CONSENSUS STATEMENT

The 2026 International Society for Heart and Lung Transplantation Consensus Statement on clinical cardiac xenotransplantation

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1. THE ISHLT 2000 CONSENSUS REPORT

In the year 2000, the International Society of Heart and Lung Transplantation (ISHLT) issued a report from the xenotransplant advisory committee on the role of xenotransplantation in the treatment of end-stage cardiac and pulmonary diseases.[1] The consensus report acknowledged the urgent need for more thoracic organs and highlighted xenotransplantation as one potential solution. Significant hurdles were identified including challenging

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immunologic barriers, uncertainty regarding whether anti-HLA antibodies might cross-react with pig antigens, and risks of transmissible infections. After taking stock of the field of xenotransplantation and of preclinical and clinical thoracic organ transplant results to date, the committee highlighted the necessity for further research before initiation of clinical trials could be considered, including specific benchmarks for preclinical heart and lung xenotransplant models. Additionally, the document addressed ethical considerations surrounding the choice of source animal and the potential for porcine organs to provide life-sustaining function in the human recipient.

After two decades, considerable strides have been made in the realm of xenotransplantation generally and of cardiac xenotransplantation more specifically, including the performance of two clinical heart xenotransplant cases. Yet, the landscape remains fraught with challenges reminiscent of those identified in the ISHLT's initial report. In this consensus blueprint for the future, we collectively redefine some previously articulated challenges, review the state of the field, and outline recommendations for moving the field forward.

2. ORIGINAL RECOMMENDATIONS OF THE 2000 ISHLT XENOTRANSPLANTATION ADVISORY COMMITTEE

Based on experimental data at the time, the committee concluded that a clinical trial of heart or lung xenotransplantation was premature. The report emphasized that achieving at least 60% survival of life-supporting pig organs in a minimum of 10 non-human primates for at least three months with good recipient condition and without life-threatening complications from immunosuppression (IS) or infection would establish an acceptable benchmark to move towards human xenotransplantation. The report recommended that patients entering a trial should either be ineligible for allotransplantation or 'destination' mechanical circulatory support (MCS), or unlikely to survive until a human organ becomes available and for whom MCS as a 'bridge' was not feasible. This report also emphasized the importance of national regulatory bodies to oversee all trials, preferably with international coordination among regulators. Infectious disease surveillance and study outcome monitoring, in the form of a registry facilitated by independent organizations like the ISHLT, was also advised. Furthermore, the authors recommended that no trial should be undertaken until infection transmission risks were minimal by experts in microbiology and the relevant regulatory. Finally, the committee recommended that the relationship between the presence of anti-HLA antibodies and anti-pig antibodies, their cross-reactivity, and the outcome of pig-organ xenotransplantation in recipients previously sensitized to HLA antigens should be closely investigated.[1]

3. IMMUNOLOGY OF PIG-TO-HUMAN ORGAN XENOTRANSPLANTATION

A successful pig organ xenotransplant must overcome both the innate and adaptive immune system hurdles as well as species-dependent biochemical incompatibilities involving the coagulation and complement regulatory pathways. The initial 'hyperacute rejection' response by a human recipient of an unmodified 'wild type' pig organ is mediated by natural (preformed) anti-pig antibodies that bind primarily to glycans (carbohydrates) on the vascular endothelial cells of the pig organ (Table 1), which activate the complement system, resulting in hyperacute rejection. A systemic inflammatory response to injury of the pig tissue augments the innate immune response and activates the coagulation system. Molecular incompatibilities between the coagulation-anticoagulation systems of human and pig either cause or amplify the development of thrombotic microangiopathy in the graft and consumptive coagulopathy in the recipient, leading to

Table 1 Glycan Xenoantigens that have been Deleted in Gene-edited Pigs

Carbohydrate (Abbreviation)	Responsible Enzyme	Gene-knockout Pig
1. Galactose-α1,3-galactose (αGal)	α1,3-galactosyltransferase (GGTA1)	*GGTA1*-KO
2. N-glycolylneuraminic acid (Neu5Gc)	Cytidine monophosphate-N-acetylneuraminic acid hydroxylase (CMAH)	*CMAH*-KO
3. Sda	β-1,4N-acetylgalactosaminyltransferase 2 (B4GALNT2/B4GALNT2L)	*B4GALNT2/L*-KO

graft failure and recipient death.[2] These immune and biochemical barriers have been tackled thus far by a combination of (i) genetic engineering (GE) of the organ-source pig and (ii) the administration to the recipient of an effective immunosuppressive regimen (e.g., blockade of the CD40/CD154 T cell co-stimulation pathway).[2,3] In the gene-edited pig, the genes responsible for the three known pig antigens (αGal, Neu5Gc, Sda), against which humans have natural antibodies, have been deleted (Table 1), and 'protective' transgenes have been introduced.[4] These include human genes for the expression of complement pathway-regulatory proteins (CPRPs; e.g. CD46, CD55, CD59), coagulation-regulatory proteins (e.g., thrombomodulin, endothelial protein C receptor, tissue factor pathway inhibitor), and anti-inflammatory proteins (e.g., heme oxygenase-1, A20, CD39). These combinations of gene knock-outs and protective transgenes are designed to prevent early rejection and improve transplant survival. However, the adaptive immune response results in high levels of *de novo* antibodies to other pig antigens. To combat this, other genetic manipulations are possible such as those targeting downregulation of autoreactive T cells by PD-L1.[5] To avoid cross-reaction of antibodies to human leukocyte antigens (HLA) with swine leukocyte antigens (SLA), SLA can be inactivated in the pig.[6] To further minimize the innate cellular immune response, which involves macrophages, and natural killer cells, additional transgenes may be introduced into the porcine genome (human CD47, HLA-E and -G1).[2,7] Current gene editing strategies alone may not be consistently sufficient to support long-term pig organ survival in nonhuman primates. To suppress systemic complement activation associated with the surgical procedure, a very short course of a complement inhibitor (e.g., a C1-esterase, C3, or C5 inhibitor), may be beneficial, as may longer-term anti-inflammatory therapy by IL-6 receptor blockade with tocilizumab.[8,9]

As the recipient will otherwise produce antibodies or cellular immune responses against porcine protein or carbohydrate antigens that are not present in the recipient, an effective immunosuppressive regimen is required to suppress the activation of T and B cells. Studies have demonstrated that combinations of conventional immunosuppressive agents (e.g. cyclosporine, tacrolimus, mycophenolate mofetil), are relatively unsuccessful in suppressing the adaptive immune response to a pig organ.[2,10] Pig hearts with relatively few genetic modifications have enabled increasingly consistent survival following orthotopic transplantation in baboons using co-stimulation pathway blocking monoclonal antibodies targeting the CD40/CD154 pathway.[11]

All in all, immunological training mechanisms remain a very active field of research and are of interest to both innate and adaptive immunity, which will likely allow for a better understanding of rejection mechanisms in xenotransplantation.

