma detection (MycoAlert) with confirmatory DAPI staining and PCR testing. While the majority of samples met safety thresholds (CFU <10; B/A <0.9), a subset revealed contamination (e.g., *Staphylococcus* coagulase-negative, Micrococcus spp. and elevated ATP signals on laboratory surfaces), prompting immediate corrective actions including retraining and protocol revision. In addition, a cytogenetic karyotype test was standardized to assess chromosomal integrity, with initial results confirming the presence of sex chromosomes and supporting the genomic stability of porcine fibroblast lines. Challenges such as early phase contamination underlined the importance of rigorous QA–QC interplay for data validity.

Funding: This work was supported by the São Paulo Research Foundation (FAPESP) – grant number 21/11872-5 and the National Council for Scientific and Technological Development (CNPq) – grant number 444075/2023-2.

P1.33 | Mapping the Evolution of Solid Organ Xenotransplantation Research: A Systematic Review

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INSERM, PARCC, Paris Institute for Transplantation and Organ Regeneration, Université de Paris Cité.

Introduction: Xenotransplantation has emerged as a promising solution to address organ shortage, generating numerous publications. To date, no studies have analyzed the dynamics of xenotransplantation research. We aimed to provide a systematic assessment of xenotransplantation publication activity.

Methods: A systematic literature search was conducted in PubMed, with a search period limited only with the end date, November 22, 2024. Publications written in English were included, with no limitation on study design or publication type. Eight independent reviewers screened the publications retrieved with a search. Studies on xenotransplantation of solid organs and islets of Langerhans from animals to humans, or perfusion with human blood and its derivatives, were included. For each included publication, following data were collected: (1) publication basic information, including title, date of publication, affiliated country of the first author; (2) publishing journal; (3) publication type; (4) organ studied; (5) donor species studied; (6) topics covered.

Results: Among 2944 publications identified, 706 met the inclusion criteria. Among these, 291 were original articles (41.2%), 290 (41.1%) reviews, 101 (14.2%) publications without original data, 11 (1.6%) case reports, 9 (1.3%) research letters, and 4 (0.6%) systematic reviews and meta-analyses. Publication activity displayed two

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peaks: first in the 1990s, driven by the advancements in genetic engineering, mainly attributed to publications with original data, and the second in the early 2020s following the first pig-to-human deceased recipient transplantation, marked by a predominance of reviews and publications without original data. The top five publishing countries were the USA with 340 (48.2%), Germany with 72 (10.2%), UK with 38 (5.4%), Sweden with 34 (4.8%), and China with 30 (4.2%) of publications. Xenotransplantation journal accounts for 19.7% of all publication activity, while six transplantation journals together accounted for 27.6%, and general medical journals for 5.4%. Islets of Langerhans were studied in 23.1% studies, and the most studied organs were the heart (21.2%), followed by kidney (17.1%), the liver (12.2%), and lung (6.2%). A total of 356 topics were identified and grouped into 20 thematic groups. The most represented thematic groups were rejection ($n = 170$), immune mechanisms ($n = 165$), challenges ($n = 118$), genetic engineering ($n = 115$), and current research and prospects ($n = 97$).

Conclusion: This study is the first systematic assessment of xenotransplantation research, underscoring its growing global interest and evolving focus areas. The low proportion of publications with original data underscores the crucial need for original studies to advance the field forward. The limited presence of xenotransplantation research in general medical journals highlights the importance of engaging a broader audience as xenotransplantation nears clinical trials.

P1.34 | Fabrication Strategy of Parathyroid Organoids and Their Applicability for Non-Clinical Primate Transplantation

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Introduction: Parathyroid transplantation is a cornerstone treatment for permanent hypoparathyroidism following thyroidectomy. However, autotransplantation faces limitations in tissue availability, while allogeneic transplantation is hindered by immune rejection (e.g., CD3+ T-cell infiltration). To address these challenges, parathyroid organoids (PTOs) have emerged as a promising alternative, leveraging their 3D structural and functional similarity to native tissue. This study reviews recent advances in PTO research and systematizes fabrication strategies.

Methods: Human Parathyroid Stem Cell-Derived PTOs: Hyperplastic parathyroid tissues were enzymatically digested using collagenase II and cultured in 3D Matrigel with conditioned media for four passages. Human-Induced Pluripotent Stem Cell (iPSC)-Derived PTOs: Skin-derived fibroblasts were reprogrammed into iPSCs, followed by a 20-day differentiation protocol to generate organoids. Tonsil-Derived Mesenchymal Stem Cell (TMSC)-Based PTOs: TMSCs were differentiated into parathyroid-like cells and cultured in a microfluidic chip-based "parathyroid-on-a-chip" system.

Results: Self-Renewal Capacity: Patient-derived PTOs maintained self-renewal through four passages (p1: 100% → p4: 18%) and showed a 31.3% reduction in PTH secretion under high calcium (3.4 mM, $p = 0.015$). Drug Responsiveness: Cinacalcet (a calcimimetic) induced significant changes in calcium and phosphate levels. Chip Model Functionality: The parathyroid-on-a-chip system regulated PTH secretion in response to calcium fluctuations. Limitations: Lack of vascularization and extracellular matrix (ECM) components reduced long-term viability.

Conclusions: Parathyroid organoids demonstrate functional mimicry and potential as drug screening platforms. However, the absence of native tissue microenvironment components remains a critical barrier. Future studies should prioritize coculture with endothelial cells and CRISPR-Cas9-based editing to enhance survival post-transplantation in non-human primates.

P2.01 | Legal Gaps in the Regulation of Xenotransplantation Using Genetically Engineered Pigs: A Case Study of the South Korean Legal Framework

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This study identifies and analyzes regulatory shortcomings in South Korea's legal framework concerning xenotransplantation research utilizing genetically engineered pigs. Focusing on key statutes such as the Animal Protection Act, the Laboratory Animal Act, the Act on the Safety and Support of Advanced Regenerative Medicine and Advanced Biological Products, and the 2024 Guidelines for the Quality, Non-Clinical, and Clinical Evaluation of Xenotransplantation Products issued by the Ministry of Food and Drug Safety, this study systematically reviews the legal and policy framework applicable to xenotransplantation research. The analysis reveals several critical structural issues, including legal ambiguities surrounding the classification and protection of source animals, the absence of clear criteria distinguishing laboratory animals from livestock, overlapping and vague standards for facility certification, and regulatory gaps concerning the transportation and handling of harvested organs. By highlighting these foundational issues, the study aims to contribute to future interdisciplinary discussions and policymaking efforts that will be necessary for the responsible governance of xenotransplantation and other biotechnology applications involving genetically modified animals.

Funding: This work was supported by a grant of the Xenotransplantation Research and Development Project through the Korea Health Industry Development Institute (KHIDI), funded by the Ministry of Health & Welfare, Republic of Korea (No. RS-2023-KH136898).

P2.02 | Profile and Attitudes of High School Students (São Paulo City, Brazil) Toward Xenotransplantation

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Table 1. Attitudes and perceptions on transplantation and xenotransplantation among high school students (n = 619).

Variable	Answer option 1	%	Answer option 2	%	Answer option 3	%	Other Answer
Age	16 years	39.61%	15 years	31.18%	17 years	20.37%	8.84%
Gender	Female	52.61%	Male	44.23%	Non-binary	1.92%	1.24%
Religion	Christianity	55.91%	Atheism/Agnosticism	28.71%	Spiritism/Syncretism	5.63%	9.75%
Do you know someone who has undergone an organ transplant?	No	38.19%	I don't remember	32.42%	Yes	29.40%	–
Are there enough organs for everyone who needs a transplant?	No	89.29%	Don't know	8.38%	Yes	2.34%	–
Have you talked to family/friends about organ donation?	Yes	43.82%	No	41.76%	I don't remember	14.42%	–
Do you consider yourself an organ donor?	Yes	54.95%	No opinion	33.52%	No	11.54%	–
Have you ever heard of xenotransplantation?	No	74.04%	Yes	15.25%	I don't remember	10.71%	–
What do you understand by xenotransplantation?	Transplantation between different species	94.59%	Don't know	3.60%	Transplantation between humans	1.80%	–
Possible benefits of xenotransplantation	Increased organ availability	47.03%	Reduced waiting time	45.66%	Don't know	4.11%	3.20%
Would you accept a xenotransplant?	Yes	46.85%	Not sure	42.34%	No	10.81%	–
Main concerns about xenotransplantation	Disease transmission between species	29.72%	Use of animals as organ sources	25.30%	Religious restrictions	24.50%	20.48%
How do you see the future of xenotransplantation?	An important medical alternative	63.96%	Alternative treatments developed without the use of animals	20.72%	Not sure	10.81%	4.51%
Do you think it is acceptable to use organs from other animal species for transplantation in order to save human lives?	Yes	47.53%	No opinion	36.26%	No	16.21%	–

FIGURE 1 | P2.02

Xenotransplantation has emerged as a potential alternative to address the global shortage of human organs for transplantation. This study investigated the attitudes of Brazilian adolescents toward xenotransplantation and the factors associated with its acceptance or rejection. The research was conducted in São Paulo, Brazil, a region where xenotransplantation research is currently advancing. A structured questionnaire was administered to 619 adolescents aged 15 to 17, collecting demographic, educational, and perceptual data regarding organ transplantation and xenotransplantation. The variable "Xenotransplantation Attitude" was constructed based on respondents' willingness to receive an animal organ and their ethical evaluation of the practice. Only participants who reported prior knowledge of xenotransplant were included in this portion of the analysis. Associations between variables were tested using Fisher's exact test for categorical variables and the Mann–Whitney U test for age, with a significance level of 5%. Most adolescents (74.04%) had never heard of xenotransplantation. Among those who had, 94.59% correctly defined it as transplantation between species. The study found that nearly half of the adolescents (47.5%) demonstrated a positive attitude toward xenotransplantation. In contrast, 5.9% showed a negative attitude, and 46.6% either had no opinion or lacked sufficient knowledge on the subject. Statistical analysis revealed that a positive attitude toward xenotransplantation was significantly associated with the participants' perception of the future of the technique and their age. Adolescents who viewed xenotransplantation as a promising medical alternative were more likely to express favorable attitudes, suggesting that trust in the potential of the technique directly influences its acceptance ($p = 0.01$). Age also proved to be a distinguishing factor, with statistically significant differences observed between groups ($p = 0.004$). Factors such as gender, religion, and knowledge of allotransplantation showed no significant association with xenotransplantation attitude. Despite limited prior knowledge, adolescents showed openness to xenotransplantation, acknowledging its potential benefits while expressing ethical and biological concerns. The high rate of uncertainty regarding both personal acceptance and ethical judgment underscores the need for educational initiatives that promote informed and critical discussion of the topic. Considering the current progress in xenotransplantation research in Brazil, especially in São Paulo, building a well-informed public perspective will be essential for the future development and societal acceptance of this medical innovation.

Funding: This work was supported by the São Paulo Research Foundation (Fapesp) – grant number 21/11872-5 and the National Council for Scientific and Technological Development (CNPq) – grant number 444075/2023-2.

P2.03 | Establishment of a Designated Pathogen-Free (DPF) Unit in Germany

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Introduction: The increasing shortage of human organ donors worldwide demands innovative solutions. Xenotransplantation - the transplantation of organs from animal donors into humans - has emerged as a promising approach. While initial procedures have already been performed in humans in the USA, their implementation in Germany faces complex legal requirements. This work explores the legal framework and practical requirements for establishing a designated pathogen-free (DPF) unit in Germany as a key structural and regulatory solution to enable safe xenogeneic organ donation.

Methods: A legal and procedural analysis was performed to assess the requirements for xenotransplantation in Germany, with special focus on animal husbandry, the use of genetically modified organisms (GMOs), and their integration into the

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production of Advanced Therapy Medicinal Products (ATMPs). Relevant frameworks include the German Animal Welfare Act (TierschG), Genetic Engineering Safety Ordinance (GenTSV), Transplantation Act (TPG), the Medicinal Products Act (AMG), and its derived requirements for EU-GMP (Good Manufacturing Practice). International guidance and scientific best practices were also reviewed.

Results: Establishing a DPF-Unit in Germany interfaces with multiple legal frameworks. However, if these frameworks are addressed and implemented to their fullest, a DPF-Unit provides a pragmatic solution by embedding secure animal housing and controlled organ explantation into the production process. This includes the housing of transgenic piglets under biosafety level 2 conditions from the pre-born stage, minimizing pathogen exposure and transport-related risks. Dedicated equipment, hygienic procedures, and staff expertise ensure the absence of designated pathogens. Cardiac extractions can be conducted on-site and delivered directly to clinical facilities, fulfilling EU-GMP Part IV requirements and enhancing patient safety while complying with ethical and environmental standards.

Conclusion: This work proposes the DPF-Unit as a central structural element for enabling xenotransplantation under current legal frameworks and offers a possible roadmap for establishing a DPF-Unit in Germany. By providing conditions that meet both animal welfare requirements and EU-GMP Part IV standards for organ extraction, the DPF-Unit serves as a reliable environment for the generation of safe donor animals and high-quality xenogeneic organs, thereby contributing substantially to overall patient safety.

P2.04 | Histocompatibility Testing in Preclinical Cardiac Xenotransplantation

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Introduction: Cardiac xenotransplantation is emerging as a highly promising strategy for addressing terminal heart diseases, with the potential to provide a solution to the global shortage of donor organs. Significant progress has been made in overcoming hyperacute rejection, a major hurdle in transitioning from preclinical to clinical application, including genetic modification of porcine donors, improved preservation techniques, and novel immunosuppressive regimens. In allotransplantation, pretransplant crossmatching is essential for identifying compatible donor-recipient pairs. Applied to xenotransplantation, crossmatching enables detection of donor-specific antibodies (DSA), assessing compatibility between porcine donors and baboon or human recipients.

Methods: This retrospective study evaluates preclinical trials in which porcine hearts were transplanted into baboons. Standard crossmatching techniques - complement-dependent cytotoxicity (CDC) and fluorescence-activated cell sorting (FACS), both considered gold standard - were adapted from clinical allotransplantation protocols to assess donor-recipient compatibility in the xenogeneic setting. Peripheral blood mononuclear cells (PBMCs) from 25 triple genetically modified German Landrace hybrid pigs and sera from corresponding baboon recipients are analyzed via FACS to detect anti-pig DSAs. Preliminary optimization included testing different media for PBMC thawing, evaluating various secondary antibodies and their optimal dilutions by titration, and cytokine stimulation with porcine IL-2 to improve cell viability in cases of sample degradation due to long-term storage.

Results: FACS crossmatch results demonstrate the technical feasibility of the adapted protocol and its ability to detect DSAs in the xenotransplantation context. A systematic evaluation is currently being conducted to investigate a potential correlation between crossmatch results and post-transplantation clinical parameters such as graft survival and signs of rejection. We aim to demonstrate that histocompatibility testing can serve as a predictive tool for transplant outcomes and for follow-up monitoring to identify de novo xenospecific antibodies potentially leading to antibody mediated rejection.

Conclusions: Although our previous preclinical trials in cardiac xenotransplantation were performed without pretransplant screening for preformed DSAs, crossmatching may serve as a valuable additional tool for donor–recipient selection and further improving outcome. In cardiac allotransplantation, crossmatching is a mandatory step to assess immunologic compatibility and reduce the risk of rejection. Implementing this approach to xenotransplantation with consideration of the findings from the retrospective study could enhance the safety and efficacy of future clinical procedures. Ongoing analyses aim to determine whether crossmatch results correlate with clinical outcomes, potentially enabling more informed donor selection in xenotransplantation.

Funding: This work was supported by the German Heart Foundation/German Foundation of Heart Research, the German Research Foundation (Deutsche Forschungsgemeinschaft, DFG) TRR 127, the Swiss National Science Foundation (CRSII5_198577), the Bavarian Research Foundation (AZ-1543-22), and the Leducq Foundation (23CVD01).

P2.05 | Ferroptosis in Pig-to-Baboon Cardiac Xenotransplantation

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Introduction: Cardiac xenotransplantation is currently the most promising alternative to cardiac allotransplantation. However, despite significant progress in recent years, some challenges remain on the path toward regular clinical application. The implementation of cold non-ischemic preservation using an extracorporeal heart preservation system – consisting, among other components, of a roller pump, a cooler/heater unit, and an oxygenated hyperoncotic cardioplegic solution with nutrients, erythrocytes and hormones - has significantly reduced ischemia-reperfusion injury and perioperative cardiac xenograft dysfunction (PCXD). Nonetheless, the intrinsic vulnerability of porcine hearts to ischemia remains a relevant hurdle. In contexts other than cardiac xenotransplantation, ischemia-reperfusion injury has been associated with ferroptosis - a form of regulated necrosis characterized by iron-dependent phospholipid peroxidation - and ferroptosis inhibitors (ferrostatins) have shown protective effects. Ferroptosis has also been implicated in allotransplantation and multi-organ dysfunction; however, no data are available on its occurrence or therapeutic targeting with ferrostatins in pig-to-baboon cardiac xenotransplantation.

