Current Status of Cardiac Xenotransplantation: Report of a Workshop of the German Heart Transplant Centers, Martinsried, March 3, 2023

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Abstract

This report comprises the contents of the presentations and following discussions of a workshop of the German Heart Transplant Centers in Martinsried, Germany on cardiac xenotransplantation. The production and current availability of genetically modified donor pigs, preservation techniques during organ harvesting, and immunosuppressive regimens in the recipient are described. Selection criteria for suitable patients and possible solutions to the problem of overgrowth of the xenotransplant are discussed. Obviously microbiological safety for the recipient and close contacts is essential, and ethical considerations to gain public acceptance for clinical applications are addressed. The first clinical trial will be regulated and supervised by the Paul-Ehrlich-Institute as the National Competent Authority for Germany, and the German Heart Transplant Centers agreed to cooperatively select the first patients for cardiac xenotransplantation.

Keywords
- xenotransplantation
- preclinical
- clinical
- cardiac

Introduction

Novel medical treatments for advanced heart failure have proven to be highly effective.[1] However, in cases where all other treatment options have been exhausted, heart transplantation (HTx) remains the preferred approach for patients with end-stage heart disease, offering a strong likelihood of extended life in good health. Unfortunately, the shortage of available human organs for transplantation has led to extensive waiting lists, with annual demand far exceeding the actual number of transplants performed.

Exploring alternative solutions, researchers have considered taking increased risks in donor selection, such as the acceptance of hepatitis C-positive brain-dead persons.[2] Another

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avenue under investigation is donation after circulatory death (DCD);[3–5] however, DCD is not permitted in Germany.

At present, mechanical assist devices serve as the primary alternative to HTx, but these devices come with a high complication rate and offer only moderate improvements in patients' quality of life. The 1- and 5-year survival rates for patients on these devices are 83 and 52%, respectively, which are significantly worse when compared to allogeneic heart transplants. After implantation of assist devices, hospital readmission rates are high, primarily due to infections and bleeding events, with 36 and 68% occurring at 3 and 12 postoperative months, respectively. The main cause of death in these cases is withdrawal of care.[6]

Encouragingly, significant progress has been made in the field of pig-to-primate cardiac xenotransplantation. This progress is attributed to genetically modified (GM) donor pigs, improved preservation techniques, optimized transplantation models, and effective immunosuppressive regimens.[7–10] A milestone was reached in January 2022 when the first compassionate use xenotransplantation (XT) of a GM pig heart into a patient with terminal heart failure took place at the University of Maryland, Baltimore.[11,12] Although the patient passed away after 2 months due to various complications, this achievement marked a crucial step in demonstrating the feasibility of clinical cardiac XT by sustaining normal heart function for over 45 days.

Subsequent to this, in June and July 2022, two orthotopic HTx were performed at New York University using the same $10 \times$ GM pigs (United Therapeutics/Revivicor, Blacksburg, Virginia, United States) as donors, allowing the hearts to beat for 72 hours without signs of rejection.[13] It is worth noting that while these short-term experiments provide valuable insights, the unstable condition of brain-dead recipients limits longer observation times.[14,15] For more reliable data, XT must be conducted in living patients.

On September 20, 2023, the Baltimore group performed a second pig-to-human heart transplant in a 58-year-old patient ineligible for an allogeneic heart transplant due to severe peripheral vascular disease and complications with internal bleeding. The patient died 40 days after transplant presumably due to initial signs of rejection.

Genetic Modification of Source Pigs to Alleviate the Pathobiology of Pig Heart Xenotransplantation

The complexity of the pathobiology in organ XT surpasses that of allotransplantation, with innate immune responses playing a more prominent role (►**Table 1**).[16] In essence, during infancy, both humans and nonhuman primates (NHPs) produce antibodies that react to carbohydrate antigens present on the surface of unaltered pig cells. Consequently, when a normal pig organ is transplanted into a human or baboon, these antibodies quickly attach to the vascular endothelial cells of the graft. This triggers the activation of the complement cascade and attracts leukocytes that infiltrate the porcine heart through various mechanisms, ultimately leading to the rejection of the graft within minutes to hours. This rapid rejection, dependent on antibodies, is known as "hyperacute rejection" and is characterized by histopathological features such as venous thrombosis, loss of vascular integrity, interstitial hemorrhage, edema, and the infiltration of innate immune cells.

Hyperacute (and subsequently acute) rejections of pig organs in humans or NHPs primarily occur due to preexisting