4. PRECLINICAL MODELS: STATE-OF-THE-ART, ISSUES AND CHALLENGES

Thoracic organ xenotransplantation has been modeled primarily in cynomolgus monkeys and baboons, with the latter prioritized in recent work. Extensive work in these nonhuman primates (NHPs) using heterotopic heart transplantation models facilitated approaches to overcome first hyperacute rejection and then 'delayed xenograft rejection' (DXR: thrombotic microangiopathy in the graft, consumptive coagulopathy in the recipient) using hearts from wild-type and GE pigs. Since the 2000 report, median survival in non-human models has gradually improved from one to two months using *GGTA1*-KO or *GGTA1*-KO.hCPRP (human complement regulatory proteins, designed to suppress complement-mediated injury) hearts. Additional expression of hTBM (human thrombomodulin, a key endothelial anticoagulant protein) on *GGTA1*-KO.hCD46 hearts was associated with consistent avoidance of DXR in both abdominal heterotopic and orthotopic heart transplant models, with recipient survivals consistently exceeding 3 months in one series.[8,12,13] Importantly, as opposed to cold static preservation, hypothermic oxygenated cardiac perfusion along with agents to reduce growth and blood pressure has produced consistent graft survival for upto 6 months, and the perfusion method was repeated with the extension of survival to 9 months.[8,13]

Triple-knockout (TKO) pigs lacking GGTA1, CMAH and B4GALNT2/B4GALNT2L have been designed and created for use in humans based on identification of Neu5Gc (synthesized by CMAH) and Sda (synthesized by B4GALNT2/L) as carbohydrate targets of preformed or elicited anti-non-Gal antibodies that are injurious to *GGTA1*-KO hearts and other organs. Work in NHP (Non Human Primate) models using hearts or kidneys from TKO pigs, with or without other gene modifications, has unexpectedly revealed that the *CMAH* gene knockout unveils a putative '4th xenoantigen' that causes a positive 'crossmatch' in all NHP species, as determined by a conventional complement-dependent cellular cytotoxicity assay. As such, preclinical results using TKO kidneys are inferior to those using *GGTA1*-KO organs, and best-in-class kidney results in a large series of multi-gene-edited (GE) cynomolgus pig kidney recipients showed that approximately 33% of graft failure within two months after transplant was largely attributable to antibody-mediated graft injury escaping control by co-stimulation-based IS.[14,15]

Refinements to the IS regimen such as increasing the dose of anti-CD154 (also known as anti-CD40L) antibody and adoption of conditioning regimens (T- and B-cell depletion, complement inhibition) that are used clinically in high-immunologic risk organ allograft recipients may prove sufficient to overcome this barrier of sensitization in the NHP model. However, most experts believe that the NHP recipient overestimates the difficulty of accomplishing long-term pig organ survival in clinical application as these studies are done in the context of a 'positive cross-match' against TKO organs in NHPs, which will not be present for TKO organs in most prescreened humans. While this problem may be addressed by using double-KO pig organs—including *GGTA1*- and *B4GALNT2*/*L*-KO but omitting the *CMAH*-KO—this approach directly conflicts with FDA guidance that preclinical success must be demonstrated using the same genotype of pigs as are intended for use in any clinical trial. For this reason, some in the field argue that consistent long-term success in NHP models should not remain a prerequisite to undertaking pilot studies or even clinical trials in carefully selected, fully informed patients who lack other viable therapeutic options.

5. HUMAN CARDIAC XENOTRANSPLANTATION EXPERIENCES

At the time of this writing, two porcine cardiac xenotransplants into humans have been performed using organs from cloned source pigs with 10 genetic modifications (10-GM): TKO to eliminate three immunodominant xeno-antigens αGal, Neu5Gc, and Sda; KO of the growth hormone receptor (*GHR*) gene to reduce intrinsic xenograft growth; transgenic expression of human CD46 (membrane co-factor protein) and CD55 (decay-accelerating factor) to mitigate antibody-dependent complement graft injury; transgenes for the human thromboregulatory proteins thrombomodulin and endothelial cell protein C receptor to compensate the inefficiencies of porcine-derived blood factors for the activation of protein C; and transgenes for the human anti-inflammatory proteins CD47 and heme oxygenase 1.[2]

6. THE FIRST CASE

The landmark first gene-edited pig-human transplant was carried out on January 7, 2022, in a 57-year-old man with advanced heart failure (HF) in the setting of non-ischemic cardiomyopathy complicated by progressive pump failure and severe ventricular arrhythmias requiring resuscitation and peripheral veno-arterial extracorporeal membrane oxygenation (ECMO).[16] He had been deemed ineligible for traditional allotransplantation due to a history of inadequate adherence to treatment. In addition to marked debility associated with his non-ambulatory status and deteriorated condition, his 6-week pre-xenotransplant course was notable for chronic bone marrow suppression with mild leukopenia and thrombocytopenia, adrenal insufficiency, gastrointestinal bleeding and treated bacteremia.

The early peri-operative course was complicated. The xenograft required a second circulatory arrest to repair a type A dissection; an endovascular stent was subsequently needed in an upper-pole left renal artery due to residual dissection. Renal replacement therapy was required due to persistent oligo-anuric acute renal failure. Significantly, planned immunosuppression protocols for B-cell and T-cell depletion induction needed to be modified in the context of his pre-transplant thrombocytopenia and leukopenia. Otherwise, maintenance immunosuppression with the anti-CD-40 ligand humanized monoclonal antibody (KPL-404, Kiniksa, Lexington), mycophenolate mofetil (MMF) and methylprednisolone (125 mg rapidly tapered to 30 mg daily) proceeded initially as planned. Overall, by the end of week 1, the clinical course looked highly promising. ECMO was discontinued on Day 4, and the xenograft was functioning well (cardiac output ranging between 5 to 6 liters per minute and a normal left ventricular (LV) ejection fraction $\geq$ 55% and LV diastolic wall thickness between 1.2–1.4 cm). Systolic blood pressure ranged between 130 to170 mmHg requiring antihypertensive treatment.

During weeks 2 to 5, the overall trajectory was stable, despite emerging surgical, immunosuppression and infection issues requiring tailored management. Abdominal pain and concerning imaging findings led to an exploratory laparotomy confirming focal small bowel ischemia complicated by suppurative multi-microbial peritonitis, which was treated with washout, antibiotics and several weeks of parenteral feeding. Maintenance immunosuppression was adjusted from week 3 onwards due to severe neutropenia, with MMF discontinued and ultimately tacrolimus initiated with a low target level (3–5 ng per ml). Despite rigorous negative testing prior to xenotransplantation, weekly porcine cytomegalovirus (PCMV)/porcine roseolovirus (PRV) microbial cell-free DNA

(cfDNA) testing became positive on day 20, with subsequent testing of the donor animal spleen and recipient's peripheral blood mononuclear cells confirming latent PCMV infection. Antiviral therapy was then changed from ganciclovir to cidofovir (it should be noted that this agent while effective against human CMV has not been known to be effective against PCMV). On the day of the 1st xenograft biopsy (day 34) all appeared satisfactory. The heart was functioning well by hemodynamics and imaging and the biopsy revealed no evidence of rejection. The patient was continuing rehabilitation.

Just after week 6 (beginning day 43), the overall course began to turn. The patient became unstable, requiring intubation and pressors; chest radiograph showed infiltrates in both lungs and bronchoscopy found diffuse shallow ulcers throughout the airways which were negative for viral inclusions. Although briefly extubated, on day 49, further deterioration was evident with a precipitous rise in serum lactate associated with hypotension, abdominal distension and confirmed low output syndrome with low mixed venous saturation (33%). Echocardiogram confirmed significantly increased LV wall thickness to 1.7 cm and reduced LV chamber size. A repeat laparotomy found no abnormalities and ECMO was reinstated later that evening. A repeat xenograft biopsy the next day did not indicate the presence of ISHLT-defined acute cellular or antibody-mediated rejection (AMR). However, edema alongside focal capillary damage and antibody staining in capillaries – negative for C3d or C4d – was seen, troponin I level was increasing, and it was later found that levels of xenograft-derived cfDNA were also peaking from this time point. Treatment for a presumed atypical AMR was started, including plasma exchange, a second dose of iv immunoglobulin (1st dose Day 43 for hypogammaglobulinemia), complement inhibition and rituximab. Repeat endomyocardial biopsy on day 56 revealed pathologic AMR grade 1 with C4d staining present, reduced edema but 40% myocyte necrosis. Echocardiogram showed hyperdynamic function with no significant improvement in wall thickness. ECMO could not be weaned to below 2 liters per minute. The patient was severely deconditioned and had experienced worsening cachexia, with weight dropped to a nadir of 62 kg from initial 85 kg. Given the overall clinical picture, together with progressive diastolic xenograft failure, the decision was reached to compassionately withdraw care (day 60).

Although hyperacute rejection was avoided, xenograft failure occurred due to diffuse endothelial injury suggesting a form of AMR. Intravenous immunoglobulin, given as a treatment for hypogammaglobulinemia and AMR, was found to bind strongly to donor endothelium, possibly causing or contributing to immune endothelial activation and injury. Reactivation and replication of latent PCMV/PRV in the xenograft could have contributed to graft damage.[17] These observations informed the management of the second case.