Methods: Plasma samples from $n = 17$ baboons undergoing orthotopic pig-to-baboon cardiac xenotransplantation were analyzed. Malondialdehyde (MDA), a biomarker of lipid peroxidation, was measured using a colorimetric assay. As perioperative procedures were consistent across all recipients, samples were initially analyzed as a single study group for the first postoperative week. Subsequently, baboons were divided into subgroups based on the course of the experiments, such as the development of pleural effusions or rejection episodes.

Results: Plasma samples from all $n = 17$ baboons showed a marked increase in free MDA levels at 1 and 6 h after cessation of cardiopulmonary bypass (CPB) following cardiac xenotransplantation. This elevation declined by the first postoperative day, with no subsequent increase observed in any animals during the first postoperative week. Preliminary analyses at later time points following xenotransplantation revealed a secondary increase in MDA levels in baboons that developed recurrent pleural effusions or exhibited signs of rejection.

Conclusion: This study provides initial insights into the occurrence of wave of lipid peroxidation following pig-to-baboon cardiac xenotransplantation, hinting for involvement of ferroptosis. Based on our findings, we hypothesize that treating recipient baboons - and potentially future patients - with ferrostatins may further reduce ischemia-reperfusion injury, as well as potential adverse effects associated with cold, roller pump-based preservation or CPB, thereby improving post-transplant outcomes. Moreover, our preliminary data suggest that ferrostatin treatment during the later postoperative course may help reduce the incidence of pleural effusions or other inflammatory processes.

Funding: This research was funded by the German Research Foundation (Deutsche Forschungsge- meinschaft, DFG) TRR 127 and—in part—by the Swiss National Science Foundation (CR-SII5_198577), the Bavarian Research Foundation (AZ-1543-22) and the Leducq Foundation (23CVD01).

P2.06 | How Important Is the Fc-Portion for CD40/CD40L Costimulation Blockade in Orthotopic Pig-to-Baboon Heart Transplantation?

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Background: Orthotopic cardiac xenotransplantation is close to a clinical pilot study. For clinical use, immunosuppression based on costimulation blockade with humanized antibodies is required. In addition to full antibodies, Fab fragments have been developed. These Fabs lack the Fc region, which has been reported to be thrombogenic in the case of first-generation anti-CD40L antibodies. It remains unclear whether the Fc region is essential for immunosuppression in xenotransplantation.

Methods: Hearts from genetically modified (GGTA1-KO, hCD46/hTBM transgenic) juvenile pigs were orthotopically transplanted into male baboons using cold, non-ischemic organ preservation. In group I ($n = 4$), costimulation blockade was performed with anti-CD40L PAS-Fab at 20 mg/kg body weight (b.w.); in group II ($n = 3$), the anti-CD40L mAb TNX-1500*, which possesses the identical variable region as anti-CD40L PAS-Fab, was administered initially at 25 mg/kg b.w. and subsequently at 20 mg/kg b.w. Additionally, all recipients were treated with an mTOR inhibitor, anti-hypertensive medication, and a fast corticoid tapering.

Results: In each group, one animal was lost due to technical failure. The mean survival of the remaining animals in group I was 33 ± 10 days ($n = 3$), and in group II, it was 181 ± 15 days ($n = 2$). In group I, all grafts were lost due to either acute humoral (Day 18; $n = 1$) or cellular rejection (Days 29 and 51; $n = 2$). In group II, one graft was lost due to acute cellular rejection (Day 196) and the other due to acute myocardial infarction caused by an embolus in the right coronary artery (Day 166).

Conclusions: With identical variable regions, the two antibodies differed only in the presence or absence of the Fc region. Therefore, we hypothesize that the Fc region or the bivalency of the full-size mAb – apart from differences in the dosing scheme – is essential for an effective anti-CD40L costimulation blockade in orthotopic pig-to-baboon xenotransplantation.

Funding: This research was funded by the German Research Foundation (Deutsche Forschungsge- meinschaft, DFG) TRR 127 and—in part—by the Swiss National Science Foundation (CR-

* TNX-1500 is an investigational new drug, its efficacy and safety have not been established and it has not been approved for any indication

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SII5_198577), the Bavarian Research Foundation (AZ-1543-22) and the Leducq Foundation (23CVD01).

P2.07 | The Endothelial Glycocalyx in Pig-to-Baboon Cardiac Xenotransplantation – First Insights

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Introduction: Cardiac xenotransplantation is a promising alternative to allotransplantation. While recent progress has led to increased survival, acute vascular rejection remains a challenge. The endothelial glycocalyx, a crucial regulator of endothelial health and inflammation, is implicated in allograft rejection. However, its role in xenotransplantation is not well understood. This study retrospectively analyzed peri- and postoperative plasma levels of hyaluronan (HA), heparan sulfate (HS), and syndecan-1 (Sdc-1) as surrogate markers for endothelial glycocalyx integrity in a pig-to-baboon cardiac xenotransplantation model.

Methods: Plasma samples from $n = 4$ baboons undergoing orthotopic pig heart transplantation were analyzed in the peri- and postoperative period using ELISA. These $n = 4$ experiments were divided into two groups in the postoperative period: experiments, that were deliberately terminated after 90 postoperative days (group I) and animals, which were tested positive for PCMV/PRV (group II). As probes were available for the postoperative period (but no perioperative probes were available any more), a fifth baboon was analyzed in this period. In this animal, the xenograft was ischemically preserved and no growth-inhibiting drugs were administered.

Results: Perioperatively, HA levels transiently increased, while HS and Sdc-1 decreased in all $n = 4$ animals, suggesting some glycocalyx shedding, albeit potentially less pronounced than in comparable human cardiac surgery settings. In contrast to the human cardiac surgery setting, no correlation was found between perioperative lactate and glycocalyx component changes. Postoperatively, group I animals showed stable glycocalyx component levels. In contrast, animals with PCMV/PRV infection exhibited a marked increase in HA and Sdc-1, and a decrease in HS. The animal with graft overgrowth also showed increased HA and Sdc-1 and decreased HS levels.

Conclusion: This study provides first insights into endothelial glycocalyx dynamics in pig-to-baboon cardiac xenotransplantation. Using current strategies of cardiac xenotransplantation, damage of the endothelial glycocalyx seems to be comparable or even less pronounced than in similar human settings. At the same time, the data from experiments where current strategies, like PCMV/PRV elimination, non-ischemic preservation and growth inhibition, could not be applied indicate that damage of the endothelial glycocalyx also plays an important role in cardiac xenotransplantation.

P2.08 | Renal Arterial Resistive Index Identifies Thrombotic Microangiopathy After Kidney Xenotransplantation in Pig-to-Baboon Model

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Background: As kidney xenotransplantation (KXTx) advances toward clinical trials, the development of effective, non-invasive methods for graft monitoring becomes increasingly essential. Renal resistive index (RRI), derived from Doppler ultrasound sonography (DUS), is widely utilized in kidney allotransplantation to assess graft dysfunction and detect rejection episodes. However, its applicability in KXTx, particularly in identifying thrombotic microangiopathy (TMA), a frequent and severe complication leading to graft loss, has not been thoroughly investigated. This study assesses the potential of RRI as an early predictive indicator of TMA in a pig-to-baboon kidney xenotransplantation model.

Methods: Five consecutive pig-to-baboon KXTx were included in this prospective study. The first three cases comprised the study group ($n = 3$), while the subsequent two served as a validation group ($n = 2$). Serial RRI measurements were performed and compared with histopathological findings from kidney biopsies, either taken concurrently with ultrasound examinations or at the subsequent biopsy following RRI assessment. Renal artery RRI measurements from naïve pigs ($n = 8$) and naïve baboons ($n = 6$) were utilized as baseline reference values.

Results: Baseline RRI values were significantly lower in the kidneys of naïve pigs compared to naïve baboons (median [IQR]: 0.597 [0.568–0.603] vs 0.640 [0.624–0.653], respectively; $p < 0.02$). In the study group, elevated RRI values were observed at the time when TMA was confirmed by biopsy (median [IQR]: 0.820 [0.696–0.883] vs 0.628 [0.575–0.717], respectively; $p < 0.02$). The optimal RRI cut-off value associated with TMA following KXTx was 0.642 (ROC AUC = 0.857, $p < 0.04$), with a sensitivity of 1.000 and specificity of 0.667.

In the validation group, RRI transiently increased above 0.70 immediately following reperfusion but subsequently declined to pre-transplant baseline levels within 1 week post-transplantation (0.50 and 0.48, respectively). However, on POD 17 and 19, the RRI again increased beyond the critical threshold of 0.642, measuring 0.71 and 0.66, respectively, despite normal serum creatinine levels. Biopsies performed at these time points indicated a suspicion of TMA without accompanying renal dysfunction, and subsequent biopsies confirmed TMA on POD 21 and 22, respectively.

Conclusion: This study suggests that non-invasive DUS-based RRI measurement may be a sensitive method for the early

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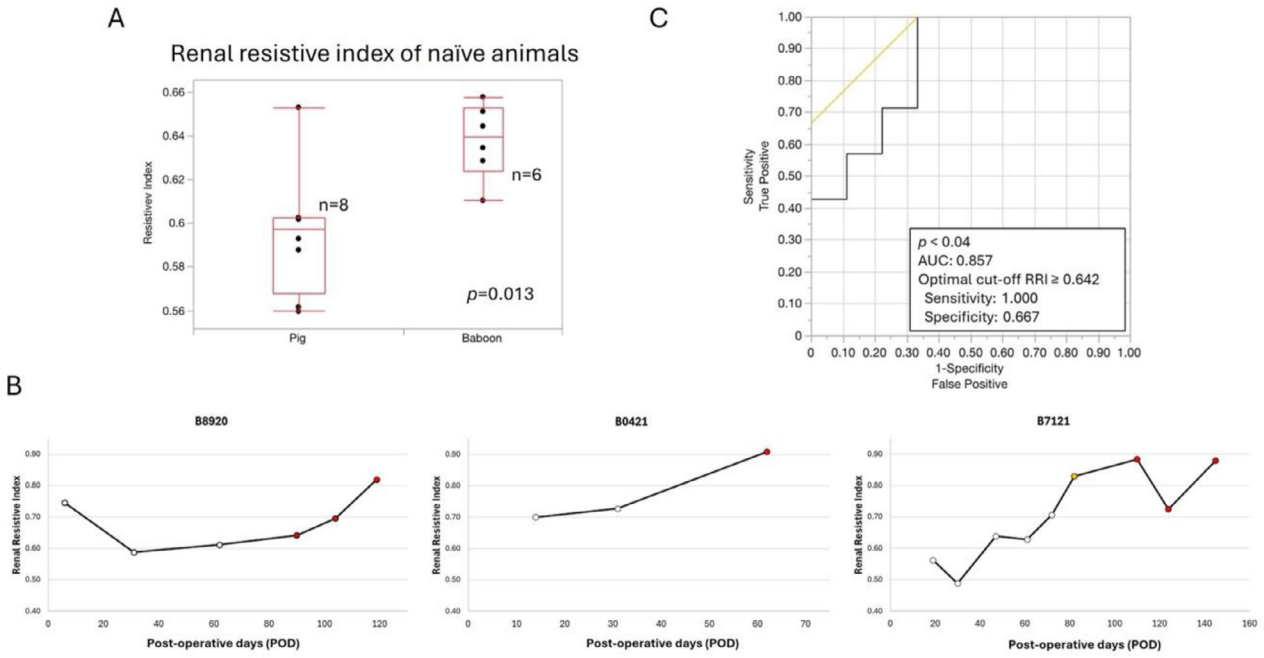


Figure 1. Renal Resistive Index (RRI) and Baseline Characteristics in Naïve Animals.
(A) Baseline RRI values in kidneys from naïve pigs (n=8) and baboons (n=6). (B) Clinical course and RRI trends in three recipient animals within the study group. The color of each dot indicates histopathological findings from kidney biopsies: white – normal histopathology; yellow – C4d deposition without TMA; red – confirmed TMA with C4d deposition. (C) Receiver operating characteristic (ROC) curve identifying an optimal RRI cut-off value of 0.642 for identifying TMA.

FIGURE 1 | P2.08

detection of commonly diagnosed TMA in kidney xenografts. An RRI value above 0.642 effectively identifies the onset of TMA, even when serum creatinine levels remain within normal ranges, indicating no apparent renal dysfunction. These results highlight the potential of RRI as a valuable monitoring tool for KXTx, potentially enabling early identification of graft complications and timely interventions that could prevent graft loss. Further research is warranted to validate the utility of RRI as a reliable biomarker in clinical xenotransplantation.

Funding: This project was funded by the National Institute of Health (NIH) 5P01AI045897 grant and an unrestricted research grant from Nefro Health and ChoironeX.

P2.09 | Cell-Free DNA Method for Monitoring Porcine-to-Human Xenotransplants

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Introduction: Xenotransplantation, the transplantation of organs of non-human origin into humans, is a potential long term solution to the chronic shortage of organs currently available for transplantation. Several xenotransplants using porcine organs from pigs that were genetically modified to remove cell-surface antigens that could lead to alloimmunity, have recently been reported, including heart and kidney, with human patients surviving the surgery for an encouraging duration. Donor-derived cell-free DNA (dd-cfDNA) has been validated as a non-invasive biomarker for the detection of rejection in solid organ transplant, and is routinely used in both a surveillance and for cause setting. A non-invasive cell-free DNA (cfDNA) method for monitoring xenotransplants is needed in anticipation of a large clinical uptake of this approach. In this proof-of-concept study, we sought to demonstrate that cfDNA can be used to measure the fraction of porcine cfDNA (pcfDNA) in plasma.

Methods: A titration series of porcine-into-human cfDNA mixtures was created from porcine and human plasma samples. Whole genome sequencing read counts mapping uniquely to porcine or human reference genomes were identified and counted to calculate the ratio of porcine cfDNA to total cfDNA in each sample. The correlation coefficient and accuracy (standard deviation; SD) was calculated.

Results: The calculated porcine-derived cfDNA (pcfDNA) ratio in the 14 porcine-human cfDNA mixtures, ranging from 100% to 0.01% pcfDNA ratio, were plotted against the intended input pcfDNA ratio, yielding a concordance of r^2 = 0.9961 and SD of 0.029. At low and very low (<2% and <0.2%) pcfDNA values, SD remained low, at 0.025 and 0.011, respectively.

Conclusions: We demonstrated that whole genome sequencing can accurately detect and quantify porcine-derived cfDNA in mixtures with human cfDNA down to <0.1% pcfDNA fraction. This method is expected to support future research and clinical applications of solid organ xenotransplantation.

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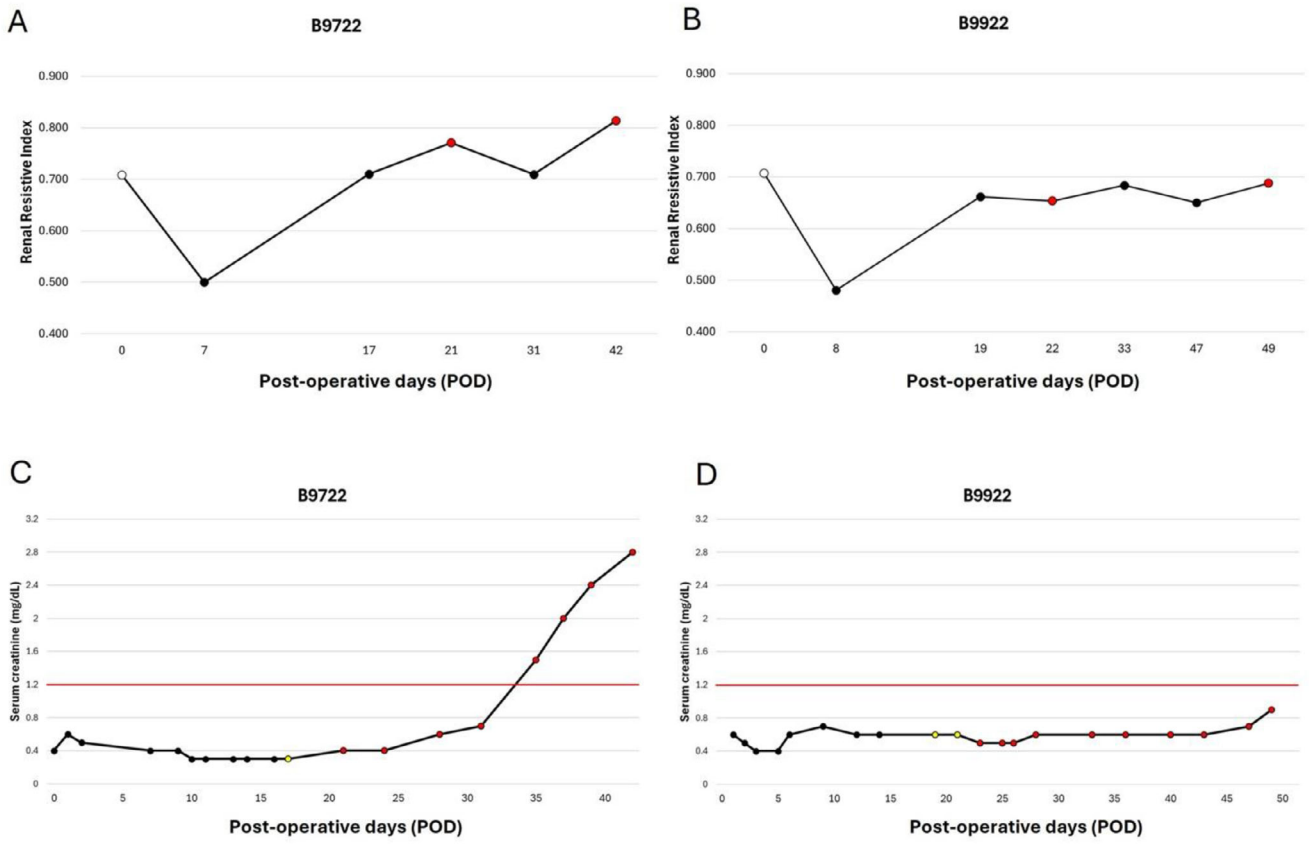


Figure 2. Clinical Application of Renal Resistive Index (RRI) in the Validation Group.
(A-B) Clinical course and corresponding RRI measurements in two recipient animals from the validation group. Dot colors indicate biopsy findings: black – Doppler ultrasound measurement without biopsy; white – normal histopathology; red – confirmed TMA. (C-D) Serum creatinine levels and corresponding RRI values during the clinical course of the validation group. Dot colors indicate clinical phases: black – Phase I (RRI < 0.642); yellow – Phase II (RRI ≥ 0.642 before biopsy-confirmed TMA); red – Phase III (RRI ≥ 0.642 after biopsy-confirmed TMA).