7. THE SECOND CASE

The second 10-GE pig heart was transplanted in a 58-year-old man on September 20th, 2023.[18] In planning for the second transplant, serological (western blot) and polymerase chain reaction assays for the detection of latent PCMV were negative at several timepoints as was tissue from the donor pig after organ recovery. The patient's serum anti-pig antibody levels were low, with screening IgG markedly lower than in the first patient. Complement-dependent cytotoxicity testing against 10-GE pig cells indicated unresponsiveness. As in the first pig heart transplant, blocking CD40L (Tegoprubart, Eledon Pharmaceuticals, Irvine) was the mainstay of immunosuppression, which was administered the day prior to surgery; it must however be mentioned that the desired serum drug trough levels were only reached on POD 8.[18] The remaining immunosuppression was the same as in the first case (including use of a C1 esterase inhibitor).

Prior to xenotransplantation, while on inotropic therapy, the patient suffered cardiac arrest secondary to ventricular arrhythmia with successful resuscitation. He developed renal dysfunction preoperatively ultimately necessitating continuous renal replacement therapy shortly after transplant surgery with persistence of anuria. In the postoperative period, he had mediastinal bleeding complications, necessitating blood product transfusion. He had a prolonged need for vasopressor therapy and required pacing to augment cardiac output, which after pacing was associated with low filling pressures but a marginal cardiac index (1.9 L/min/m^2). On day 13, an endomyocardial biopsy suggested antibody-mediated rejection (AMR) which was treated with therapeutic plasmapheresis (and replacement fresh frozen plasma, later found to have anti-pig antibodies). He ultimately suffered graft failure associated with AMR in the biopsy and by day 31 required mechanical circulatory support. There were no porcine infectious complications. On postoperative day 40, withdrawal of care and life sustaining measures was requested by the patient and his family.

The immunohistochemistry screening of three endomyocardial biopsies and terminal xenograft samples demonstrated immunoglobulin (predominantly IgG than IgM) and complement (C3d&C4d) binding, despite low anti-non-Gal antibody levels and troponin values in recipient serum. The histology showed contraction band necrosis, an anterior transmural infarction, which may have been attributed to vasoactive use, exogenous calcium infusions, and continuous pacing. Transthoracic echocardiography showed preserved systolic function for the first 3 weeks. Cell-free porcine DNA analysis showed a progressive increase after 30 days, and post-mortem molecular profiling demonstrated a pattern of AMR and a potential role of innate immunity in graft dysfunction.

The second 10-GE pig heart transplant provides further clues about the mechanism of graft dysfunction and xenograft AMR. Immunosuppression, especially in the early postoperative phase, and exposure to exogenous anti-pig antibody infusion through blood product administration may need to be carefully adjusted based on specific mechanisms contributing to the loss of graft function. Overall, the patient's clinical condition, AMR, and innate immune response likely drove xenograft dysfunction. Infection surveillance utilizing microbial cell free DNA evaluation was successful in establishing absence of zoonoses.[18] Moreover, the expression levels of the protective human transgenes have not been evaluated, and questions remain whether gene expression declines under some circumstances and if that influences outcomes.

8. SURGICAL, ANESTHESIOLOGIC AND PERIOPERATIVE CONSIDERATIONS

The surgical experience in two human heart recipients was informed by experiments in baboons. Pigs posture is unguligrade while the primates are orthograde. This drives the pig heart to orient antero-posteriorly with the apex pointed to the sternum. An oversized donor pig heart risks compression that is less common with similarly sized orthograde hearts in which the apex rests laterally along the left hemidiaphragm. A large left azygous vein enters the left side of the pig's coronary sinus and must be oversewn, preferably before implant of the heart. A bi-atrial implant technique in NHP was less technically challenging than the bi-caval anastomoses preferred in human heart transplantation. In pigs, the inferior vena cava (IVC) enters the right atrium at a perpendicular angle to the superior vena cava (SVC), unlike the linear, in-axis orientation in primates. Early efforts to perform bi-caval anastomoses in this setting frequently resulted in kinking and outflow obstruction, which were challenging to detect and correct intraoperatively.

As pigs grow, their hearts lag variably in scale. Female pigs have 5–10% smaller hearts than male counterparts. Echo images for LV mass are difficult due to pig chest anatomy obscured by donor animal obesity due to *GHR*-KO. Stroke volume can substitute as a surrogate. It is reproducibly calculated from RV outflow tract area velocity timed index (VTI). While the donor hearts were removed on site with the human operating rooms, the hearts were perfused in the XVIVO box (Gothenburg, Sweden) for less than 45 min with an oxygenated, 8 °C Steen solution perfusion system. By using this technique, donor preservation perfusion was extended to over 3 h and the two human patients avoided the need for inotropic support. In other experiments, preservation of donor hearts destined for transplantation in baboons was less effective, even with cold ischemic time of below 30 min.

Human xeno-heart recipients present atrial mismatches which can be accommodated by small pig suture bites and the removal of human atrial tissue from the roof of the left atrium and lateral aspect of the right atrium. The great vessels of the pig are at most 50% of the size of the patient's vessels and may require resizing during implantation. The separation from cardiopulmonary bypass has not generally required inotropic support (which seems poorly tolerated by the pig heart) early after its reperfusion.

Preoperative estimation of donor organ size may be useful to avoid mismatch and can be done by cardiac imaging (e.g. CT scan or echocardiography) when screening for heart/valve anomalies prior to explantation from the donor pig. In the second case, the donor heart may have been undersized and weighed 273 gms.[18]

General anesthesia should be induced and maintained (by either total intravenous anesthesia with propofol or inhalation anesthesia) as defined by the individual clinical standard for allotransplantation. Monitoring should at least include pulse oximetry, continuous electrocardiography, invasive blood pressure measurement, urinary catheter, cardiac output (CO) measurement and transesophageal echocardiography. As pulmonary artery catheters may be difficult to place due to anatomical differences, the use of transpulmonary thermodilution should be considered. All inotropic substances and vasopressors may be used for weaning the xenograft from CPB but as indicated earlier, should be avoided as the donor organ does not tolerate such agents well due to susceptibility

to arrhythmias. Antiarrhythmic therapy (e.g. amiodarone, lidocaine) should be commenced early as indicated. Electrolyte imbalances may occur during surgery requiring careful substitution (especially hypocalcemia and hypopotassemia).

Postoperative management strategies require engagement of the heart team and should be multi-disciplinary (including cardiac surgeons, cardiologists, anesthesiologists, intensivists, immunologists, pharmacists and infectious disease specialists); a team experienced in preclinical xenotransplantation should be briefed regularly and stand by for consultation. Besides general practices equivalent to standard procedures defined for cardiac allotransplantation, it is imperative that blood products for transfusion should generally be avoided since most such products contain undetected anti-pig antibodies. If transfusions are inevitable, prior screening for anti-pig antibodies is advisable and such processes should be available in a timely manner; elimination of IgG antibodies with imlifidase (a cysteine protease derived from the immunoglobulin G-degrading enzyme of *Streptococcus pyogenes*) may be considered although this has not been used in the human cases.[19] The use of intraoperative cell salvage (ICS) should be mandatory; preoperative autologous blood donation should be considered.

In the post-operative setting, attention should be paid to monitor heart function/rejection, pulmonary effusions, xenozoonoses (e.g., PCMV) and overgrowth of the donor organ including occurrence of myocardial edema. If the recipient develops arterial hypertension postoperatively, antihypertensive drugs are mandated to prevent triggering of myocardial hypertrophy; mTOR inhibitors may be considered if cardiac overgrowth occurs despite genetic growth inhibition (e.g. *GHR*-KO).