FIGURE 1 | P2.08

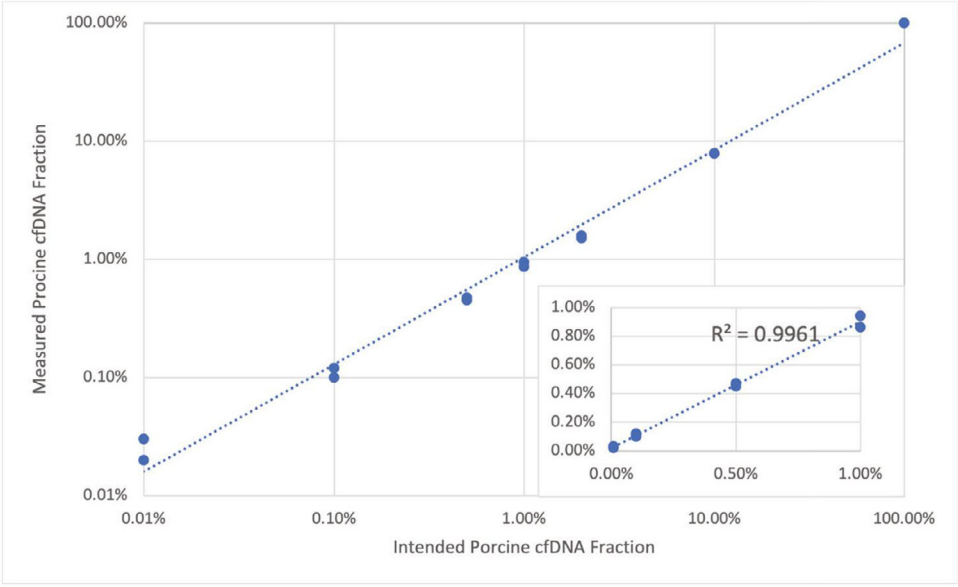


FIGURE 1 | P2.09

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Table 1: Titration series samples.

Sample number	% porcine DNA	Human DNA (ng)	Porcine DNA (ng)
1, 2	100%	0	10
3, 4	10%	18	2
5,6	2%	19.6	0.4
7,8	1%	19.8	0.2
9,10	0.5%	19.9	0.1
11,12	0.1%	20	0.02
13,14	0.01%	20	0.002

FIGURE 2 | P2.09

P2.10 | Selectin/Integrin Blockade and Macrophage Depletion Mitigate Injury in Pig Lung Xenoperfusion With Human Blood

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Purpose: This study evaluates whether selectin and integrin blockade, when combined with donor organ gene editing (GE) strategies, can reduce neutrophil and platelet sequestration, RBC damage or inflammatory cytokine elaboration observed during ex-vivo pig lung perfusion with human blood.

Method: GE lungs with 3 carbohydrate antigen knockouts and 6 human transgenes [GalKO.CMAHKO.β4GALKO.hCD46.hCD55.hTBM.hEPCR.hHO1.hCD47] +/- growth hormone receptor (GHR) (10GE, $n = 16$), and GalKO lungs ($n = 8$) were perfused with heparinized fresh human blood. Pigs received DDAVP to deplete vWF. The perfusate was treated with thromboxane synthase inhibitor (1-BIA) and histamine blockers; in some experiments aGPIb Fab was added. Treated lung perfusate received P and E selectin and integrin antagonists (rPSGL1.Fc, GMI1687, IB4), and porcine sialoadhesin blocker (mAb1F1) (10GE, $n = 8$; GalKO, $n = 4$). In seven cases, 10GE left lungs (two with additional HLA-E) were transplanted into baboons, while right lungs were perfused with rPSGL1.Fc, GMI1687, IB4, αGPIb-treated treated human blood; four of these pigs received liposomal clodronate (LC) to deplete resident tissue macrophages. Functional, blood, and tissue analyses were performed at set intervals.

Results: The combined treatment delayed but didn't prevent neutrophil and platelet sequestration. MCP-1 levels were lower, and RBC fragments and free Hgb were reduced in treated GalKO and 10GE lungs at 4hrs of perfusion compared to controls (Figure 1A–E).

Lungs from LC-treated pigs showed significantly lower neutrophil sequestration during the first 60 min and lower histamine levels throughout the perfusion compared to other 10GE lungs (Figure 2A,B).

Transplanted 10GE and 11GE lungs showed less parenchymal damage and lower IL-6/IL-8 levels than lungs with fewer genetic modifications, with the longest survival (33 days) observed in a recipient of an 11GE lung from an LC-treated pig.

Conclusion: Selectin and integrin blockade is associated with delayed neutrophil and platelet sequestration and reduced hemolysis and cytokine levels by GE lungs. Pulmonary macrophage depletion with LC may enhance protection and further extend lung xenograft survival.

P2.11 | Blockade of CD40 With or Without CD11b Versus CD154 Blockade in GTKO and hCD55 Transgenic Pig-to-Macaque Kidney Xenotransplantation

Bryar Hansen, Andre Souffrant, Katsuhiro Tomofuji, Avery Wilson, Amin Arnaout, Tatsuo Kawai

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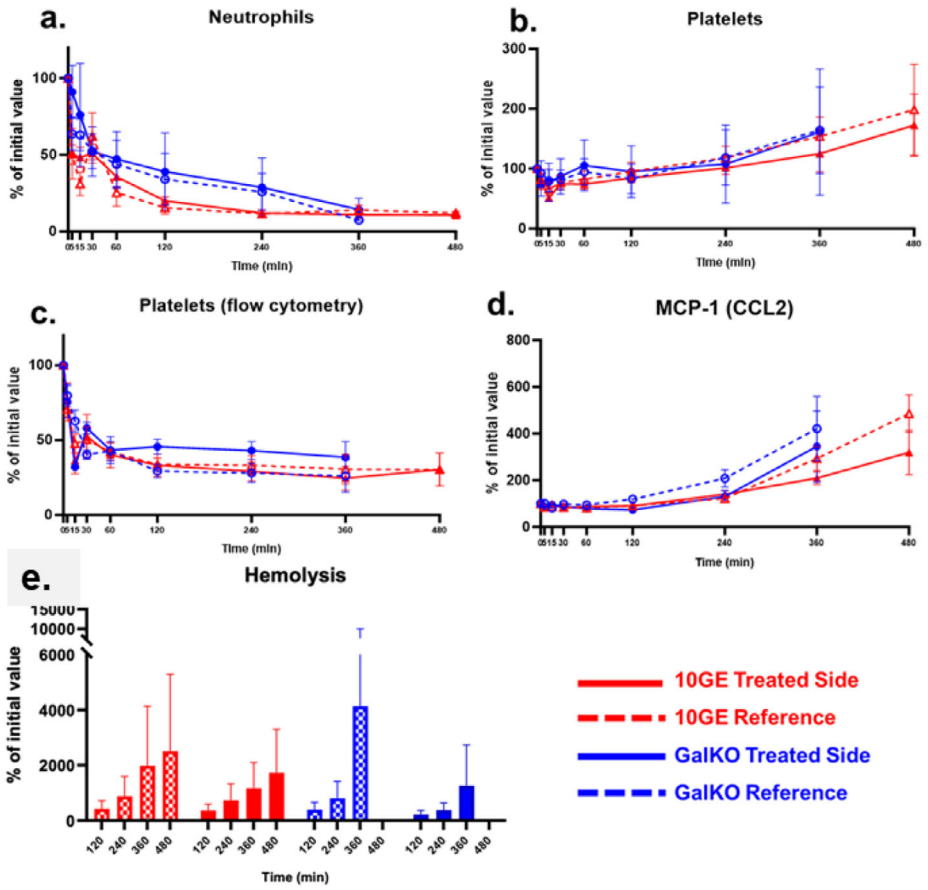


FIGURE 1 | P2.10

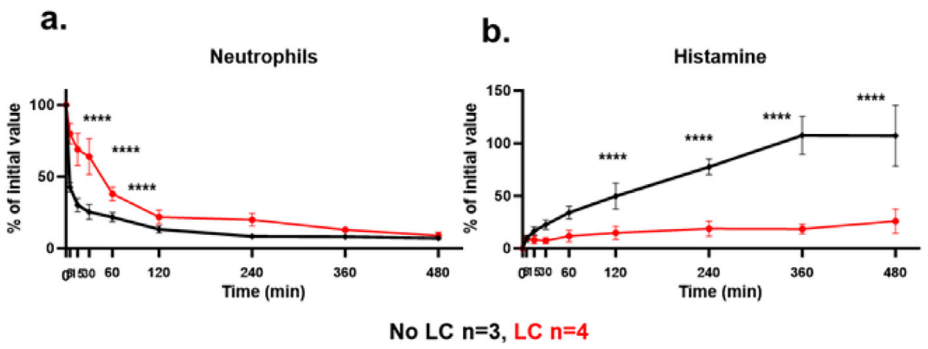


FIGURE 2 | P2.10

Center for Transplantation Sciences, Massachusetts General Hospital, Boston, Massachusetts, USA

Introduction: CD11b is a transmembrane glycoprotein that is highly expressed on innate immune cells such as neutrophils and macrophages and regulates a broad range of immune responses in xenotransplantation. We evaluated inhibition of innate responses in pig-to macaque kidney xenotransplantation by mAb107 (aCD11b), a unique non-agonizing mAb that blocks the archetypal innate immune receptor integrin CD11b/CD18 (CD11b), in combination with CD40 blockade.

Methods: Using a genetically modified pig donor (GTKO and hCD55 insertion), kidney grafts were transplanted into NHP recipients. One study arm received weekly infusions of anti-CD40 monoclonal antibody with monthly infusions of aCD11b, while another arm received aCD40 alone. These results were compared to a cohort receiving weekly infusions of aCD154 alone. Along with the standard postoperative immunosuppressive regimen, we examined how survival outcomes changed with the experimental inhibition of the CD11b pathway. Biopsy samples in all arms were collected from the xenograft 1 h after reperfusion and at time of necropsy. Protocol biopsies were obtained every 1–3 months in recipients with stable function, and when a rise in serum creatinine was detected. FFPE sections were used for routine pathologic studies as well as transcriptomic analyses using the NanoString platform.

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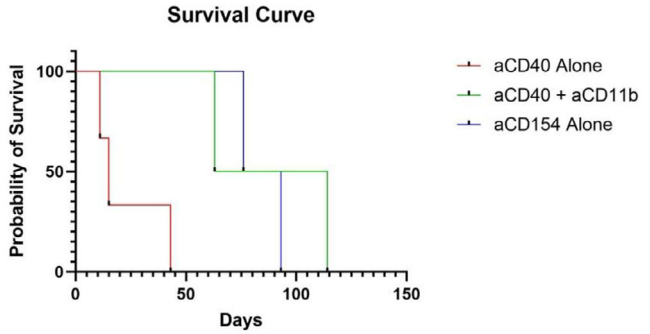


FIGURE 1 | P2.11

Results: Three cynomolgus monkey recipients that received aCD40 mAb alone rapidly lost their xenografts either due to rejection, TMA, or thrombotic graft loss at Days 11, 15, and 43, respectively. Two recipients receiving aCD154 survived longer, being euthanized on Days 76 and 93 due to antibody-mediated rejection and weight loss (without rejection), respectively. Two recipients receiving aCD40 plus aCD11b survived to 63 and 114 days before euthanasia due to severe proteinuria and subsequent peripheral edema, both with diagnosis of CAMR.

At 1-h post-reperfusion biopsy, recipients receiving aCD40 alone showed no signs of rejection but did display more diffuse acute tubular injury than the cohort receiving aCD40 plus aCD11b. Recipients receiving aCD154 treatment showed no evidence of rejection.

Transcriptomic analysis of biopsies and plasma cytokine analysis are in progress to investigate how the treatment modalities influence the microenvironment in renal xenografts.

Conclusion: In renal xenotransplantation, blockade of CD40 alone was not sufficient in preventing rapid xenograft loss. With the addition of CD11b blockade, there is survival prolongation that is comparable to survival of recipients receiving aCD154, suggesting the importance of blocking CD11b in this setting.

P2.12 | Prognostic Factor Analysis for Xenocorneal Anterior Lamellar Transplantation Using Genetically-Modified Pig

Sun Ae Hwang[1], Kyoung Sik Park[1], Wan Seop Kim[2], Hyunil Kim[3], Jeong Ho Hwang[4], Yu Rim Ahn[5], Eun Yeol Yang[1], IK Jin Yun[1], Ki Cheul Shin[5]

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Introduction: Xenocorneal transplantation has emerged as a promising alternative to address the global shortage of corneal donors. This study analyzes prognostic factors influencing survival rates in xenocorneal anterior lamellar transplantation using a genetically modified pig to non human primate.

Methods: In this study, various gene-modified pig corneas were used, including GTKO (α1,3-galactosyltransferase gene-knockout; n = 1), GTKO/CD46 (n = 10), GTKO/CD46/CD73 (n = 2), GTKO/CD46/TBM (Thrombomodulin; n = 3), TKO (GGTA1/CMAH/B4GalNT2; n = 1), TKO/CD46/TBM (n = 1), and QKO (TKO+iGb3S)/CD46/TBM (n = 2). For minimal immunosuppression, a subconjunctival injection of dexamethasone (1.5 mg/0.3 mL) was administered, together with 0.5% levofloxacin and 1% prednisolone acetate eye drops for two months. If signs of rejection, such as graft edema or initial corneal opacity, were observed, the initial immunosuppressive regimen was re-administered. The occurrence and timing of rejection, as well as the graft survival period after rejection, were compared by dividing the subjects into long-term and short-term survival groups. Histopathological analysis was also performed.

Results: In this study, consistent long-term survival was not achieved. Analysis of survival rates in xenogeneic partial-thickness corneal transplantation revealed that the long-term survival group (>180 days) had a mean survival period of 585.33 days (±135.10 days, n = 9), while the short-term survival group (<180 days) showed a mean survival period of 51.82 days (±10.45 days, n = 11). The frequency of subconjunctival dexamethasone injections and the pattern of steroid eye drop usage showed an association with graft survival. In the long-term survival group, initial intensive immunosuppressive therapy and continuous immunosuppression were found to be effective in extending graft survival. Histopathological analysis confirmed minimized tissue damage in the long-term survival group.

Conclusions: Xenocorneal anterior lamellar transplantation using genetically-modified pig demonstrates clinical potential. However, to achieve consistent long-term survival further genetic modifications, optimized immunosuppressive protocols, and deeper understanding of survival mechanisms is required.

Funding: This work was supported by the Korea Health Industry Development Institute (KHIDI) [grant number RS-2023-KH136889].

P2.13 | Immunotherapy Strategies for Clinical Approval in Kidney Xenotransplantation

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Introduction: Xenotransplantation research has relied on agents like anti-C10, which lacks clinical approval, and Cobra Venom Factor (CVF), which is unsuitable for human use. These drugs remain significant barriers to clinical translation. Therefore, revising immunomodulatory strategies and identifying clinically viable alternatives are essential steps toward clinical xenotransplantation.