9. IMMUNOSUPPRESSION IN HUMAN XENOTRANSPLANTATION

Current genetic modifications addressing complement and coagulation regulation, and natural antibodies have led to a reduced risk of hyperacute xenograft rejection.[2] Although an effective immunosuppression strategy for human xenotransplantation has not yet been demonstrated, conventional immunosuppression alone is not deemed sufficient as seen in models of both pig hematopoietic progenitor cells and kidneys from genetically modified pigs into baboons.[20,21] This was also reported in an early porcine kidney xenotransplant in a brain-dead human, where despite the use of anti-thymocyte globulin and anti-CD20 mAb with CNI, mycophenolate, and steroid-based immunosuppression, severe coagulopathy occurred in the recipient.[22] More recently, six-month survival of a 10-GM pig kidney in a baboon with only FDA approved immunosuppression was reported, however, all kidneys in this study went through rejection soon after this period.[23]

Blocking the CD40/CD40L pathway has been shown to improve graft survival in NHP models.[3,13] Administration of anti-CD154 (CD40L) mAb successfully prevented acute rejection and improved graft survival in several animal models. However, thromboembolic complications (thought to be related to CD154 which is expressed on platelets) were reported in a NHP kidney model which could be partially prevented by the administration of heparin and more completely by use of ketorolac tromethamine alone.[24] An immunoglobulin G4 anti-CD154 (anti-CD40L) monoclonal antibody, TNX-1500, which retains the fragment antigen binding region of ruplizumab (humanized 5c8, BG9588), was modified by protein engineering to decrease Fc binding to Fc-gamma receptor IIa, while retaining certain other effector functions and pharmacokinetics comparable with natural antibodies. Lassiter and colleagues reported that treatment with TNX-1500 is not associated with platelet activation in vitro *and* consistently inhibits kidney allograft rejection in vivo without clinical or histologic evidence of prothrombotic phenomena.[25]

Targeting the CD154-CD40 pathway using the anti-CD40 mAb as shown in xenotransplant models were used in the first two genetically modified pig to human heart transplants.[16–18] Although not commercially available, several formulations are under investigation in preclinical trials. Mohiuddin et al. reported heterotopic pig heart xenograft survival in NHP of over 1 year when recipients were treated with a chimeric anti-CD40 mAb weekly in addition to mycophenolate twice a day, anti-thymocyte globulin and anti-CD20 mAb in addition for induction, and steroids for the first 4–6 weeks.[26]

The complement pathway is an essential target in xenotransplants to eliminate the risk of hyperacute rejection from complement-dependent cytotoxicity. One or more human complement pathway regulatory proteins (CD46, CD55, CD59) can be expressed in GE source pigs to address this vulnerability.[2] However, the use of complement inhibitors as part of the immunosuppressive strategy may also be beneficial. The anti-C5 antibody, Tesidolumab was given weekly for 70 weeks in 7 pig kidney xenotransplants in NHP versus 10 controls.[27] Those animals that

received anti-C5 antibody therapy showed delayed graft loss from antibody-mediated rejection and improved graft survival compared to controls. Another group gave 2 doses of the C5 inhibitor, eculizumab, in 2 of 3 cases of porcine kidney xenotransplantation in brain-dead humans. The 2 decedents that received eculizumab had no evidence of thrombotic microangiopathy (TMA) or membrane attack complex (MAC) on biopsies POD1 and 3, whereas the no eculizumab decendent's POD1 biopsy showed early TMA and rare MAC that became more diffuse by POD3.[28] The first and second genetically modified pig to human cardiac transplants were treated with a C1 esterase inhibitor at the time of the transplant.[16–18]

As stated earlier, the use of human intravenous immune globulin and transfused blood products may introduce anti-pig antibodies and therefore should be avoided unless adsorbed against donor cells or pig cells or organs from the donor's genotype. Plasmapheresis and use of other therapies, such as protease inhibitors or complement inhibitors, have not been fully tested. Plasmapheresis and complement inhibitors were unsuccessful to reverse cardiogenic shock or massive cardiac hypertrophy in either human case.[16–18]

10. IMMUNE MONITORING AND PATHOLOGY AFTER XENOTRANSPLANTATION

After allotransplantation, surveillance for cellular and antibody mediated rejection are achieved via a combination of antibody detection of donor-specific antibodies (DSAs) quantification, gene expression profiling in peripheral blood lymphocytes, cell-free donor DNA monitoring, and histologic or molecular assessment of endomyocardial biopsy. The presence of high titers of 'natural' preformed antibodies (nAbs) to porcine antigens (xenoantigens) triggers an immediate destructive immune response termed hyperacute (HAR), typically by triggering activation of complement pathway cascade, in which proteins amplify activity of and damage to endothelial cells and leading to platelet aggregation and microvascular thrombosis. Most of human-anti-pig Abs are against porcine carbohydrate antigens that are not found in humans, such as galactose-α1–3 galactose (αGal; the most predominant, at around 85%), Sda and Neu5Gc.[2] If not prevented by effective immunosuppression, elicited Abs against non-Gal-antigens also play a major role in posttransplant thrombotic microangiopathy, consumptive coagulopathy, and antibody-driven graft injury, which collectively are termed acute humoral xenograft rejection (AHXR) or delayed xenograft rejection (DXR).[2] To this effect, pathology of the first genetically modified porcine-to-human cardiac xenotransplantation demonstrated scattered myocyte necrosis, interstitial edema and red-cell extravasation without microvascular thrombosis, findings distinct from "typical" allograft rejection, but commonly observed in preclinical cardiac xenograft models.[16,17] Characterization of the mechanisms driving various rejection-associated pathologies in the setting of xenotransplantation may therefore require further work.

A collection of non-invasive tools such as gene expression profiling and donor-derived cell free DNA in blood are emerging in allotransplantation for surveillance of immunological challenges however their value remains underexplored in xenotransplantation.[29–31] An approach using genetically engineered porcine endothelial cells to evaluate human immune responses in vitro has been developed.[32,33] In this technique five genes including *GGTA1*, *CMAH*, *B4GALNT2*/*L*, *SLAI* α chain, and β2-microglobulin (*B2M*) that are responsible for the production of major xenoantigens (αGal, Neu5Gc, Sda, and SLA-I) were sequentially disrupted in immortalized porcine endothelial cells using CRISPR/Cas9 technology which dramatically reduced the antigenicity of the porcine cells, though the cells still retained their ability to provoke human natural killer cell activation. This method could be further developed to measure specific xeno-immune responses in vivo although will require focused investigation.[33] Detection of the proportion of porcine cell free DNA in human recipient blood may hold promise in xenotransplantation as a marker of ongoing graft injury or immune processes.[16–18] A quantitative real-time PCR (qPCR) was used to monitor immune rejection in xenotransplantation through circulating pig-specific DNA (cpsDNA) in blood samples in pig-to-mouse cell transplantation models and pig-to-monkey artery patch transplantation models. This method was suggested to be useful for its simplicity, convenience, and low cost and may hold promise in immune surveillance.[33,34] The molecular microscope diagnostic system (MMDx), which is used to separate AMR, cellular rejection, mixed rejection or other causes of injury could be modified in xenotransplantation for diagnosis of immune responses, but this will require much greater experience in understanding the diverse phenotypes and their specific implications.[35] X-ray phase contrast imaging may offer a non-destructive way to assess the full myocardial sample in three dimensions, which may improve the accuracy of detecting acute cellular rejection.[36] How these newer methods may aid in understanding of detection and monitoring for rejection in xenotransplantation remains to be elucidated.

Endomyocardial biopsies that are performed should include standard formalin-fixed, paraffin-embedded tissue for H&E and immunohistochemistry, a piece fresh/frozen for potential immunofluorescence to look for antibody/complement/fibrin deposition, and a piece in glutaraldehyde to evaluate the ultrastructure of the microvasculature by electron Microscopy. The appropriate cadence of biopsies remains uncertain and initially could mimic the strategies advocated for allotransplantation.