Methods: To address the limitations of non-approved drugs, two approaches are possible: using regimens based on already

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approved agents, or developing new drugs suitable for clinical application. We have chosen to focus on the development of novel immunomodulatory agents that could gain clinical approval, rather than relying on drugs currently used in allotransplantation protocols.

Results: Recent studies have evaluated PG-405 as a substitute for the non-approved antibody C10. PG-405 contains the same Fab fragment as C10, conjugated to interleukin-10 (IL-10), an immunosuppressive cytokine expected to enhance regulatory T-cell activity and suppress immune responses. MD-3 (anti-ICAM-1) is a new agent that modulates immune suppression by inhibiting T-cell differentiation and affecting dendritic cells. Crovalimab, a complement 5 (C5) inhibitor, has been used as an alternative to CVF and functions by blocking C5 activity. In three recent studies using transgenic pigs, kidneys treated with PG-405, MD-3, and Crovalimab demonstrated graft survival periods of 61 and 44 days. Additional therapies under development include anti-CD154/CD28 bispecific antibodies and CAR-Treg cell therapy, which aim to overcome side effects such as thrombosis.

Conclusions: The development and clinical approval of new immunotherapeutic agents will facilitate clinical trials in xenotransplantation and may also lead to innovative immunomodulation protocols applicable to allotransplantation, ultimately advancing the field of transplantation medicine.

Funding: This work was supported by the Korea Health Industry Development Institute (KHIDI) [grant number RS-2023-KH135254].

P2.14 | Effect of High-Dose Steroid Therapy on Graft Survival and Inflammatory Markers in Nonhuman Primate Recipients of Genetically Modified Porcine Kidney Xenografts

Hee Jung Kang[1], Eun Mi Park[1], Haneulnari Lee[1], Keon Bong Oh[2], Jeong Ho Hwang[3], Joohyun Shim[4], Hyunil Kim[4], Ik-Jin Yun[5]

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Introduction: Since the hyperacute rejection in xenotransplantation was overcome by using alpha-1,3-galactosyltransferase gene knockout (GTKO) pigs as donors, porcine kidneys from pigs with further genetic modifications have been transplanted in nonhuman primate (NHP) recipients, of which the results have varied widely. Commonly observed in rejected porcine kidneys are thrombotic microangiopathy and microvascular inflammation characterized by endothelial cell activation, yet the precise mechanisms of graft failure remain unclear. Previously, we noted an early increase in interferon-gamma (IFNg) in the circulation of NHP recipients of xenotransplantation, which can initiate subsequent inflammatory responses in endothelial cells. Glucocorticoids is well known to suppress proinflammatory downstream signaling of IFNg. In this study, we explored whether a high dose of steroid therapy could mitigate vascular inflammation and extend the survival of the xenografts in NHP recipients.

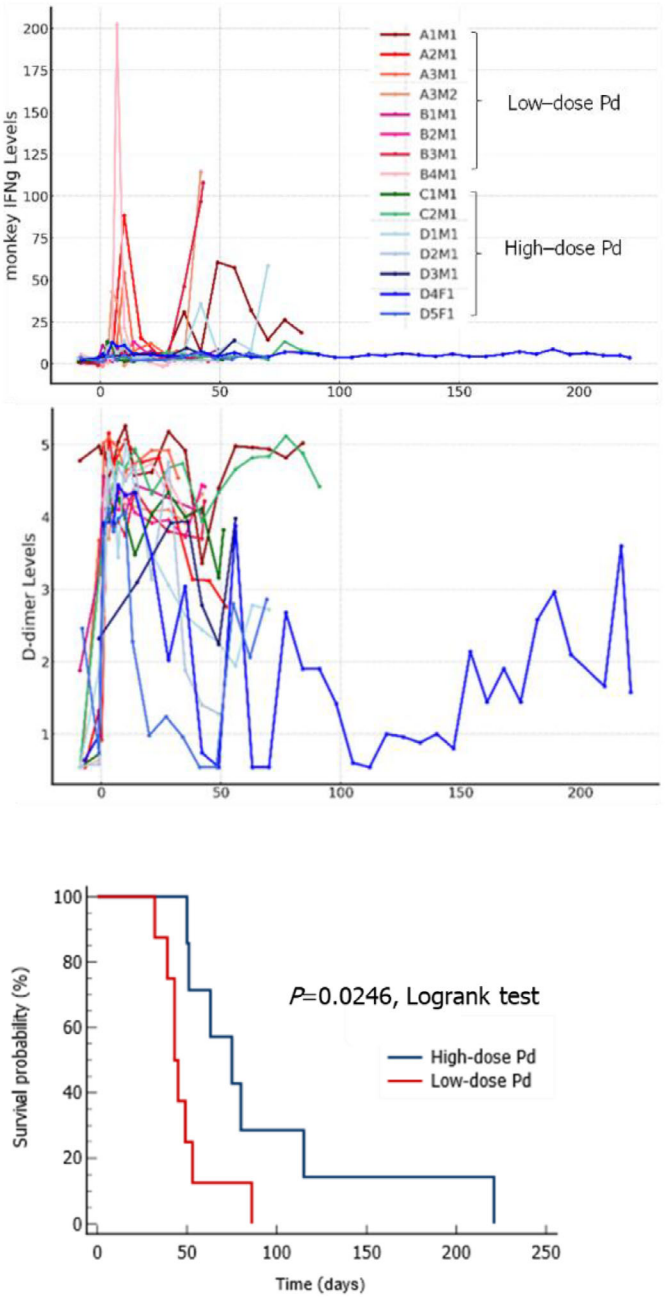


FIGURE 1 | P2.14

Methods: We transplanted kidneys from GTKO pigs with additional genetic modifications into cynomolgus monkeys ($n = 15$). The monkeys received a regimen of anti-CD154, rituximab, antithymocyte globulin, rapamycin (or tacrolimus), mycophenolate mofetil, and methylprednisolone (Pd) before and after transplantation. Pd was administered at either 4 mg/kg/d (low-dose, $n = 8$) or 10 mg/kg/d (high-dose, $n = 7$) and tapered over 5 weeks. Blood samples were taken at regular intervals until graft failure to measure D-dimer, inflammatory cytokines, anti-pig cell IgG and IgM antibodies, and to compare these markers with graft survival across the different Pd dosage groups.

Results: D-dimer levels were initially high post-transplantation, decreased gradually, and then increased again at graft rejection. The time-weighted average median level of D-dimer was significantly lower in the high-dose Pd group compared to the low-dose

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group (2.87 vs 4.00 u/mL, $p = 0.0140$). Regarding IFNg, an early post-transplant elevation was observed in the low-dose Pd group within the first 1–2 weeks but not seen in the high-dose group. The time-weighted average median level of IFNg was also lower in high-dose pd group compared to low-dose group (2.62 vs 10.13 pg/mL, $p = 0.0140$). No differences were observed in the levels of other measured cytokines or antibodies. The median survival of porcine kidney xenografts was significantly prolonged in the high-dose group compared to the low-dose group (75 days vs. 43 days, $p = 0.0246$).

Funding: This work was carried out with the support of the "Cooperative Research Program for Agriculture Science and Technology Development" [Project No. PJ015607], Rural Development Administration, Republic of Korea. This research was partly supported by Hallym University Research Fund.

P2.15 | Enhanced Graft Survival in Full-Thickness Corneal Xenotransplantation From Glycan or Complement Gene-Edited Pigs to Non-Human Primates

Chung Young Kim[1,2], Dong Hee Choi[2], Jin Suk Ryu[2], Won Young Jeong[3], Jong-Min Kim[3,4], Sang-Ik Cho[5], Eun-Jee Oh[6], Chang Ho Yoon[1,2], Chung-Gyu Park[3,7], Joohyun Shim[8], Hyunil Kim[8], Mee Kum Kim[1,2,3]

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Introduction: Given the persistent shortage of donor corneas in East Asia, porcine corneas are being explored as alternative graft sources. While early genetically engineered (GE) models demonstrated limited graft survival without substantial immunosuppression, recent multi-gene-edited pigs are being developed to overcome these limitations. This study aimed to evaluate the safety and efficacy of full-thickness corneal xenotransplantation from pigs with targeted disruption of four glycan-related genes—GGTA1, CMAH, β4GalNT2, and iGb3s (QKO)—alongside transgenic expression of two human immune-regulatory genes, CD55 and CD39.

Methods: The QKO genotype was designed to eliminate key xenoantigens such as Galα(1,3)Gal, Neu5Gc, β4GalNT2, and iGb3s. To further improve immune modulation, hCD55 and hCD39 were introduced to inhibit complement activation and regulate inflammatory signaling. Full-thickness corneas from either QKO or QKO.hCD55.hCD39 knock-in (KI) pigs were transplanted into six cynomolgus monkeys (two received QKO grafts, four received QKO.hCD55.hCD39 grafts). Postoperatively, systemic and topical immunosuppressive regimens were administered. Ocular assessments included rejection index, central corneal thickness, intraocular pressure, and C3a levels in aqueous humor. Systemic monitoring included body weight/temperature, CBC, renal and liver function tests, tacrolimus trough levels, T/B cell analysis (flow cytometry), and donor-specific IgG/IgM (ELISA)

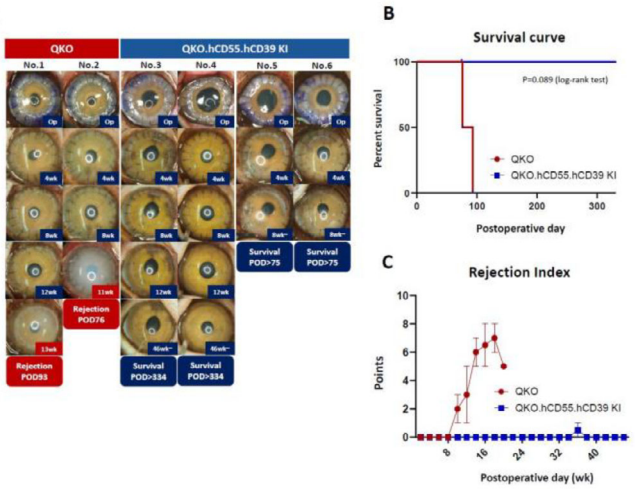


FIGURE 1 | P2.15

Results: QKO grafts survived for 10 and 14 weeks, respectively. One case experienced rejection 2 weeks after tacrolimus withdrawal due to weight loss. In both cases, highly increased levels of IFN-γ–secreting CD8+ T cells were detected 1 week before rejection. Concurrently, donor-specific IgG/IgM antibodies and aqueous humor C3a levels were significantly elevated at the time of rejection. In contrast, QKO.hCD55.hCD39 KI grafts demonstrated faster resolution of corneal edema and survived without rejection for up to 12 months. Although IFN-γ–producing CD4+/CD8+ T cells and memory T cell subset concentrations increased up to 6 months post-transplantation, levels of those cell concentration were not high compared with those of rejected group and immune activity substantially declined thereafter. Concentration of donor-specific IgG and IgM antibodies also increased during the first six months, but showed individual variation beyond that period. Aqueous humor C3a levels were well controlled.

Conclusion: QKO.hCD55.hCD39 KI corneas showed significantly improved biocompatibility and prolonged graft survival compared to QKO controls. Transgenic expression of hCD55 and hCD39 effectively enhanced immune regulation and reduced rejection in corneal xenotransplantation. Systemic T and B cell suppression was critical for the first 6 months post-transplantation, and ongoing B cell control beyond this period should be individualized based on recipient immune profiles.

P2.16 | Impact of Increased Dose of Anti-CD154 Monoclonal Antibody on Graft Survival in Pig-to-Baboon Kidney Xenotransplantation

Kohei Kinoshita[1], Akihiro Maenaka[1], Maho Terashita[1], Yuji Hidaka[1], Ivy A. Rosales[2], Gweneth E. Lavalla[1], David Ayares[3], Seth Lederman[4], Robert B. Colvin[2], Richard N. Pierson[1], Tatsuo Kawai[1], David K.C. Cooper[1].

[1]Center for Transplantation Sciences, Department of Surgery, Massachusetts General Hospital, Boston, Massachusetts, USA; [2]Immunopathology Research Laboratory, Department of Pathology, Massachusetts General Hospital, Boston, Massachusetts, USA; [3]Revivicor

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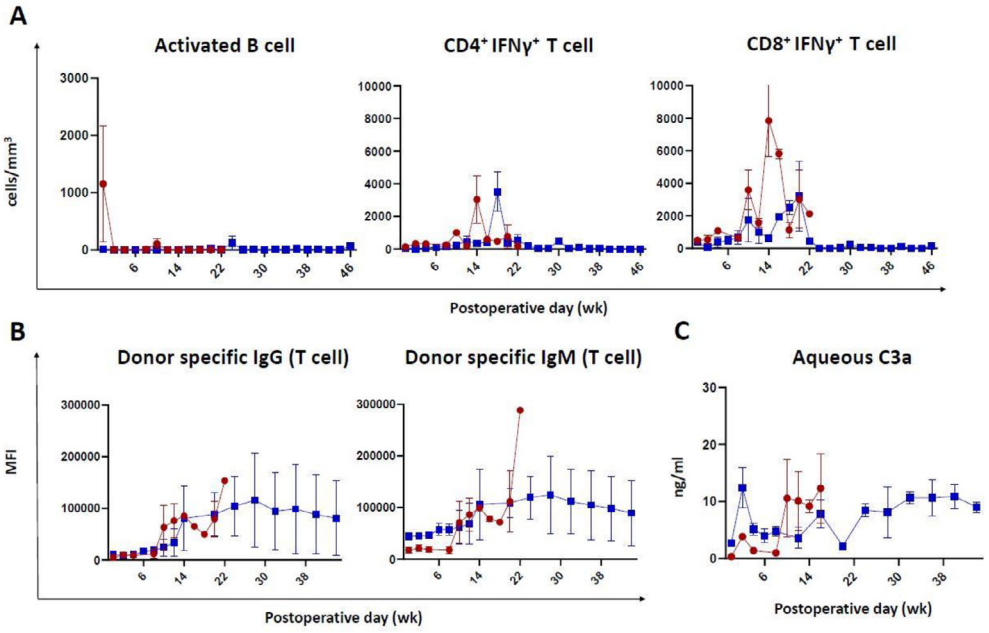


FIGURE 2 | P2.15

Inc., Blacksburg, Virginia, USA; [4]Tonix Pharmaceuticals Holding Corp., Chatham, New Jersey, USA

Purpose: This study aimed to evaluate the efficacy of an optimized immunosuppressive (IS) regimen, including a higher dose of anti-CD154mAb (TNX-1500), in prolonging graft survival in pig-to-baboon kidney xenotransplantation.

Methods: Nine baboons received kidneys from gene-edited pigs (n = 7; either triple-knockout or double-knockout with growth hormone receptor knockout and six human transgenes - hCD46, hCD55, hTBM, hEPCR, hHO-1, and hCD47; Revivicor Inc). Group A (n = 3) received induction therapy with anti-thymocyte globulin (ATG), anti-CD20mAb (rituximab), and C1-esterase inhibitor, followed by maintenance therapy with TNX-1500 (20mg/kg, Tonix Corp), rapamycin, methylprednisolone, and IL-6 receptor blockade (tocilizumab). Group B (n = 6) received the identical regimen except for an increased TNX-1500 dose (30mg/kg). All animals were confirmed CMV-negative. Monitoring included blood counts, flow cytometry, serum and urine analyses, ultrasound examinations, and renal biopsies.

Results: In Group A, survival was 59, 86, and 120 days (median 86 days, mean 86 days), with AMR at the time of graft failure. In Group B, graft survival was significantly prolonged (median 214 days, mean 248 days, $p < 0.05$): >1 year ($n = 2$), >6 months ($n = 2$), and >3 months ($n = 2$). One baboon developed antibody-mediated rejection (AMR) after IS reduction (Day 439); the remaining five were euthanized with functioning grafts for complications believed to be unrelated to anti-CD154mAb therapy (on Days 100, 117, 203, 225, and 405), though one had arterial thrombosis (Day 117) and one had unexplained chronic diarrhea and weight loss (Day 225).

In Group B, five of six recipients developed mild focal thrombotic microangiopathy (TMA) between 3–6 months post-transplant, although serum creatinine levels remained normal or near-normal. No histopathological features of AMR were observed at 3 months post-transplant. All of the biopsies taken at >6 months demonstrated features of transplant glomerulopathy and/or progression of TMA. Despite these pathological changes, serum creatinine levels remained relatively stable in all four baboons surviving >6 months.

Flow cytometric analysis in Group B showed that both CD4+ and CD8+ T cell numbers remained suppressed <50% of baseline at 6 months. Notably, the number of regulatory T cells (Tregs) gradually recovered from ATG, and the percentage of Tregs increased compared to baseline for >3 months.

Conclusions: An anti-CD154mAb constitutes a key component of the IS regimen. Increasing its dose resulted in significantly prolonged graft survival with few associated complications. The combination of CD40/CD154 co-stimulation pathway blockade with rapamycin and tocilizumab may exert synergistic effects in suppressing effector T cells and expanding Tregs, offering a promising strategy to enhance long-term xenograft survival.

Funding: This work on xenotransplantation in DKCC's laboratory is supported in part by NIH NIAID U19 grant AI090959 and in part by a Kidney X Prize from the US DHHS and the American Society of Nephrology.

P2.17 | Dual-Purpose Xenogeneic Lung Perfusion: Towards Xeno- and Allo-Lung Transplantation

Robert Ramm[1,2,3], Klaus Höffler[1,2,3], Sophie Kruszona[1,2,3], Nathalie Roters[1,2,3], Saleh Tavil[1,2,3], Janin Schob[1,2,3], Konrad Fischer[4], Inga Brüsch[5], Philipp Felgendreff[6], Jörg Mengwasser[6], Reinhard Schwinzer[6], Heiner Niemann[7], Arjang Ruhparwar[1,2,3], Patrick Zardo[1,2,3], Fabio Ius[1,2,3], Tobias Goecke[1,2,3]

[1]Department for Cardiac, Thoracic, Transplantation and Vascular Surgery, Hannover Medical School, Hannover, Germany; [2]Lower Saxony Center for Biomedical Engineering, Implant Research and Development, Hannover, Germany; [3]Biomedical Research in End Stage and Obstructive Lung Disease Hannover (BREATH), DZL, Hannover, Germany; [4]Chair of Livestock Biotechnology, School of Life Sciences Weihenstephan, Technical

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University of Munich, Freising, Germany; [5]Institute for Laboratory Animal Science, Hannover Medical School, Hannover, Germany; [6]Department of General, Visceral and Transplant Surgery, Hannover Medical School, Hannover, Germany; [7]Department of Gastroenterology, Hepatology, Infectious Diseases and Endocrinology, Hannover Medical School, Hannover, Germany

Introduction: Despite significant progress in heart and kidney xenotransplantation, long-term survival after pig-to-NHP lung xenotransplantation has not yet been achieved. The underlying causes of rapid lung failure in most experimental transplantations are not yet fully understood. In contrast, ex vivo organ perfusion of human donor lungs has been established in a few centers worldwide, allowing for constant monitoring of lung function and the possibility to precondition donor lungs using treatments such as cold perfusion and specific gene or cell therapeutics. Here, we used a clinical-grade ex vivo lung perfusion (EVLP) system to simulate xenotransplantation of pig organs and to establish a hypothermic lung perfusion as a conditioning treatment to improve transplantation outcome.

Methods: Eight lungs (four wild-type (wt) and four triple-knockout (GGTA1, CMAH, B4GALNT2)) were surgically explanted and preserved using clinical-grade cold perfusion. After cold ischemia of up to 2 h, the lungs were connected to an EVLP system (XPS, XVIVO) and perfused at 10°C for 2 h using cell-free Steen solution. The perfusate was then exchanged for human blood, which was prepared from erythrocyte concentrates, fresh frozen plasma, and a buffy coat obtained from the Hannover Medical School blood bank. Coagulation was inhibited by adding heparin, and calcium was added to maintain functionality. The blood was warmed to 37°C, and porcine lungs were perfused and ventilated for up to 6 h. In addition to functional parameters, total blood count, D-Dimer, CRP, and IL-6 were measured. Lung edema was quantified at the time of explantation.

Results: Triple-KO porcine lungs could be perfused for 6 h, whereas the wild-type (wt) lungs could only be perfused for 4–5 h due to accumulation of pulmonary edema (up to 300% of initial weight). The oxygenation capacity of wt lungs was lower compared to triple-KO lungs, with a high variability observed between individual lungs. IL-6 and CRP levels were barely measurable in both groups, while wt lungs showed a significant higher increase in D-Dimer concentration compared to triple-KO lungs.

Conclusion: The clinical XPS EVLP system was successfully used to simulate pig-to-human xeno- lung transplantation for up to 6 h, including hypothermic preconditioning. The triple-KO lungs maintained good lung function, but a rise in D-Dimer and weight indicated ongoing processes of rejection and coagulation processes.

Off-Note: During the time of experimental xeno- lung perfusions, the team performed the first human EVLP using the XPS system at Hannover Medical School, emphasizing the direct transfer of knowledge between xeno- and allo- lung transplantation.

P2.18 | Exploring Macrophage Responses in Porcine—Human Lung Xenotranplantation: Interplay of Cytokine Signalling and Glycan Dynamics

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Introduction: Xenogeneic lung transplantation with genetically engineered pig lungs is an emerging potential solution to the global shortage of human donor organs. Immune rejection and ischemia-reperfusion injury (IRI) are major barriers to clinical success. IRI induces oxidative stress and innate immunity activation by damage-associated molecular patterns (DAMPs), potentially furthered by species-specific immune differences. The objective here is to Understand how porcine immune components such as, cytokines (IL3 and MCSF) and surfactant protein D (SP-D), affect human monocytes as it is pivotal for implementing potential immunomodulatory therapies.

Methods: In phase 1 compatibility of porcine IL-3 and M-CSF for human monocyte survival and differentiation to that of human IL3 and MCSF. Human Monocytes were cultured and differentiated to macrophages over 14 days using Human/Pig or Mixed IL-3 and M-CSF and viability was measured through live –dead assays.Phenotypic shifts were analyzed by investigating Surface markers (CD11b, CD80, CD206) via flow cytometry. Phase 2 was conducted to analyze the effect of porcine SP-D on human monocyte polarization and morphology. Human Monocytes were treated with increasing doses of pig SP-D and polarization patterns were analyzed by flow cytometry on Days 2, 8, and 14. Immunostaining of surface markers cd80, cd206 and CD11b was used to visualize phenotypic differences between human or pig SP-D treated human monocytes to get a preliminary understanding of the protein's immunomodulatory effects. Dose-response experiments are ongoing to determine the most suitable concentration for comparative analysis

Results and Conclusion: Preliminary data suggests F Porcine IL-3 combined with human MCSF/Pig MCSF is suppressive to human monocyte viability, perhaps secondary to receptor incompatibility, while porcine M-CSF when combined with human IL3 promote survival but is unable to induce effective differentiation. Additionally, porcine SP-D stimulates a pro-inflammatory phenotype(CD80) in human monocytes that is contrary to the usually immunoregulatory role of the human homolog. These findings suggest important species-to-species differences that potentially may contribute to graft rejection. It will be crucial to delineate and prevent such differences so that specific immunomodulatory strategies can be developed to further graft acceptance and advance the therapeutic potential of xenogeneic lung transplantation.

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P2.19 | To Investigate the Changing Trends in Cardiac Function Following Xenogeneic Heterotopic Heart Transplantation of Multi-Gene Edited Pig Hearts and Assess the Impact of Recipient Immune Responses on Donor Heart, Laying Experimental Groundwork for the Clinical Application of Gene Editing Technology

Zhipeng Ren, Huan Wang, Ziqiang Dai, Dianyuan Li
Cardiac and large vascular surgery, Suzhou Municipal Hospital, Suzhou, People's Republic of China.

Chengdu Zhongke Aoge Biotechnology Co., Ltd. Surgical methods of heart transplantation. The changing experience of xenogeneic heart transplantation in China.

If I am lucky enough to be selected for this oral presentation, I will give a presentation on three different simultaneous heart xenotransplantation procedures, including many empirical problems arising from transplantation. For example, is there not enough volume for two hearts in the chest?Since December 16, 2023, a research team in China has conducted five xenogeneic heterotopic heart transplants between pigs and rhesus monkeys. These procedures involved three parallel experimental models designed to assess graft function and recipient immune response under postoperative stress.

Among these cases, one recipient survived for 46 days post-transplant, demonstrating the feasibility of simultaneous xeno-transplantation. This marks a significant step forward, and the experience gained through these trials may offer valuable insights for colleagues in the field working toward improving xenotransplantation outcomes.

In this particular case, the transplanted pig heart was functionally integrated, partially supporting both pulmonary and systemic circulation in the recipient. The team also worked on optimizing the immunosuppressive protocol and surgical techniques specific to xenotransplantation. Notably, this case set a new national record for the longest survival time following a xenogeneic heart transplant in China.

While advancements in gene editing technologies have opened new possibilities for xenotransplantation, further research is necessary before these approaches can be translated into clinical practice.

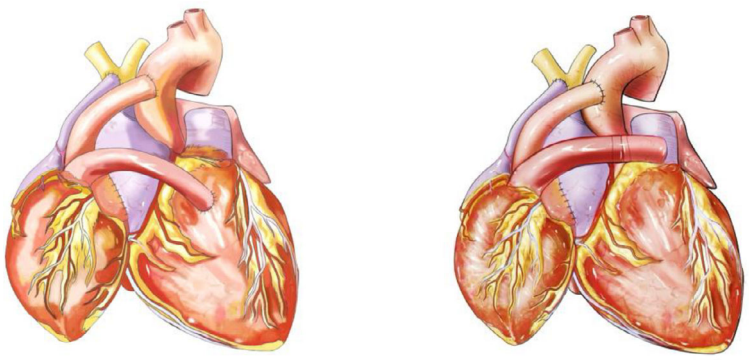


FIGURE 1 | P2.19

P2.20 | Influence of Gene Knockouts and Human Transgenes on the Interaction Between Human Blood and Porcine Lung-Derived Endothelial Cells in the Context of Pulmonary Xenotransplantation

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Introduction: In recent years remarkable progress has been achieved for heart/kidney xenotransplantation (XTx) in primates and humans. But there are still difficulties, especially for lung xenografts. In pig-to-primate xeno lung transplantation (XLTx), porcine lungs often fail immediately with maximum survival of one month so far [1, 2]. To overcome this hurdle, it is assumed that lung-specific incompatibilities need to be addressed. This project aims to identify genetic modifications in donor pigs in vitro that could have a positive impact on the tolerance in porcine-to-human XLTx. Endothelial cells (EC) were chosen as model for the first contact between xenograft and recipients' blood: I) Lung-specific and systemic ECs with different genetic modifications were isolated. II) EC subtypes and genotypes were characterized and functionally analyzed. III) ECs will be compared and exposed to human blood with an in vitro flow chamber model to investigate the lung-specific effects of genetic modifications.

Methods: To obtain primary lung-derived ECs, different isolation protocols were tested and compared, allowing the isolation of ECs from systemic and pulmonary arteries (PA), veins or microvasculature (MV) of porcine lungs. After confirmation of their EC characteristics (CD31, cobble stone morphology), the cells were tested for their EC subtype expression profile of DARC, CA4 and GJA5 via flow cytometry or immunofluorescence. They were also microscopically analyzed for endothelial-specific functionality by standard methods (scratch assay). Some of the ECs were selected for immortalization to obtain lung-specific cell lines with distinct genotypes for further analyses.

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Results: By perfusing larger vessels and MV, ECs from different tissue origins and with variants of genetic modifications (GGTA1-KO/+CMAH-KO, ß4GALNT2-KO (TKO)/+SLAI-KO (4KO) ± hCD46.hCD55.hCD59.hHO-1.hA20) were obtained. High proportions (>90%) of the isolated cells exhibited a cobble stone morphology and stained positive for CD31. The isolated systemic cells from aorta and vena cava served as controls for the arterial ($DARC^{+}$, $CA4^{++}$, $GJA5^{-}$) and venous ($DARC^{++}$, $CA4^{+}$, $GJA5^{++}$) subtype. Both PA and MV ECs tend to be more like aortic than venous cells but showed slight differences in their expression levels: PA ($DARC^{-}$, $CA4^{++}$, $GJA5^{-}$) and MV ($DARC^{+}$, $CA4^{++}$, $GJA5^{-}$).

Conclusion: Various subtypes of lung specific ECs have been obtained by isolating them from different vessels and genetically modified pigs. These subtypes can be distinguished by their DARC, GJA5, and CA4 expressions. The isolation and use of the lung-specific ECs (cell lines) under flow conditions could help to gain insides in how different genetic modifications of the donor cells can have organ-specific impacts on the outcome in XLTx.

References:

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P2.21 | Xenoperfusion of Triple Glycan Knock-Out, Seven Human Transgene Insertion Porcine Livers With Human Whole Blood

Alex Sagar[1], John Fallon[1], Mohamed Elzawahry[1], Syed Hussain Abbas[1], Leanne Lanieri[2], Kathryn Stiede[2], Kristen Getchell[2], Paula Hamilton[2], Carson Sutter[2], Amanda Marett[2], Michael Etter[2], Cassandra Miller[2], Kayla Janke[2], Rafferty Wyatt[2], Raquel Castro[2], Chris Morris[3], Dan Fower[3], Shawn Welch[3], Lydia Lamriben[2], Eric Brass[4], Gregory Hendricks[5], Kelly Cederquist[6], Frederick Vyas[6], Peter Abt[6], Charles Abrams[7], Abraham Shaked[6], Susan Low[2], Peter Friend[1,3]

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Introduction: Short-term xenografting of transgenic porcine livers holds promise as a therapy to temporarily support patients with acute and acute-on-chronic liver failure until recovery of native liver function or allotransplantation. Ex situ xenoperfusion is a technique in which a xenograft is perfused with human whole blood under physiological conditions outside the body. This may provide a platform, in parallel to in vivo experiments, to investigate xenograft viability, erythrophagocytosis, thrombocytopenia and drug metabolism.

Methods: Porcine livers (EGEN-5784) were procured from Yucatan minipig clones with the following genetic edits: (i) triple glycan knockout; (ii) hemizygous insertion of seven human transgenes; (iii) functional inactivation of porcine endogenous retrovirus pol gene (3KO.7TG.RI). The EGEN-5784 livers underwent normothermic perfusion with pooled human whole blood on a metra (OrganOx, Oxford, UK).

Five livers (3KO.7TG.RI: $n = 4$; 3KO.7TG: $n = 1$) underwent xenoperfusion for 48 h to assess xenograft viability and erythrophagocytosis. During xenoperfusions 4 and 5, CYP3A4 and

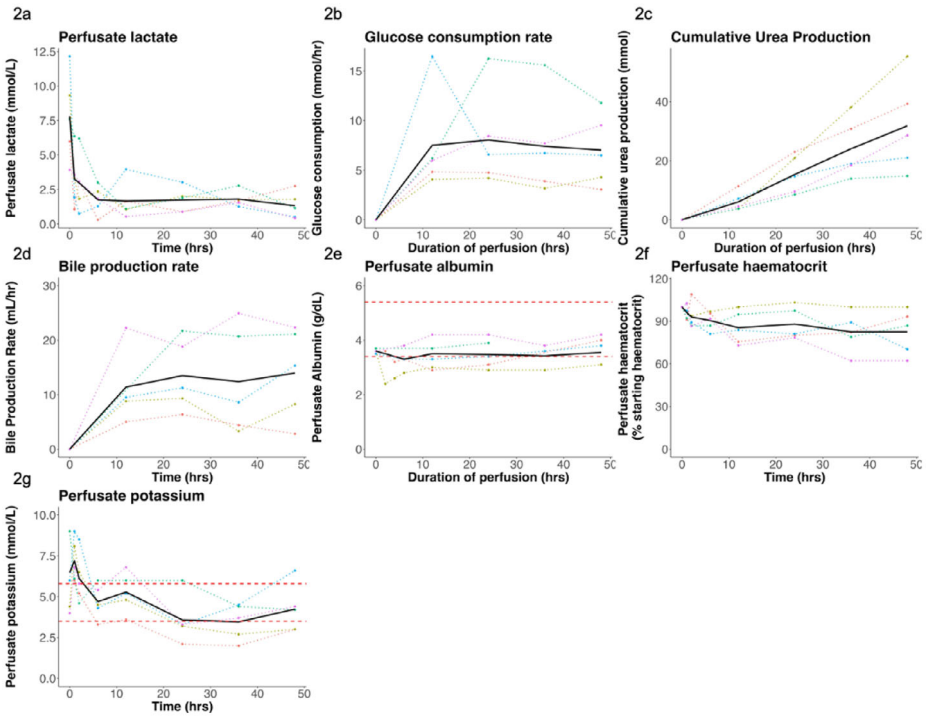


FIGURE 1 | P2.21

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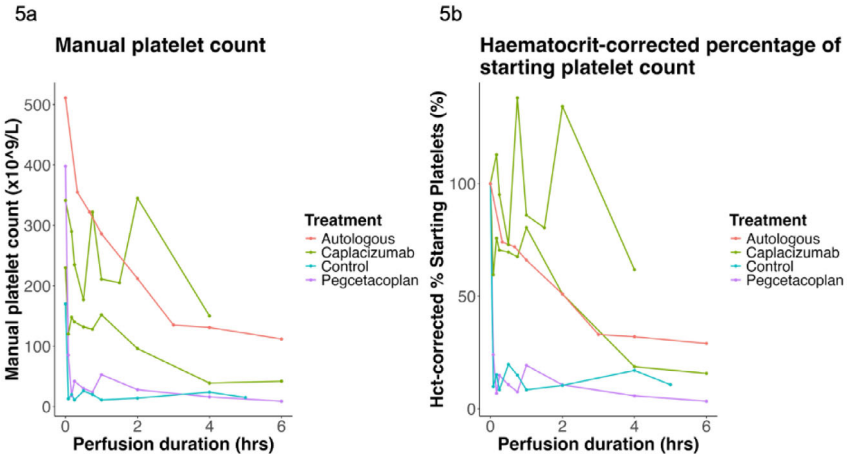


FIGURE 2 | P2.21

CYP2C19 activity was assessed by measuring midazolam and omeprazole clearance.