11. INFECTIOUS DISEASE CONSIDERATIONS

Xenotransplantation carries the risk of transmission of microorganisms from the donor to the immunosuppressed recipient.[37] As for allotransplantation, this risk is determined by the microbiology of the source animal, the recipient, as well as environmental exposures.[38] Thus, infection due to both porcine and human pathogens would be expected to be amplified in the human xenotransplant recipient. Some porcine zoonotic viruses are known, i.e., viruses inducing disease in humans including Hepatitis E virus genotype 3 (HEV3) or swine influenza.[37]

Other viruses such as the porcine cytomegalovirus, a porcine roseolovirus (PCMV/PRV), is not zoonotic per se, in that it does not infect human cells, but may cause systemic manifestations when introduced into the recipient with a xenotransplant.[39,40] Such viruses are termed "xenozoonotic". PCMV/PRV induces systemic coagulopathy, inflammation and graft rejection in nonhuman primates transplanted with a PCMV/PRV-positive pig organ.[40] The presence of PCMV/PRV DNA was demonstrated in the first human recipient receiving a pig heart and was deemed to have contributed to morbidity and death of the patient.[17,18] In the absence of effective therapy for PCMV infection, breeding of PCMV-free swine requires serological and molecular assays and early weaning of newborns from latently infected sows.

Porcine circoviruses (PCV1–4) are ubiquitous but have not been associated with human disease. PCV3 replication was observed in baboon recipients of porcine cardiac xenografts.[41] Recent reports of a human circovirus associated with clinical hepatitis in an organ transplant recipient, and exacerbation of PCV-infection by other pathogens common in transplantation, suggests that PCV should be monitored or excluded in pigs bred as organ donors.[42,43] Porcine lymphotropic herpesviruses (PLHV) are gamma herpesviruses associated with lymphoproliferative disease in immunosuppressed mini-swine but do not appear to infect human or NHP cells in vitro.

A potential infectious risk of xenotransplantation is the porcine endogenous retroviruses (PERVs).[2] PERV-A and PERV-B are present in variable copy numbers in the germ line of all pigs while PERV-C is absent in some strains. Recombinants between PERV-A and PERV-C, PERV-A/C, were found in the genome of somatic pig cells, indicating ongoing recombination activity of PERVs in vivo.[44] Human cells have receptors for PERV-A, -B and A/C, but not for PERV-C. In vitro, PERV infects few, generally transformed, human cell lines.[45] Infection of primary human cells is rare, and PERV has never been transmitted in preclinical or clinical trials of pig tissues. Assays for PERV in recipients require correction for false-positive testing due to microchimerism.[46] Several strategies have been developed to prevent PERV transmission, such as selection of pigs lacking PERV-C (thereby avoiding the more aggressive PERV-AC recombination), or those that express low levels of PERV A or B, vaccines, CRISPR/Cas-based genomic editing and antiretroviral drugs. In vitro data show that reverse transcriptase inhibitors zidovudine (AZT), tenofovir (TDF), and adefovir (ADF), as well as the integrase inhibitors raltegravir and dolutegravir can inhibit PERV.[37,47]

To detect these viruses, protocolized surveillance strategies and sensitive and specific molecular and immunological assays are required. Metagenomic or next generation sequencing (NGS) or cell free DNA sequencing may supplement standard assays for unknown pathogens. Transmission of most porcine viruses may be prevented by early weaning, Cesarean delivery, or embryo transfer.[48] These strategies do not apply for PERV, but micro-chimerism must be ruled out in recipients of pig organs.

The microbiological safety of clinical xenotransplantation is determined by organisms in the donor pig or in the recipient, environmental exposures, and the nature and intensity of immunosuppression.[37] Management of infectious risk requires monitoring and surveillance of donor animals to limit potential microbial transmission and to assure animal health, product release criteria, and surveillance in recipients. Regulatory guidelines for breeding and for clinical trials must consider microorganisms likely to be important for immunocompromised recipients.[49]

Designated pathogen-free (DPF) herds must be maintained in barrier-controlled facilities with strict microbiological safety practices to prevent the introduction of organisms from other species into donor animals and the organs intended for human transplantation. In the absence of specific organisms in donor swine, transmission will not be likely to occur.

Given limited experience with, and validation of, microbiological assays for pig pathogens in humans, caution is merited. Advanced testing of close contacts of xenotransplant recipients and hospital staff might be reserved for symptoms or demonstrated infection in the recipient.

12. CARDIAC XENOTRANSPLANTATION IN CHILDREN

Neonates and infants with complex life-threatening congenital heart disease (such as those with a borderline single right ventricular flow dynamic and (palliative) Fontan circulation), are a patient population for whom pig heart xenotransplantation might fill a critical unmet need.[50] Based on the observation that early life ABO-incompatible heart transplantation results in tolerance to the mismatched donor blood group antigens it seems likely that the relatively immature immune system of neonates and infants will permit similar effects after xenotransplantation. Perhaps relatedly, thymectomy as performed during neonatal heart transplantation may play a role in mitigating the pathogenic immune response. Theoretically, transplantation of vascularized pig thymic tissue at the time of cardiac xenotransplant could also contribute to induction of immunological tolerance to a bioengineered pig heart.

Growth of a pig heart at a pace appropriate to the physiologic maturation of the donor pig may be problematic after transplantation in neonates and infants if heart growth is disproportionately rapid compared to the recipient's somatic growth: left ventricular hypertrophy diminishes its cavity, and together with a sub valvular muscular outlet stenosis, ends in diastolic pump failure and demise of the recipient animal.[2] Growth of pig organs can be reduced by deletion of the growth hormone receptor gene or by use of a miniature pig. Furthermore, inclusion of rapamycin in the immunosuppressive regimen restrains growth of orthotopic pig organ xenografts, however if emphasized may curtail the duration of support that a xenotransplant may enable. Re-transplantation might be needed if human growth rates of the porcine heart cannot be achieved but appear feasible (just as multistage operations are done today).[8]

Non-ischemic preservation during heart transplantation appears advantageous to prevent perioperative cardiac xenograft dysfunction in preclinical models and was associated with excellent initial heart function in the 2 human heart xenograft recipients.[16,18] For pediatric heart xenografts, perfusion devices will need to be modified for smaller hearts. As in adult cardiac xenotransplantation induction immunosuppression with B cell depletion and T cells reduction using polyclonal antithymocyte globulin as well as peri-transplant complement inhibition are advocated. As an initial step in clinical xenotransplantation, the implantation of a pig heart in neonatal or infant congenital heart patients as a bridge to human heart transplantation is compelling, either as a primary operation or after failed initial palliative surgery.[50]

13. REGULATORY CHALLENGES IN XENOTRANSPLANTATION – A GLOBAL PERSPECTIVE

The World Health Organization (WHO) has played a pivotal role, particularly through its engagement with the International Xenotransplantation Association (IXA).[51,52] In 2003, WHO convened meetings focusing on ethical, access, and safety issues in transplantation, culminating in World Health Assembly Resolution WHA57.18.[53] This resolution urged member states to allow xenotransplantation trials contingent upon effective national regulatory oversight mechanisms led by individual National Health Agencies.

An example of this can be seen in Australia's regulatory journey in xenotransplantation which began before the WHA57.18 Resolution was released. Research involving humans and animals in Australia is overseen by institutional committees—the Human Research Ethics Committee (HREC) and the Animal Ethics Committee (AEC). In 1994, the National Health and Medical Research Council (NHMRC) established the Gene and Related Therapies Research Advisory Panel (GTRAP) to advise on cutting-edge technologies including gene therapy.[54] Importantly in 1999, GTRAP expanded its mandate to include xenotransplantation. In 2001 the NHMRC formed the Xenotransplantation Working Party (XWP) comprising members including external experts, the XWP was tasked with developing guidelines for assessing animal-to-human transplantation trials and conducting public consultations with initial guidelines published in 2002 and issuance of a 5-year moratorium on xenotransplantation.