To assess the mechanism of early xenograft-associated thrombocytopenia, a further four livers (3KO.7TG.RI: $n = 4$) underwent 4–6-h xenoperfusion under three different conditions: (i) control: standard normothermic liver perfusion ($n = 1$), (ii) capalacizumab pre-treatment (von Willebrand factor inhibitor) ($n = 2$), and (iii) pegcetacoplan pre-treatment (complement C3 inhibitor) ($n = 1$). A single whole blood autologous wild-type pig liver perfusion was used as an 'autoperfusion' control.

Results: Viable xenoperfusion was maintained for 48 h for all five livers. The livers maintained physiological hepatic artery (0.27± 0.11 L/min) and portal vein (0.97± 0.04 L/min) flows, and demonstrated hepatic function throughout perfusion. The 48-h perfusate haematocrit was 83 ± 16 % of the starting value.

Intrinsic xenograft clearance of midazolam was 16.14 ± 2.83 L/min/kg and 10.85 ± 2.24 L/min/kg for xenoperfusions 4 and 5, respectively. Intrinsic xenograft clearance of omeprazole was 2.36 ± 0.87 L/min/kg and 1.89 ± 0.76 L/min/kg for xenoperfusions 4 and 5, respectively.

Control and pegcetacoplan pre-treated xenoperfusions demonstrated a rapid, profound decline in perfusate platelet count. Capalacizumab pre-treatment delayed the onset of thrombocytopenia. In these xenoperfusions, progressive platelet aggregation occurred in the hepatic sinusoids within the 1st hour, associated with sinusoidal endothelial von Willebrand factor staining.

Conclusions: Viable xenoperfusion of EGEN-5784 porcine livers can be maintained for 48 h, with evidence of preserved hepatic function and CYP450 activity. Early xenograft-associated thrombocytopenia appears to be mitigated ex situ by von Willebrand factor inhibition; this warrants further investigation in vivo.

P2.22 | Management of Pleural Effusions Following Orthotopic Cardiac Xenotransplantation in a Pediatric Non-Human Primate Model

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Objective(s): Xenotransplantation of genetically engineered porcine hearts (GEPH) has been utilized as destination therapy in adult human subjects under FDA expanded access criteria. We envision this technology can save the lives of human infants with critical congenital heart disease who are currently dying on the cardiac allotransplant waiting list. A successful animal model is necessary to study this application and prove concept.

Methods: Three olive baboons aged 29, 15, and 29 months and weighing 6.9, 4.8, and 6.1 kg respectively, underwent orthotopic cardiac xenotransplantation from size matched Yucatan miniature swine. Animals were maintained on immunosuppression (IS) centered on CD40 ligand blockade (Tegoprubart, Eledon Pharmaceuticals) for up to 8 months. The function of the xenograft was tracked on weekly echocardiogram and invasive hemodynamic monitoring. Immunologic responses were followed with flow cytometry. Xenografts were removed and surgically replaced with allografts from prospectively crossmatched, ABO compatible, size-matched donor baboons. Immunosuppression following this was transitioned to tacrolimus and mycophenolate mofetil. Functional and immunologic outcomes following allotransplantation were monitored in the aforementioned fashion.

Results: All animals survived >4 months with xenografts that exhibited normal systolic function up to the time of explant. There was no evidence of anti-pig antibody above pre-transplant levels nor was there evidence of same species sensitization (Figure 1). One out of three survived the allotransplant operation. The first animal was allowed to retain a small portion of porcine inferior vena cava (IVC) to facilitate repeat transplant. The second succumbed following a surgical error made in attempt to remove all porcine tissue. The third succumbed to volume overload during induction therapy prior to allotransplantation. Baboon #1 developed acute rejection 71 days following allotransplant and survived 105 days before elective euthanasia. Anti-pig antibodies increased in the setting of conventional immunosuppression and retained pig IVC.

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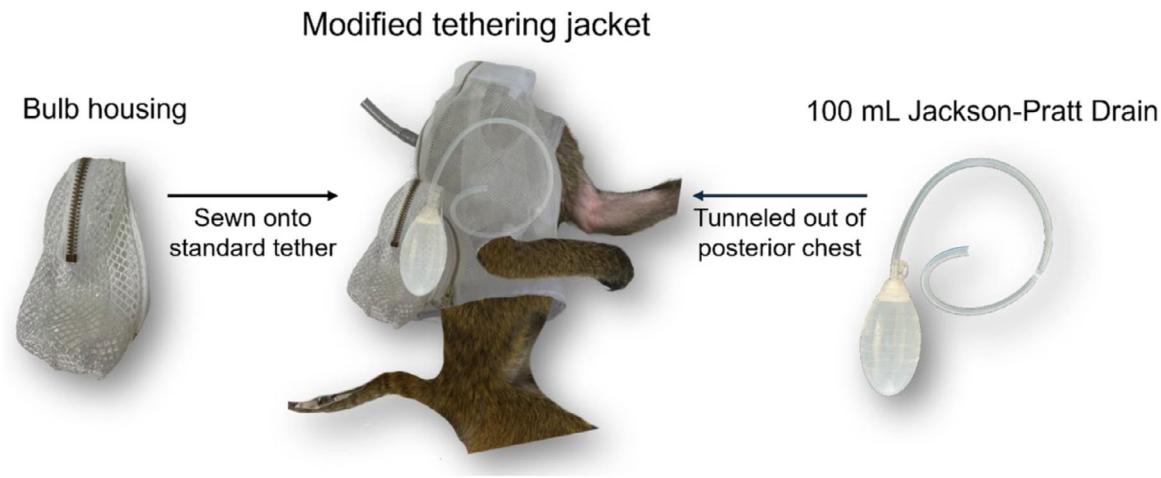


FIGURE 1 | P2.22

Conclusion: Herein is proof of concept that bridge to cardiac allotransplantation with a cardiac xenograft is feasible. Prolonged exposure to GEPH does not cause same species sensitization, but conventional immunosuppression is not capable of preventing reactivity to xeno-antigens. All pig tissue must be removed prior to transition to conventional IS regimens and surgical strategies must be tailored to allow for this. Further study is warranted in the animal model.

P2.23 | Experimental Study on Transplantation of Transgenic Edited Pig-Primate Transplantation of Heterotopic Hearts

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Introduction: To explore the changing trend of cardiac function after allogeneic heterotopic heart transplantation with transgenic edited pig hearts, and the influence of the receptor's immune response on the donor heart, providing an experimental basis for the application of gene editing technology in the clinical field.

Methods: From December 16, 2023 to March 17, 2025, four cases of allogeneic and ectopic heart transplants between pigs and rhesus monkeys were completed. The functional status of transplanted plants under the load state after transplantation and the immune indicators of the recipients were observed.

Results: The longest case of a recipient monkey has survived for 46 days, and no hyperacute or acute immune rejection reactions have occurred. Pathological analysis showed minor myocardial damage in the monkey heart but more severe damage in the porcine heart, suggesting chronic immune rejection.

Conclusion: Transgenic editing technology offers the possibility of xenotransplantation, but its clinical application still requires further in-depth research.

P2.24 | Comparison of Three Double Costimulatory Blockade Regimens for Suppressing Xenograft Rejection in Pig-to-Nonhuman Primate Kidney Xenotransplantation

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Introduction: Costimulatory blockade is one of the key immunosuppressants in kidney xenotransplantation. We investigated optimal combinations of double costimulatory blockade including two forms of anti-CD154 mAb (5C8, PG405), abatacept (CTLA-4Ig), and anti-ICAM-1 mAb (MD3) in pig-to-nonhuman primate kidney xenotransplantation.

Methods: Kidneys from six or eight genetically-engineered pigs (knock-out for GGTA1, CMAH, iGb3s, or B4GalNT2; knock-in for human CD39, CD46, CD55, or thrombomodulin) were transplanted into five cynomolgus monkeys. Common immunosuppressive regimen consisted of thymoglobulin, rituximab, anti-C5 inhibitor (crovalimab), etanercept, and triple maintenance immunosuppressants including prednisolone, tacrolimus, and mycophenolate mofetil. Additionally, three groups received different combinations of costimulatory blockade. Group A ($n = 3$) received modified anti-CD154 mAb with Fc modification and IL-10-conjugation (PG405) and anti-ICAM-1 mAb (MD-3). Group B ($n = 1$) received modified anti-CD154 mAb (PG405) and abatacept with anti-IL-6R mAb (tocilizumab). Group C ($n = 1$) received original anti-CD154 mAb (5C8) and abatacept with tocilizumab. Contralateral native nephrectomy was performed at 4 weeks after transplantation. Kidney xenograft biopsies were performed at 4 weeks post-transplantation and at later timepoints.

Results: The serum levels of BUN and creatinine after native nephrectomy were higher in groups A and B compared to those in group C. Furthermore, serum levels of proinflammatory cytokines (IL-6, TNF-α) and complement activation (membrane attack complex [MAC]) were lower in group C compared to those in group A. Serum levels of TNF-α were markedly increased, while those of IL-6 and MAC were low in group B. Notably,

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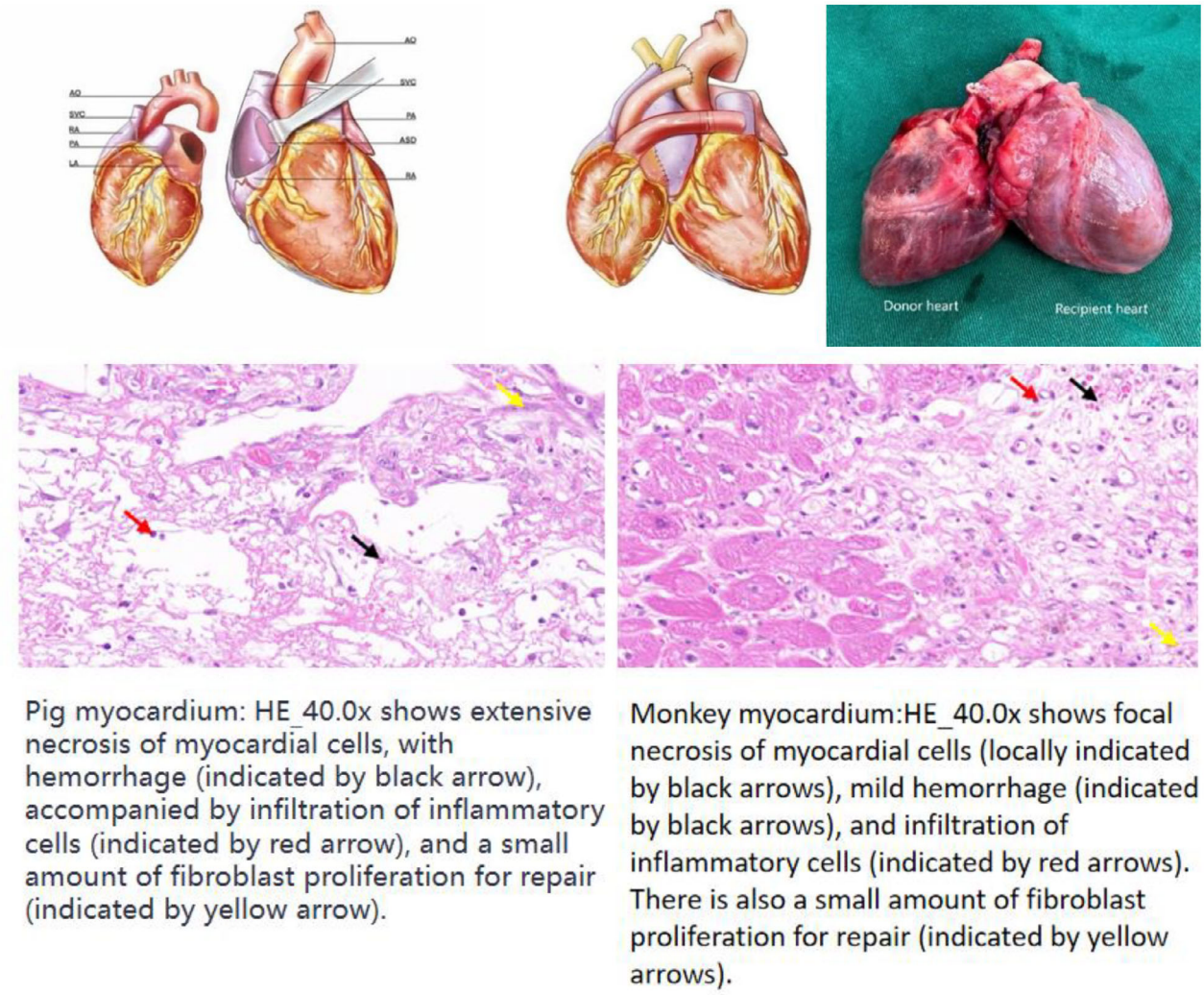


FIGURE 1 | P2.23

de novo anti-pig-specific antibodies were detected in groups A and B, whereas they were not in group C. However, anti-pig-specific T cell responses were not significantly different among three groups. Number of memory T cells, monocyte, and NK cells in peripheral blood were kept low in group C. In parallel, kidney xenograft biopsies showed that antibody-mediated rejection occurred in all monkeys of groups A and B, whereas it did not in group C. Groups A and B exhibited mesangiolysis, thrombotic microangiopathy, microvascular inflammation, and vasculitis. Additionally, immunohistochemistry staining showed that groups A and B had significant intragraft infiltration of CD3+ T cells, CD68+ macrophages, and CD61+ platelets with deposition of IgG, C4d, or MAC, whereas group C showed minimal intragraft infiltration of immune cells without significant IgG or complement deposition.

Conclusion: Combination of 5C8 clone of anti-CD154 mAb and abatacept seemed to suppress kidney xenograft rejection better than combination of a modified anti-CD154 mAb and anti-ICAM-1 mAb. Therefore, we need to develop new double costimulatory blockade strategies, including safer anti-CD154 mAb without thromboembolic complications.

P2.25 | Development of Proteinuria Following 4GE Pig-to-Rhesus Monkey Kidney Xenotransplantation Leads to Loss of Anti-CD154 Antibody and Accelerates the Process of AMR

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Introduction: The occurrence of proteinuria after xenotransplantation of gene-edited (GE) pigs to non-human primates (NHP) is relatively common and may have a serious adverse impact on the prognosis of the xenograft. Our team performed six cases of GTKO/βGalNT2KO/hCD55/TM (4GE) pig-to-rhesus monkey kidney xenotransplantation in 2024, during which anti-CD154 antibody treatment was administered. We observed the occurrence of proteinuria and its influence on the levels of anti-CD154 antibodies and antibody-mediated rejection (AMR).

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Methods: Six rhesus monkeys with low levels of preformed antibodies against donor pigs received kidney transplants from 4GE pigs. The recipient monkeys received induction therapy with anti-thymocyte globulin (ATG), C1-esterase inhibitors, and anti-CD20mAb (rituximab) and maintenance therapy with anti-CD154 antibody, tacrolimus, tocilizumab, and low-dose corticosteroids. Monitoring of the monkeys included frequent complete blood counts, serum metabolic panel (including serum creatinine, blood urea nitrogen, serum total protein, and albumin), and tacrolimus levels, with urine protein levels measured at intervals. Serum and urine levels of anti-CD154 antibody and anti-4GE pig IgM/IgG levels, and CDC were measured retrospectively.

Results: Six recipients survived for 57 (Case 1), 184 (Case 2), 61 (Case 3), 45 (Case 4), >184 (Case 5), and > 134 (Case 6) days. Among them, 5 cases developed moderate to severe proteinuria (500–8000mg/L) within 15 to 150 days. Except for Case 2 that proteinuria occurred about 5 months, others occurred about 1 month. After the occurrence of proteinuria, the serum creatinine can still remain within the normal level. However, by detecting the concentrations of anti-CD154 antibodies in serum and urine through flow cytometry, it was found that (1) A significant decrease in the concentration of anti-CD154 antibodies occurred with proteinuria simultaneously; (2) A large amount of anti-CD154 antibodies were detected in the urine, which was positively correlated with the level of proteinuria. With the loss of anti-CD154 antibodies, the levels of anti-donor pig IgM and IgG antibodies in 3 cases (Cases 2, 3, and 4) showed a significant increase. Pathological examination after graft failure revealed that Case 1 rapidly lost its kidney xenografts due to thrombotic microangiopathy (TMA), while three cases showed significant AMR. In addition, all five cases were accompanied by hypoproteinemia, with significant decreases in both albumin and total protein levels occurring simultaneously.