Today, Australia's regulatory framework for xenotransplantation is primarily guided by the National Statement on Ethical Conduct in Human Research 2007, updated in 2018.[55] Chapter 3.4 of this document specifically addresses animal-to-human xenotransplantation, outlining stringent guidelines for HRECs across three key elements: research scope, participant recruitment, and informed consent. These guidelines ensure ethical justification, risk minimization, long-term monitoring, and voluntary consent in clinical trials. The Australian Therapeutic Goods Administration (TGA) plays a critical role by regulating biologicals containing live animal cells, tissues, or organs under specific standards and guidance.[56] Likewise, The Administración Nacional de Medicamentos, Alimentos y Tecnología Médica of the Argentine Republic (ANMAT), Chinas National Medical Products Administration (NMPA), Japan's Pharmaceuticals and Medical Devices Agency (PMDA), Korea's Ministry of Food and Drug Safety (MFDS), and the New Zealand Medicines and Medical Devices Safety Authority (MEDSAFE) all base their regulatory standards on international bodies guidance's from the IXA, WHO, European Medicines Agency (EMA), and US Food and Drug Administration (FDA). With extensive cross sharing of regulatory oversight including ongoing consultation with the IXA it ensures safety and efficacy in clinical trials worldwide. For example, xenogeneic cell-based products adhere to the EMA's guidelines the TGA has adopted the EMA Guideline on Xenogeneic Cell-Based Medicinal Products, 2010 which was developed with significant consultation and representation from the IXA.[51]

The regulatory framework for xenotransplantation has evolved over 25 years, integrating global best practices guided by organizations including: the EMA, FDA, TGA, ANMAT, NMPA, PMDA, MFDS, and MEDSAFE with oversight and guidance from international bodies including the IXA and the WHO.[51]

In the European Union (EU), a regulatory framework for Xenotransplantation (XTx) is based on guidelines and ordinances on advanced therapy medicinal products (ATMP), pharmacovigilance and clinical trials. The fundamental rights of both animals as donors and humans as recipients of organs, tissues, and cells are adequately protected by the framework. Moreover, in the 27 EU member states, national laws may be implemented, such as those on genetic engineering, protection against infection and medicinal products, e.g. the German Medicinal Products Act. The ATMP regulation on XTx displays some limitations since animal organs are not explicitly mentioned, even though they may be derived from genetically modified animals. Therefore, those animal organs would be substantially manipulated compared with organs that are harvested from wild-type animals. In the ATMP regulation, the definition of somatic cell therapeutics, as well as the definition of tissue-engineered products of animal origin, is based on tissues or cells; however, it excludes organs. Naturally, organs derived from gene-edited animals contain tissues and cells.

To this end, the European Medicines Agency (EMA, Amsterdam, Netherlands) has published the guideline on xenogeneic cell-based medicinal products. Central elements of the ATMP regulation include (a) designation of the EMA to grant marketing authorizations for xenotransplantation products within the EU, (b) requirement for traceability of xenogeneic organs, tissues and cells, from creation through clinical use and ultimate disposition, and (c) hospital exemption for medicinal products that are not routinely prepared. In the EU, regulatory pathways to yield marketing authorizations for medicinal products, including ATMP, are based on data that cover product quality, nonclinical assessment (i.e., preclinical trials), as well as clinical trials.

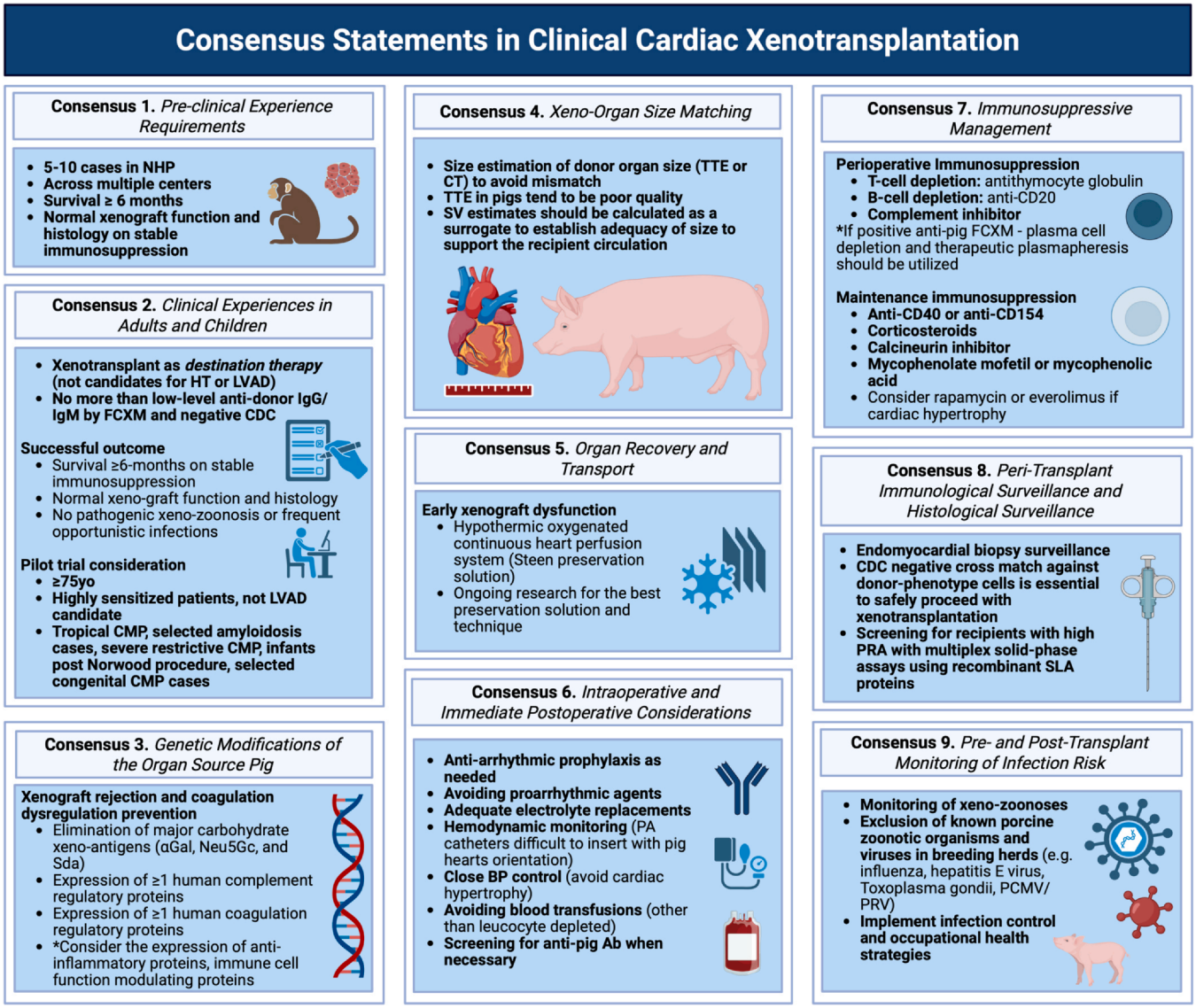
Typically, data must be summarized by the applicant, often the pharmaceutical entrepreneur working in partnership with clinical investigators and their medical institution(s), in dossiers including an internationally standardized set of Common Technical Documents (CTD). The application is evaluated by the European National Competent Authorities (NCA, in Germany the Paul-Ehrlich-Institut, Langen) that are nominated as rapporteur and co-rapporteur by EMA. The CTD are expected to show consistent data on the quality, safety, and efficacy of the product.

Public engagement has been crucial, reflecting diverse perspectives and ensuring regulations are responsive to ethical, safety, and scientific advancements. Moving forward, ongoing updates and refinements continue to shape the regulatory approach to xenotransplantation, balancing innovation with rigorous ethical and safety standards, underpinned by robust regulatory oversight and global collaboration.

14. CONSENSUS STATEMENTS IN CLINICAL CARDIAC XENOTRANSPLANTATION

Here we present 'consensus' statements regarding the scientific basis for proceeding with both additional 'pilot', exploratory single cases and with IND-qualifying clinical trial design. (Figure 1).

Figure 1 Ab: antibody, αGal: α1,3-Galactosyltransferase, BP: blood pressure, CDC: complement-dependent cytotoxicity, PCMV: porcine cytomegalovirus, CMP: cardiomyopathy, CT: computed tomography, FXCM: flow crossmatch, HT: heart transplant, IgG: immunoglobulin G, IgM: immunoglobulin M, LVAD: left ventricular assist device, Neu5Gc: N-glycolylneuraminic acid, NHP: non-human primate, PA: pulmonary artery, PRA: panel reactive antibody, PRV: pseudorabies virus, Sda: Sialyl-D-galactosyl epitope, SLA: swine leukocyte antigen, SV: stroke volume, TTE: transthoracic echocardiography.



14.1. Consensus 1: Pre-clinical experience requirements

A body of evidence in pre-clinical models must exist in support of transition to a human IND/IMPD-qualifying trial. We believe that should include an experience of at least 5–10 cases in NHP models across multiple centers that achieve survival for a minimum of 6 months along with demonstration of normal xenograft function and histology while on a stable maintenance immunosuppression.

14.2. Consensus 2: Recommendations for clinical experiences in adults and children

a) In tandem with accumulating additional preclinical data, we recommend that individual 'expanded access' cases should continue to be brought forward to contribute to the clinical experience in an ethical and careful manner.

Initial adult candidates could include individuals unable to receive either durable mechanical circulatory support or allotransplantation and for whom the xenograft represents a definitive 'destination' therapy. To avoid otherwise predictable early antibody-mediated xenograft injury, initial patients should exhibit no more than low-level anti-donor IgG or IgM by Flow cross match (FCXM) and a negative complement-dependent cytotoxicity (CDC) 'crossmatch'. Graft and patient survival beyond 6-months on stable, well-tolerated immunosuppression with normal xeno-graft function and histology in an ambulatory patient without detection of pathogenic xeno-zoonosis or frequent opportunistic infections would represent a successful outcome. Furthermore, in adults, a heterotopic intrathoracic xeno-transplant could be considered in carefully selected situations such as elevated, fixed pulmonary hypertension. Infant xenotransplantation may be pursued as a bridge to allo- but also to a further xeno-transplantation.

b) Based on results of 'compassionate' initial experiences, a pivotal/pilot trial in 'destination' patients may be considered. The patients suitable for such initial and pivotal experiences could include the elderly (e.g. > 75 years), highly sensitized individuals with or without an artificial heart pump option, those with cancer of recent onset but currently with no evidence of active disease, individuals with tropical cardiomyopathies, amyloidosis unable to receive immunoablative therapy (but without severe non-cardiac organ dysfunction), those with severe restrictive cardiomyopathy and small chamber size who are unable to be supported with mechanical circulatory support. Additionally, infants after a Norwood procedure and those with complex congenital heart disease without other options could be considered (a comprehensive list may be found in Konstantinov et al).[50] Another perspective also discusses some of these potential use cases, with which we agree.[57] (Table 2, these should be considered suggestive and not exhaustive in scope)
Ethical issues are beyond the scope of this document: the Society has issued a separate statement in this respect.[58]

14.3. Consensus 3: Genetic modifications of the organ source pig

Organ source pigs must be genetically modified to prevent xenograft rejection and coagulation dysregulation. At a minimum, these genetic modifications should include the elimination of major carbohydrate xeno-antigens (αGal, Neu5Gc, and Sda), the expression of one or more human complement regulatory proteins, and the expression of one or more human coagulation regulatory proteins.[59] Additional modifications—such as the expression of anti-inflammatory proteins or proteins that modulate immune cell function—may offer further protective benefits.

14.4. Consensus 4: Xeno-organ size matching

Estimation of donor organ size is needed to avoid mismatch and can best be done by cardiac imaging (e.g. CT scan or echocardiography) when screening for heart/valve anomalies in the donor pig prior to explant. Transthoracic echocardiography images in pigs are typically poor quality and thus uninformative however, measurement of stroke volume estimates (derived from the right ventricular outflow tract and velocity timed index) should be calculated as a surrogate to establish adequacy of size to support the recipient circulation.

14.5. Consensus 5: Organ recovery and transport

In adults, use of a hypothermic oxygenated continuous heart perfusion system with the Steen preservation solution has been used consistently to avoid early xenograft dysfunction. Whether isolated controlled hypothermia using cardiac preservation solutions other than those previously tested, such as del Nido solution (especially in children), or alternative continuous perfusion techniques such as normothermic organ perfusion in lieu of the hypothermic oxygenated continuous heart perfusion approach have not been fully evaluated. The appropriate solution and technique for preservation remains a matter of ongoing scientific inquiry.

14.6. Consensus 6: Intraoperative and immediate postoperative considerations

a) The porcine heart is prone to arrhythmias and either prophylactic or early use of antiarrhythmic therapy must be considered. Use of proarrhythmic catecholamines, inotropic agents or vasopressors should be avoided, and electrolytes (especially calcium, magnesium, and phosphate) must be carefully repleted.

b) Pulmonary artery catheters may be difficult to place due to the orientation of a pig heart. Alternative hemodynamic monitoring strategies such as use of transpulmonary thermodilution (that measures the temperature

Table 2 Recommendations for Clinical Experiences in Adults and Children

Section	Key Recommendations	Eligible Patient Populations	Outcome Targets / Considerations
(a) Individual Expanded Access Cases	• Continue ethically approved individual "expanded access" xenotransplant cases in parallel with additional preclinical data acquisition. • Ensure meticulous patient selection and immunologic compatibility before proceeding.	• Adults unable to receive durable mechanical circulatory support (MCS) or allotransplantation, for whom xenograft is intended as destination therapy. • Initial candidates should exhibit no more than low-level anti-donor IgG or IgM by flow crossmatch (FCXM) and negative complement-dependent cytotoxicity (CDC) crossmatch. • Adults with fixed, elevated pulmonary hypertension may be considered for heterotopic intrathoracic xenotransplant. • Infants may be considered as bridge to allo- or subsequent xeno-transplantation.	• Successful outcome defined as >6-month graft and patient survival on stable, well-tolerated immunosuppression. • Preservation of normal xenograft function and histology in an ambulatory patient, without pathogenic xenozoonosis or recurrent opportunistic infections.
(b) Early Pivotal / Pilot Trials ("Destination" Patients)	• Following encouraging "compassionate use" results, consider a pivotal/pilot trial in destination therapy candidates.	• Elderly (>75 years). • Highly sensitized individuals (with or without artificial heart pump option). • Patients with prior malignancy (recently treated, no active disease). • Tropical cardiomyopathies. • Amyloidosis unable to receive immunoablative therapy (but without severe non-cardiac dysfunction). • Severe restrictive cardiomyopathy with small ventricular chambers not amenable to MCS. • Infants post-Norwood procedure or with complex congenital heart disease lacking other options.	• Establish feasibility, safety, and immunologic durability of xenografts in diverse high-risk groups. • Data from these early experiences to inform subsequent phase trials and refinement of immunosuppressive and selection strategies.

change between a central venous catheter and a systemic arterial thermistor) or even implantable hemodynamic monitors (that can measure pulmonary artery pressures) may be useful and should be tested in preclinical studies.

c) Blood pressure controls may be essential to avoid cardiac hypertrophy: rapid appearance of hypertensive heart disease may evolve when blood pressure is not tightly controlled, and this may be difficult to distinguish from rapid natural growth of the porcine heart.

d) Blood transfusions, especially transfusions of products other than leukocyte depleted PRBCs (including factor concentrates and immunoglobulin), should be avoided due to concerns for pre-existing anti-pig antibodies in these products unless products are screened and administered based on low anti-pig antibody titers.

e) We recommend that resources and processes for screening for anti-pig antibodies in any administered products should be available to the clinical teams in a timely manner.