Conclusions: The changes of proteinuria after kidney xenotransplantation occur earlier than those of renal function and are important indicators that must be monitored regularly. Furthermore, the occurrence of proteinuria is associated with a significant loss of therapeutic anti-CD154 monoclonal antibodies. The loss of anti-CD154 antibodies leads to insufficient immunosuppression and the production of new antibodies, thereby accelerating the AMR process.

P2.26 | Endocrine Function of Life-Supporting 10-Gene-Edited Porcine Kidneys in a Nonhuman Primate Xenotransplantation Model

Daniel Eisenson[1], Alexander Schulick[1], Michelle Santillan[1], Kasra Shirini[1], Saghar Babadi[1], Du Gu[1], Kristy Koenig[1], William Clarke[2], Marc Lorber[3], David Ayares[3], Kristina DeSmet[3], Leigh Peterson[3], Hayato Iwase[1], Kazuhiko Yamada[1]

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Introduction: Xenotransplantation using genetically engineered pigs offers a potential solution to the organ shortage. While survival and immunologic compatibility are critical, the endocrine function of xenografts, specifically their role in erythropoiesis, electrolyte regulation, and the renin-angiotensin-aldosterone system (RAAS), remains poorly characterized. This study evaluated the ability of the 10-gene-edited (10GE) pig kidney to maintain physiologic endocrine functions in a nonhuman primate (NHP) model of life-supporting kidney xenotransplantation.

Methods: Twelve baboons received life-supporting 10GE Xenokidneys provided by United Therapeutics Corporation. Endocrine function was evaluated through serial measurements of hemoglobin, reticulocyte counts, serum electrolytes, calcium-parathyroid hormone-vitamin D axis markers, renin, and aldosterone. In vitro assays were used to assess cross-species binding of porcine erythropoietin (EPO) to the human EPO receptor. The immunogenicity of porcine EPO and renin was assessed using tiered total and neutralizing antibody assays. Blood pressure was monitored longitudinally. Baboons who received immunosuppression without transplantation were used as controls for comparison.

Results: 10GE Xenokidneys maintained creatinine clearance and electrolyte homeostasis until the onset of xenokidney failure. PTH levels were appropriately suppressed in all recipients, and 25- and 1,25-hydroxyvitamin D levels remained within physiologic range in all but three animals. Porcine EPO bound to the human EPO receptor in vitro. Only one animal required exogenous EPO, and no differences in reticulocyte counts were observed between transplant recipients and controls. Renin was undetectable in all recipients, though aldosterone remained measurable and physiologic blood pressure was maintained throughout the study. No animals developed anti-EPO antibodies. One animal developed transient anti-renin antibodies, which interfered with receptor binding in vitro but did not persist on subsequent testing.

Conclusion: The 10GE Xenokidney maintained critical endocrine functions in the NHP model, including calcium-PTH-vitamin D axis regulation and support of erythropoiesis. While renin was undetectable, downstream RAAS activity, as evidenced by stable aldosterone levels and normal blood pressure, was preserved. The absence of sustained anti-EPO or anti-renin antibody responses supports the immunologic safety of porcine endocrine protein expression in this context. These findings support the physiologic viability of the 10GE Xenokidney and inform future clinical application.

Funding: This study was supported by a grant from United Therapeutics Corporation.

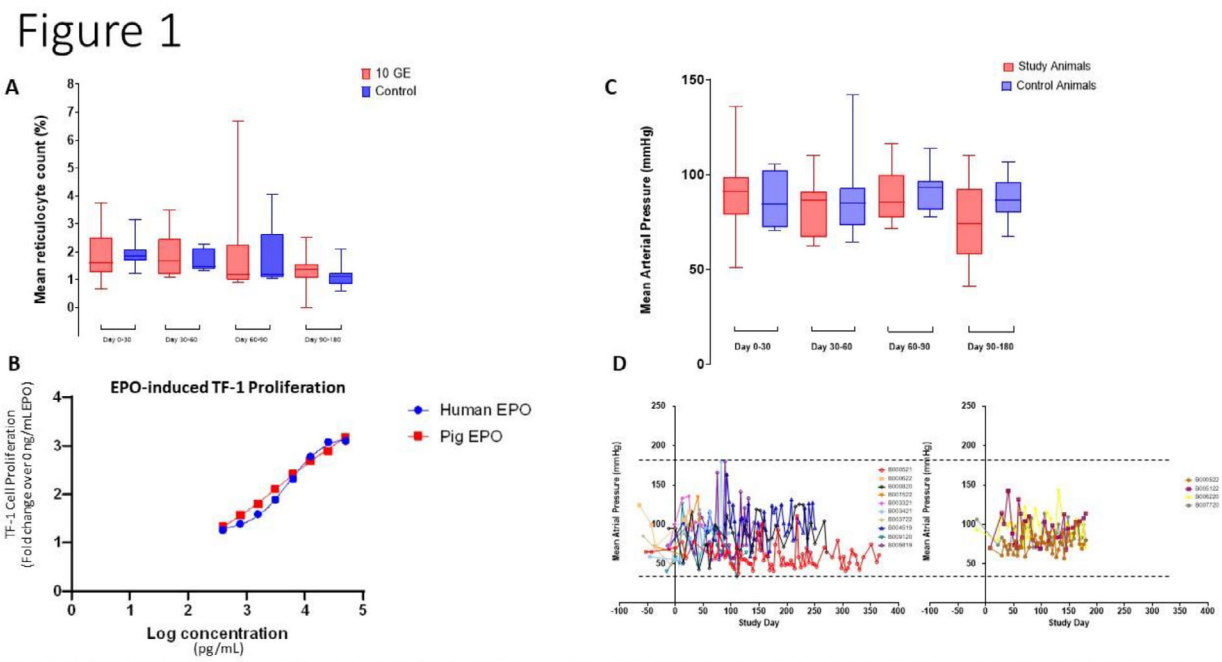
P2.27 | Source Specific Peripheral Blood Mononuclear Cells May Be More Sensitive Than Aortic Endothelial Cells for Detection of Anti-Pig Antibodies in Pig-to-Baboon Xenotransplantation

June Jones[1], Michelle R. Santillan[2], Alexander C. Schulick[2], Daniel Eisenson[2], Kasra Shirini[2], Saghar Babadi[2], Du Gu[2], Hayato Iwase[2], Donna Lucas[1], Kristina DeSmet[3], Leigh Peterson[3], David Ayares[3], Suyapa Ball[3], Daniel Warren[2], Andrew Cameron[2], Maria Bettinotti[1], Kazuhiko Yamada[2]

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Introduction: Xenotransplantation is an imminent clinical reality with clinical trials sponsored by two companies at multiple institutions. However, consensus is lacking regarding the

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Physiologic maintenance of reticulocyte count (A) and blood pressure (C and D) after life-supporting xenokidney transplantation as compared with immunosuppression control animals. *In vitro* model of EPO function utilizing both human and porcine EPO and human EPO receptor demonstrates comparable TF-1 proliferation (B).

FIGURE 1 | P2.26

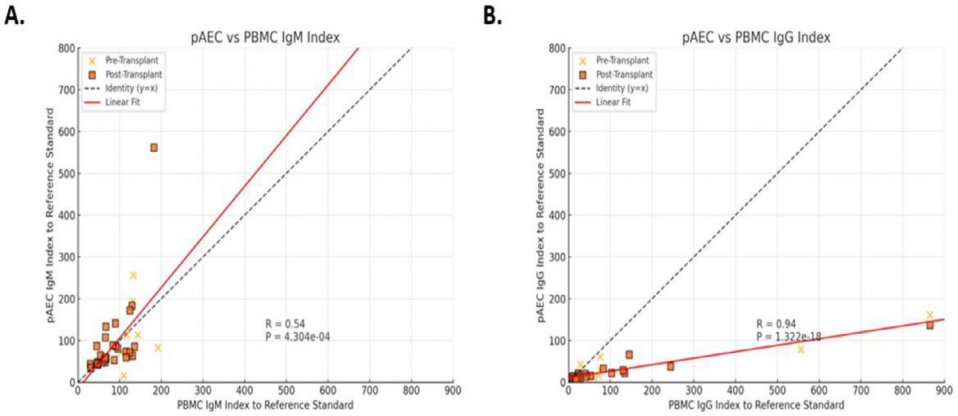


Figure 1: Comparison of IgM (A) and IgG (B) antibody binding between PBMCs and pAECs. Each point represents a pre- (yellow "×") or post- (orange square) transplant serum sample. Dashed lines denote the line of identity (y = x), and red lines indicate linear regression fits.

FIGURE 1 | P2.27

optimal screening strategy for prospective recipients and the choice of cellular substrate—peripheral blood mononuclear cells (PBMCs) versus porcine aortic endothelial cells (pAECs)—for post-transplant monitoring of donor-specific antibodies (DSAs). This study compares the sensitivity of each substrate using flow crossmatch in baboon xenograft recipients before and after pig-to-baboon kidney xenotransplantation.

Methods: Serum samples were collected from 10 baboon recipients of genetically engineered (10GE) porcine kidneys at defined pre- and post-transplant timepoints and indexed relative to a known reference standard. pAECs were isolated from the ascending aorta of the source pig, cultured, and frozen for testing. Both pAEC and PBMC testing used frozen cell preparations. Flow cytometry-based crossmatches were performed using source-specific pAECs and PBMCs to assess median fluorescence intensity (MFI) of IgM and IgG. Background MFI from unstained or no-serum controls was subtracted from each sample. DSA responses were quantified as index values normalized to a reference standard. Comparative analyses of IgM and IgG indices were conducted between pre- and post-transplant samples. SLA class I and II expression was characterized on both PBMCs and pAECs.

Results: Indexed IgM binding demonstrated near-parity between PBMCs and pAECs, with a moderate correlation ($R = 0.54$, $p < 0.0001$) and minimal deviation from the line of identity (Figure 1A). In contrast, indexed IgG binding to pAECs was

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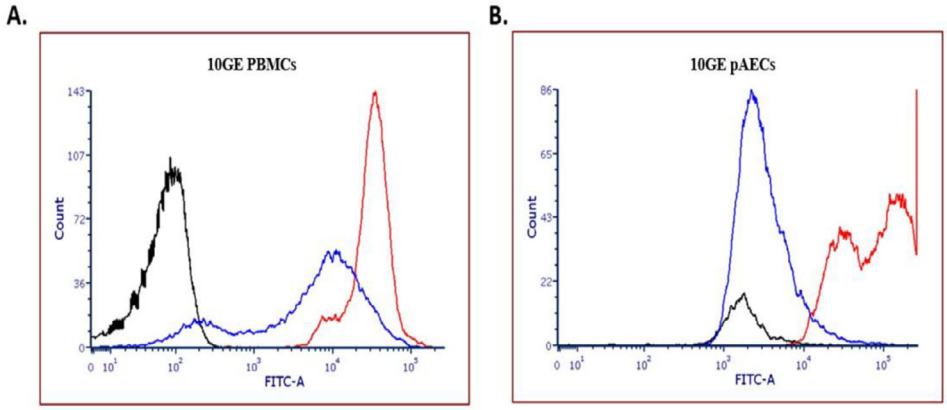


Figure 2: Expression of SLA Class I and II on 10GE PBMCs (A) and 10GE pAECs (B). Red lines correspond to SLA Class I expression and blue lines correspond to SLA Class II expression, while black lines correspond to unstained negative control.

FIGURE 2 | P2.27

consistently lower than to PBMCs, with a strong correlation ($R = 0.94$, $p < 0.0001$) and a regression line below the line of identity (Figure 1B). To help explain these differences, flow cytometric analysis of swine leukocyte antigen (SLA) Class I and II expression was performed. SLA class I (SLA-I) expression was comparable between PBMCs and pAECs, whereas SLA Class II (SLA-II) expression was markedly reduced on pAECs (Figure 2).

Conclusion: These findings suggest that IgM-targeted epitopes are consistently preserved across PBMCs and pAECs, while IgG-targeted epitopes may be absent on pAECs or selectively lost during endothelial culture. The diminished IgG binding observed on pAECs, combined with reduced SLA-II expression, suggests that cell surface marker expression may influence the sensitivity of pAEC-based assays for detecting IgG-dependent xeno-reactivity. These results highlight the importance of including PBMC-based assays alongside pAEC-based assays for comprehensive antibody testing in upcoming kidney xenotransplant trials. Accrual of these data will be crucial not only for immunologic risk assessment and post-transplant monitoring but also for the discovery of clinically relevant xenoantigens.

Funding: This study was supported by a grant from United Therapeutics Corporation.

P2.28 | HLA-Sensitized Human Sera Retain Cross-Reactivity to Genetically Modified Porcine Cells Via SLA Epitopes

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Introduction: Genetically modified pigs are promising organ sources for xenotransplantation, but the cross-reactivity of pre-existing anti-human leukocyte antigen (HLA) antibodies against pig antigens remains a key barrier. This study evaluated the humoral immune response of human sera classified by HLA antibody reactivity to genetically modified porcine peripheral blood mononuclear cells (PBMCs), aiming to characterize cross-reactive antibody reactivity patterns.

Methods: Porcine PBMCs were isolated from wild-type (WT), α1,3-galactosyltransferase knockout (GTKO), triple-knockout (TKO), and quadruple-knockout (QKO) pigs (Optipharm Inc.) using density gradient centrifugation. Human sera from 61 kidney transplant patients were classified into four HLA antibody reactivity groups: I+II+ ($n = 10$), I+ ($n = 10$), II+ ($n = 10$), I–II– ($n = 31$) using single-antigen bead (SAB) assays. Flow cytometry crossmatch (FCXM) measured IgG/IgM reactivity via median fluorescence intensity (MFI). Complement-dependent cytotoxicity (CDC-NIH/AHG) assays assessed functional antibody activity. Red blood cell (RBC) absorption removed major carbohydrate xenoantigens, while antibody elution from QKO PBMCs identified bound cross-reactive HLA antibodies via SAB. HLA eplet compatibility with swine leukocyte antigen was analyzed using structural analysis.

Results: Human sera showed strong IgG/IgM reactivity to WT PBMCs, while TKO and QKO showed markedly reduced antibody binding. For QKO pigs, sera from HLA class I+II+ patients demonstrated the highest IgG reactivity, with significantly higher median MFI ratios compared to HLA I–II– sera ($p < 0.05$). IgM responses to QKO were low across all groups. RBC absorption abolished reactivity to WT PBMCs but not QKO PBMCs, confirming elimination of major carbohydrate xenoantigens (α-Gal, Neu5Gc, Sda) in QKO. CDC assays revealed stronger cytotoxicity in HLA-reactive sera, correlating with FCXM results. Eluted antibodies from QKO PBMCs included HLA-A*01:01, B*44:02, and B*15:12, but not high-MFI alleles like A*02:01, indicating selective cross-reactivity. Structural analysis confirmed HLA eplets 144KR and 163LS/G as potential mediators of SLA cross-reactivity.

Conclusion: Histocompatibility assays, including optimized FCXM, effectively reflect immunological compatibility. HLA antibody profiles critically influence residual humoral responses

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to genetically modified pig cells, suggesting the need for comprehensive pre-transplant assessment in xenotransplantation.

P2.29 | Transforming Swine Leukocyte Antigen (SLA) Genotyping: Rapid and Accurate Determination of SLA Alleles by Long-Read Third-Generation Sequencing

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Introduction: Xenotransplantation with genetically modified pig organs represents a promising approach to address the shortage of human donor organs. Xenocompatibility between recipient human leukocyte antigens (HLA) and donor swine leukocyte antigens (SLA) plays a key role in the immune response to xenografts. Accurate SLA genotyping in donor pigs is essential for assessing potential cross-reactivity with HLA antibodies and understanding its clinical relevance. This knowledge is vital for informing targeted genetic modifications to reduce immunogenicity, improve xenocompatibility, and support long-term graft survival. Here, we introduce a rapid and accurate method for high resolution SLA genotyping using third-generation long-read sequencing technology.

Methods: Sequencing was performed on the Oxford Nanopore Technologies MinION Mk1B platform. Data analysis and typing assignments were conducted using a research prototype of the GenDx NGSengine-Turbo software pre-configured with all officially designated SLA sequences and alleles from the IPD-MHC database v3.12.0.0.

Results: Locus-specific primers were designed to amplify all classical SLA genes (SLA-1, -2, -3, -DRA, -DRB1, -DQA, -DQB1). Whole-gene amplification was achieved for all loci except DRB1, where exon 1 was excluded due to the presence of an unusually long intron 1. A total of 65 samples, sourced from both outbred commercial farm pigs and inbred research miniature pigs, were included in the validation and optimization process. Typing results were cross-referenced with previously determined low-resolution genotypes, as well as high-resolution haplotypes and alleles inferred from published SLA nomenclature reports. The method demonstrated a high level of concordance and successfully identified novel alleles that had previously been missed by prior low-resolution typing approaches. The sample cohort encompassed 46 distinct SLA haplotypes (comprising 30 unique Class I and 22 unique Class II haplotypes) with over 130 alleles, representing over 30% of the 426 officially designated alleles across the 7 targeted SLA genes. Our protocol enables multiplex sequencing and analysis of up to 24 samples per run, with a typical turnaround time of under 12 h.

Conclusion: In summary, we have developed a rapid, high-resolution SLA typing system leveraging third-generation long-read sequencing to address the growing demand for improved xenotransplantation strategies. By enabling accurate characterization of SLA alleles in donor pigs, this system enhances our understanding of xenocompatibility and facilitates more precise matching between donor pigs and human recipients, ultimately promoting the success and long-term survival of xenografts. The ability to process multiple samples per run with a fast turnaround time underscores the system's efficiency, practicality, and scalability for both research and clinical applications.

P2.30 | Characterization of SLAs in Pig Xenotransplant Donors for Personalized Patients Histocompatibility

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One of the major challenges in organ transplantation is reducing the risk of rejection, which is especially critical in xenotransplants. Studies have identified key molecules targeted by preformed antibodies in recipients of pig xenotransplants. These xenoantigens include cell surface glycans such as alpha-Gal, SDa, and Neu5Gc. Pigs lacking these xenoantigens were produced by somatic cell nuclear transfer (SCNT) using fibroblasts that were edited to remove the genes responsible for these molecules, followed by implantation of knockout embryos into synchronized surrogates. Eliminating these glycans reduced hyperacute rejection and enabled the identification of additional antigenic molecules, particularly Swine Leukocyte Antigens (SLAs), the pig orthologs of Human Leukocyte Antigens (HLAs). Some patients awaiting organ transplants have pre-formed antibodies against SLAs, which can jeopardize transplant safety and recipient survival. Therefore, we aimed to characterize the SLAs present in pig fibroblasts used as donors for SCNT. This would allow us to express these specific SLAs on the surface of lymphoblastoid cells lacking HLAs and assess immunoreactivity. Our results will be essential to determine if a patient is more suitable to receive an organ from a particular clonal group of our edited donor pigs. First, SLA genes from fibroblasts of five different pigs were amplified by PCR from isolated genomic DNA. The PCR products were cloned using the pGEMT system and sequenced by Sanger sequencing. In parallel, SLA mRNA was amplified by RT-PCR from isolated RNA and sequenced. Finally, sequences were assembled and aligned to compare them and determine their identity. Amplification of a region including SLA exons 2 and 3 from genomic DNA yielded two bands: one 495 base pairs (bp) long and another 545 bp long. Amplification of a larger region including exons 2 to 4 yielded a single 1345 bp band. BLAST alignments confirmed these sequences as SLAs. Our analysis showed that the main difference between the 495 bp and 545 bp products lies in the intron sequence. Notably, the 495 bp product showed low similarity to both the 1345 bp product and the transcripts, suggesting it may be a pseudogene. The 545 bp and 1345 bp sequences matched each other well and aligned with the transcripts, confirming amplification of SLA class I genes. Finally, the SLA sequences from the five pigs were grouped into three distinct SLA clusters. In conclusion, three different SLA sequences should be considered when immunologically testing if a patient is more likely to receive an organ from one pig group or the other.

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P2.31 | Immune Modulation in Xenolung Transplantation: Microfluidic Glycan Reduction and Surfactant D Effects on Human Monocytes

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Purpose: Xenoantigenic glycan structures on porcine endothelial cells (pECs) represent a major immunological hurdle in pulmonary xenotransplantation. Due to the lung's extensive endothelial interface, hyperacute rejection (HAR) risk remains high despite genetic modifications. While key glycans such as αGal and Neu5Gc are known, additional tissue-specific structures are less well characterized. Chemical glycan oxidation may help reduce such unknown immunogenic barriers. In parallel, porcine surfactant protein D (SP-D), a collectin with immunomodulatory roles in host defense, may influence human innate immune responses via receptors like SIRPα and calreticulin, potentially affecting macrophage polarization in a xenogeneic context.

Methods: pECs were isolated from porcine aorta and pulmonary arteries and cultured under flow in a 48-well microfluidic system. Oxidation was performed using sodium periodate at 10°C, followed by lectin staining (Isolectin IB4, Sambucus Nigra, RCA I) to assess glycan removal. Cell viability was measured via immunofluorescent live/dead staining, and hemocompatibility was tested using human serum. In additional experiments, SP-D was isolated from porcine bronchoalveolar lavage fluid and purified. Human monocytes were incubated with various SP-D concentrations (1, 15, 50, 100 μg/mL), and surface marker expression (CD14, CD80, CD206, CD11b) was analyzed by flow cytometry to explore phenotypic changes

Results: Oxidation led to a marked reduction in αGal and Neu5Gc expression, with improved pEC viability and reduced human serum-mediated damage. Surfactant experiments showed human monocyte exposure to porcine SP-D resulted in dose-dependent changes in surface marker expression. While the interpretation remains preliminary, repeated experiments suggest a tendency toward M2-like polarization. These findings raise the possibility that porcine SP-D may influence innate immune activation in the xenogeneic interface.

Conclusion: This study presents two complementary strategies to modulate early xenogeneic immune responses: (1) chemical glycan reduction on pECs and (2) porcine SP-D-mediated modulation of human monocytes. While promising, the observed SP-D effects require further validation. Planned follow-up experiments include functional CD80 activity assays and cytokine profiling (e.g., IL-2, IL-6, TNF-α, IFN-γ) to clarify immunological consequences and potential downstream effects on T cell recruitment. Translation of these findings to ex vivo lung perfusion (EVLP) models may support future applications in xenolung transplantation.

Keywords: EVLP, glycan oxidation, microfluidic system, monocyte polarization, surfactant D, xenotransplantation

P2.32 | Innate Immune–Driven Fibrotic Remodeling as a Key Driver of Chronic Kidney Xenograft Injury

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Introduction: Kidney xenotransplantation is emerging as a promising solution to the organ shortage crisis. However, the molecular mechanisms underlying xenograft failure remain poorly understood. To realize the clinical potential of kidney xenotransplantation, it is critical to elucidate the molecular and cellular characteristics that drive xenograft failure.

Methods: Life-supporting renal xenotransplantations were performed using genetically engineered pigs with targeted coagulation modifications (GE6 to GE8) into cynomolgus monkeys. The immunosuppressive regimen comprised multiple agents, including anti-CD154 monoclonal antibody, abatacept, tocilizumab, crovalimab, corticosteroids, tacrolimus, mycophenolate mofetil, and etanercept. Renal biopsies were obtained at predefined intervals and at euthanasia. Integrative molecular profiling was conducted using bulk RNA sequencing ($n = 14$), single-nucleus RNA sequencing (snRNA-seq, $n = 15$), and spatial transcriptomics (Visium, $n = 12$). Single-cell resolution cell type expression profiles derived from snRNA-seq were mapped onto spatial transcriptomics data using cell2location. Intercellular communication analysis was performed using LIANA and NicheNet to delineate molecular interactions and signaling events.

Results: Histopathological analysis showed that chronic injuries were exclusively identified after week 4 (Table 1), which was accompanied by a transcriptional transition identified through integrative analysis of bulk and single-nucleus RNA sequencing datasets. snRNA-seq data identified two noticeable degenerative stromal populations—dFIB and dVSMC/P—marked by high FN1, ACTA2, COL3A1, and COL1A1 expression. Enrichment analysis identified extracellular matrix organization and collagen biosynthesis as the predominant pathways in these stromal populations, reflecting active and pathological extracellular matrix remodeling driven by excessive collagen production and assembly by degenerative stromal cells. Intercellular-communication modeling revealed a directional axis from nonclassical monocytes (ncMonocyte) and M2 macrophages to these stromal cells, with TGF-β1 and SPP1 as principal ligands. Spatial transcriptomics showed TGF-β target genes concentrated around glomeruli (median distance < 500 μm), indicating a periglomerular fibrotic niche driven by innate immune–mediated signaling (Figure 1).

Conclusions: Our findings reveal that innate immune cell–mediated fibrotic remodeling underlies chronic injury in kid-

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Table. Longitudinal Comparison of Banff Scores at Sequential Biopsy Time Points

Chronic score		D0	2W	≥4W	Acute score		D0	2W	≥4W
ct***	0	8 (100.0)	4 (100.0)	1 (8.3)	g*	0	8 (100.0)	4 (100.0)	4 (40.0)
	1	0 (0.0)	0 (0.0)	4 (33.3)		1	0 (0.0)	0 (0.0)	5 (50.0)
	2	0 (0.0)	0 (0.0)	2 (16.7)		2	0 (0.0)	0 (0.0)	1 (10.0)
	3	0 (0.0)	0 (0.0)	5 (41.7)	v	0	8 (100.0)	3 (100.0)	5 (41.7)
ci***	0	8 (100.0)	4 (100.0)	1 (8.3)		1	0 (0.0)	0 (0.0)	4 (33.3)
	1	0 (0.0)	0 (0.0)	2 (16.7)		3	0 (0.0)	0 (0.0)	3 (25.0)
	2	0 (0.0)	0 (0.0)	1 (8.3)	ptc	0	8 (100.0)	4 (100.0)	10 (83.3)
	3	0 (0.0)	0 (0.0)	8 (66.7)		1	0 (0.0)	0 (0.0)	1 (8.3)
cg*	0	8 (100.0)	4 (100.0)	4 (36.4)		2	0 (0.0)	0 (0.0)	1 (8.3)
	1	0 (0.0)	0 (0.0)	5 (45.5)	t	0	8 (100.0)	4 (100.0)	4 (66.7)
	2	0 (0.0)	0 (0.0)	1 (9.1)		1	0 (0.0)	0 (0.0)	1 (16.7)
	3	0 (0.0)	0 (0.0)	1 (9.1)		2	0 (0.0)	0 (0.0)	1 (16.7)
t_IFTA*	0	8 (100.0)	4 (100.0)	4 (33.3)	i	0	8 (100.0)	4 (100.0)	4 (80.0)
	1	0 (0.0)	0 (0.0)	4 (33.3)		1	0 (0.0)	0 (0.0)	1 (20.0)
	2	0 (0.0)	0 (0.0)	4 (33.3)					
i_IFTA***	0	8 (100.0)	4 (100.0)	1 (8.3)					
	1	0 (0.0)	0 (0.0)	9 (75.0)	Fisher exact test *p<0.05, **p<0.01, ***p<0.001				
	2	0 (0.0)	0 (0.0)	2 (16.7)					

FIGURE 1 | P2.32

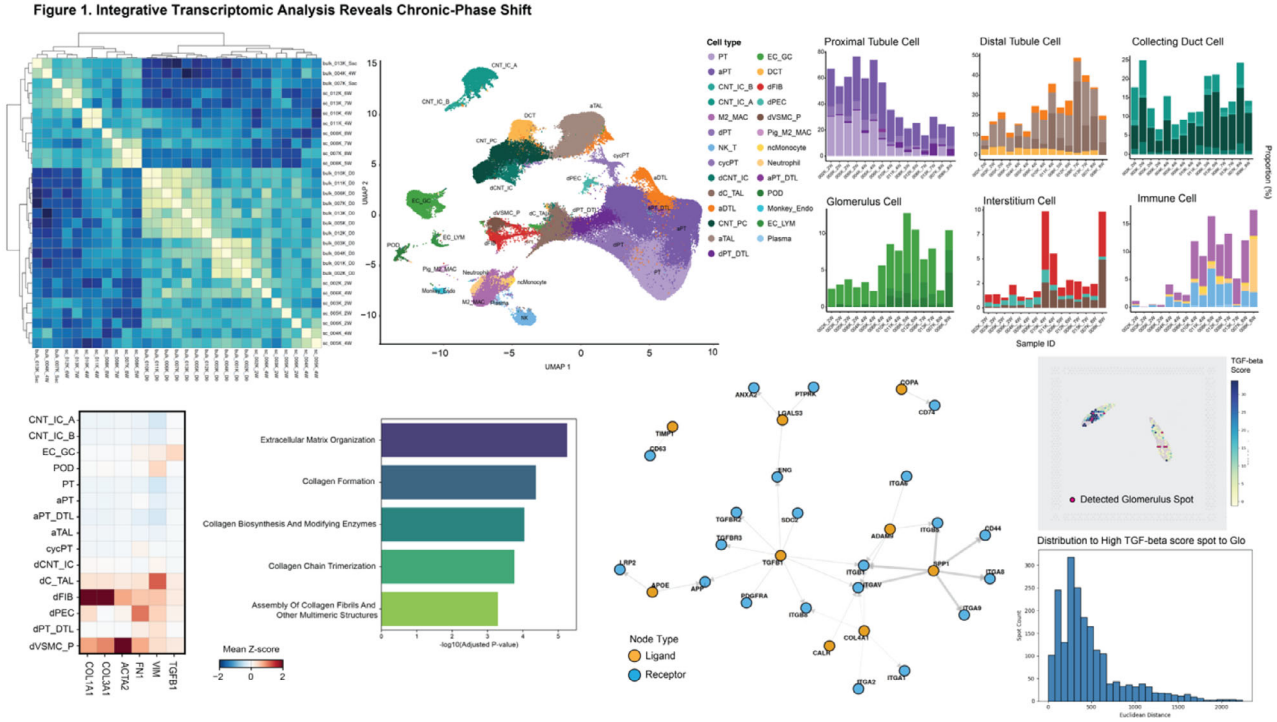


FIGURE 2 | P2.32

ney xenografts, with the periglomerular region serving as a focal site of pathological extracellular matrix activation. These results highlight TGF-β1–driven signaling as a pivotal mechanism of chronic graft failure and suggest that targeting innate immune–fibrotic interactions may enhance long-term xenograft survival.

Funding: This research was supported by a grant from the Korea Health Technology R&D Project through the Korea Health Industry Development Institute (KHIDI), funded by the Ministry of Health & Welfare, Republic of Korea (grant number: HI23C0417). This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT) (RS-2024-00341570).

P2.33 | Nursing Care for Brain-Dead Patients in Xenotransplantation and Xenotransfusion Trials: Practical Insights and Challenges

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Background: From 2021 to 2024, our hospital conducted two cases of genetically-engineered (GE) pig kidney xenotransplantation and one GE pig red blood cell (pRBC) xenotransfusion in brain-dead human subjects as preclinical xenotransplantation

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models. The specialized care provided by nursing staff played a critical role in these trials, requiring adaptation to unique challenges. This study aims to explore practical insights gained from the care of brain-dead subjects undergoing xenotransplantation and xenotransfusion, with the goal of educating nursing staff and identifying the essential components of specialized nursing care for these procedures. The focus is on optimizing care strategies and addressing key challenges related to infection prevention, hemodynamic management, and pressure ulcer prevention.

Methods: Nursing care protocols were developed to meet the specific needs of these patients, with an emphasis on pressure ulcer prevention through frequent repositioning and the use of specialized bedding. Infection prevention strategies, particularly targeting respiratory infections, were reinforced by a 2-week antibiotic regimen. Hemodynamic stability was ensured through continuous monitoring and the careful adjustment of vasopressors and fluid administration according to the patient's condition.

Results: In the two kidney xenotransplantation cases, rejection occurred after 10 days, while the xenotransfusion case concluded as planned after 14 days. The nursing team successfully implemented preventive measures, minimizing pressure ulcers and infections. Hemodynamic stability was maintained through timely interventions using vasopressors and fluids. Care optimization: In order to prevent the occurrence or further spread of pressure sores, in the nursing process, in addition to frequent turning over and reducing the pressure time in the same place, air cushion bed and hydrocolloid excipients can be used to protect the pressure part, and joint massage and other measures can effectively reduce the occurrence of pressure sores.

Challenges: In the process of nursing, the biggest challenge we face is the management of fluids and the principle of fluid rehydration according to the positive and negative balance mechanism of patients. However, the recessive intake and recessive water loss of patients have not been calculated more accurately.

Conclusion: Nursing care for brain-dead subjects involved in xenotransplantation and xenotransfusion trials presents unique challenges and requires specialized approaches. The practical insights gained from these cases underscore the critical role of nursing in supporting advanced xenotransplantation research. These insights are invaluable for preparing for future preclinical trials involving brain-dead subjects and lay the foundation for the eventual clinical application of xenotransplantation in living human patients.

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