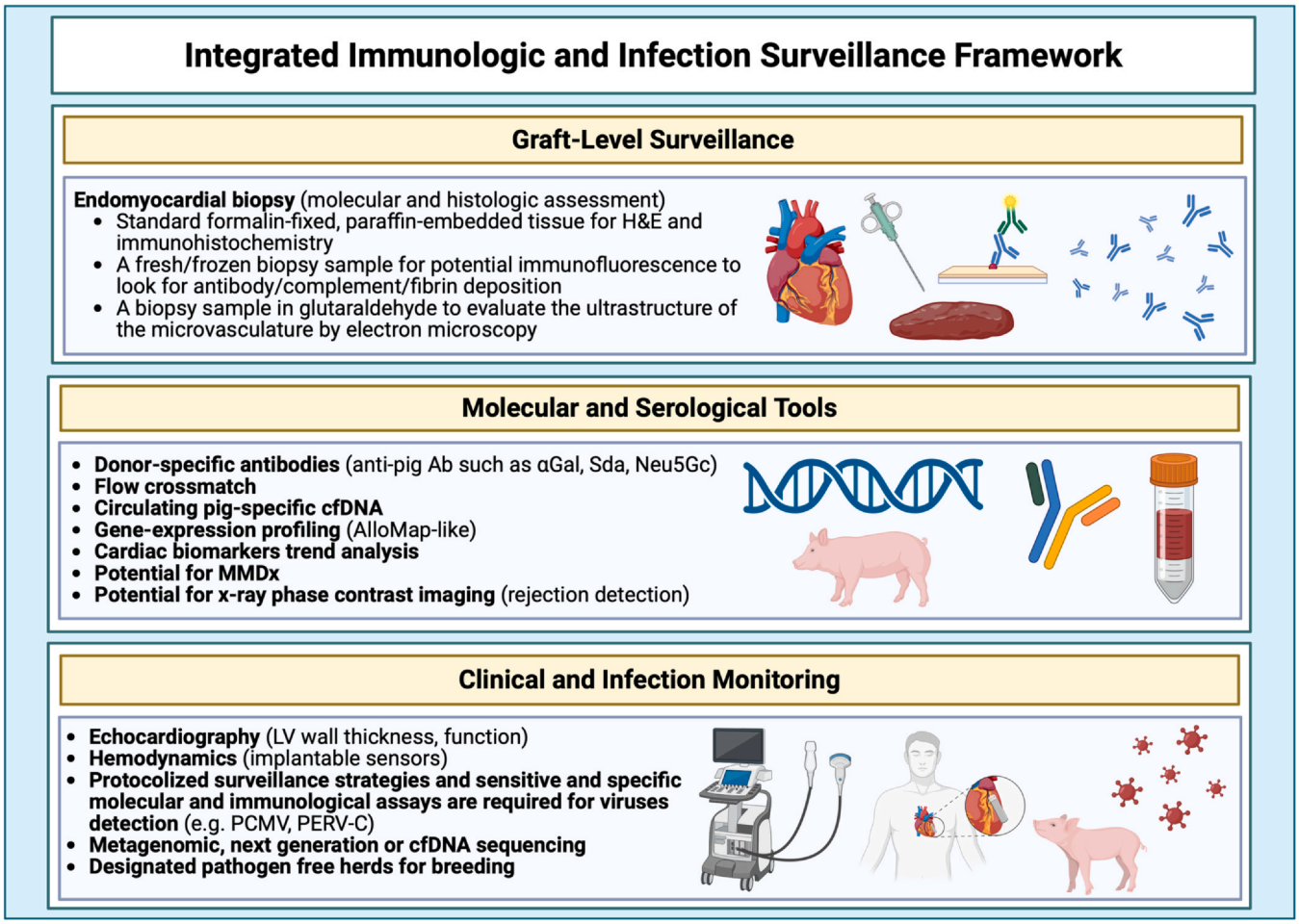
14.7. Consensus 7: Immunosuppression management

a) Perioperative immunosuppression with T cell (antithymocyte globulin) and B cell (anti-CD20) depletion therapy plus a complement inhibitor should be utilized for all adult cases. In case of a positive anti-pig FCXM, plasma cell depletion and therapeutic plasmapheresis should be utilized.

b) Use of costimulatory blockade, such as an anti-CD40 or anti-CD154 drug at the time of transplant and as part of maintenance immunosuppression regimen with steroids plus a conventional combination of calcineurin inhibitor and mycophenolate mofetil should be considered to support long-term successful survival of xenografts.

c) Proliferation signal inhibitors such as rapamycin or everolimus and intensified blood pressure lowering should be considered if cardiac hypertrophy is observed.

Figure 2 Ab: antibody, αGal: α1,3-galactosyltransferase, cfDNA: cell-free DNA, H&E: Hematoxylin and Eosin, LV: left ventricle, MMDx: Molecular Microscope Diagnostic System, Neu5Gc: N-glycolylneuraminic acid, PCMV: Porcine cytomegalovirus, PERV-C: porcine endogenous retrovirus C, Sda: Sialyl-D-galactosyl epitope.



14.8. Consensus 8: Peri-transplant immunological surveillance and histological monitoring

a) The strategy of immunological graft surveillance remains uncertain. Regular endomyocardial biopsies are likely to prove essential for directing clinical decision making and for detecting subclinical immune or other injury to the graft by molecular techniques.

b) A CDC negative cross match against donor-phenotype cells is essential to safely proceed with xenotransplantation. An appropriate threshold for considering that the FCXM to be 'negative' remains uncertain; parameters used in clinical cardiac allotransplantation represent a reasonable starting guideline in the absence of evidence in cardiac xenotransplantation.

c) Multiplex solid-phase assays that use recombinant SLA proteins would potentially offer a reliable, reproducible approach to screen potential xenograft recipients who have high levels of anti-HLA antibodies (high PRA) and be useful to monitor recipients for the development of donor-specific antibodies post-transplant. In the absence of a validated anti-SLA assay system, FCXM should detect clinically important levels of human anti-donor antibody directed at SLA and other pig antigens. Genetically modified donor pigs with a defined, homogeneous genetic background will be of special interest in that regard, since they should be less difficult to match.[59–61]

d) Although characterization of xenograft rejection may be histologically like that observed in allogeneic transplantation, vigilance in pathological advances in understanding findings outside of conventional established

criteria of humoral and cellular rejection are required as the entity of rejection may be distinctive. In this regard, electron microscopic findings may aid in greater understanding of the pathology that characterizes xenograft injury and eventual failure.

e) Research tools and biomarkers such as monitoring for circulating pig-specific DNA or serial measurements of troponin may be useful to assess absence of rejection or presence of graft injury. Such techniques will require focused investigation to establish their sensitivity and specificity regarding usefulness as optimal surveillance strategies as their utility even in allotransplantation remains less optimally established.

14.9. Consensus 9: Pre- and Post-transplant monitoring of infection risk

a) Validated, specific, and sensitive detection methods and testing strategies for Xeno-zoonoses are required for the monitoring of source animals and recipients.

b) Known porcine zoonotic organisms should be excluded from the breeding herds (e.g., influenza, hepatitis E virus, Toxoplasma gondii).

c) Strategies are required to exclude pig-specific viruses from breeding herds that have the potential to cause systemic syndromes in the human recipient. This will require both serologic and molecular testing, e.g., for porcine cytomegalovirus/porcine roseolovirus (PCMV/PRV).

d) Specific strategies are required for the porcine endogenous retroviruses (PERVs) depending on the pig strain utilized as a source animal (e.g. genetic or pharmacological inactivation of active viruses). Ideally donor animals should be PERV-C negative to avoid possibly dangerous PERV-AC recombination. Micro-chimerism should be excluded when testing recipients.

e) Infection control and occupational health strategies are required to manage infectious risk at centers pre-forming clinical xenotransplantation.
(Figure 2 Integrates an immunological and Infectious Surveillance suggested framework).

14.10. Conclusion

In conclusion, this consensus statement summarizes a combination of evidence-based and theoretically founded but as-yet-untested recommendations for the selection and management of cardiac xenograft recipients, and informed guidance regarding the design and oversight of near-term clinical work in the field. It is our collective hope that the various clinical considerations with respect to pre-qualification, surgical and anesthetic management principles, immunosuppressive challenges and post implant monitoring for immunological, clinical and infection surveillance may provide a blueprint to advance this field of cardiac xenotransplantation forward.

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We also wish to recognize that several original references may not have been credited, and we have, in many cases, used a review to decrease the overall number of references. However, any such omission is not intentional, and we apologize to any author whose original work may not have been cited.

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