Table 1 Genetic modifications of clinically available genetically modified pigs

Genetic modifications	Rationale	Reference
Knockout of α-1,3-galactosyltransferase (GGTA1-KO)	Knockout to prevent hyperacute rejection, as galactose-α-(1,3)-galactose (αGal) is the major xenoantigen causing hyperacute rejection in pig-to-human/primate xenotransplantation	19
Knockout of cytidine monophosphate-N-acetylneuraminic acid hydroxylase (CMAH-KO)	CMAH is the enzyme responsible for the synthesis of Neu5Gc. Knockout removes the major non-αGal xenoreactive antigen, against which humans have an innate immune response	20,21
Knockout of β-1,4-N-acetyl-galactosaminyl transferase 2 (B4GALNT2-KO)	Removes the glycan resembling the human Sd(a), against which humans/primates develop preformed antibodies	22
Expression of human CD46[a]	CD46 is a complement regulatory protein (CRP), downregulating complement activation. Express to suppress complement activation	23
Expression of human CD55[a]	CD55 is a CRP, similar role as CD46	24
Expression of human CD59[a]	CD59 is a CRP, similar role as CD46	25
Expression of human thrombomodulin (hTBM)	Human TBM is an anticoagulant protein, necessary to overcome coagulation incompatibilities after pig-to-primate/human xenotransplantations	29
Expression of human endothelial protein C receptor (hEPCR)[b]	Human EPCR is an anticoagulant protein, supports the formation of the TBM-thrombin complex	30

[a]Probably one CPRP (complement pathway regulatory protein) is sufficient.
[b]additional **hEPCR to hTBM is not necessary.**

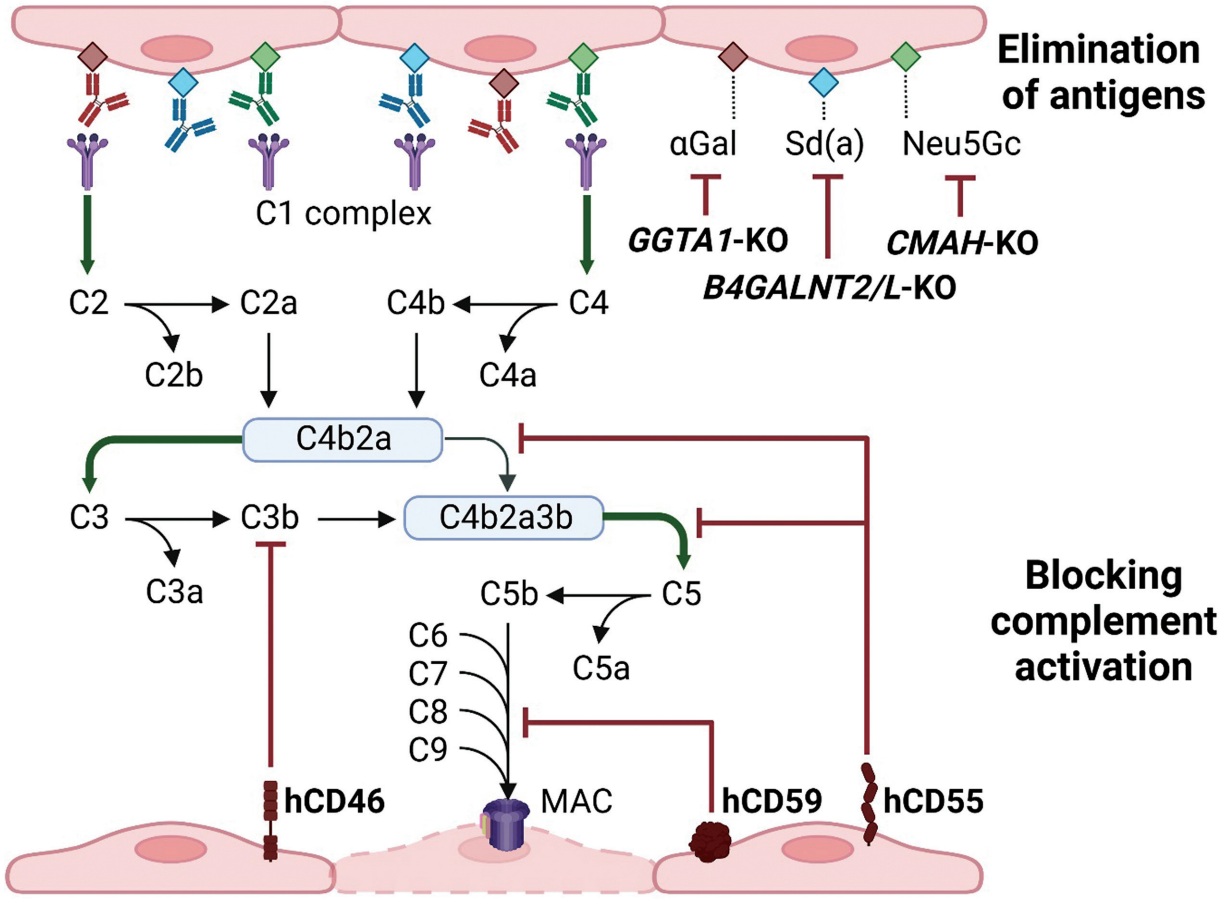

Fig. 1 Mechanisms of hyperacute xenograft rejection and strategies to overcome them. Created with BioRender.com.

antibodies targeting galactose-α-(1,3)-galactose (αGal). Humans also have natural antibodies against N-glycolylneuraminic acid (Neu5Gc) and a glycan resembling the human Sd (a) blood group antigen (often referred to as β4Gal). In contrast, NHPs only exhibit anti-αGal and anti-Sd(a) antibodies.[17,18]

To eliminate the αGal, Neu5Gc, and Sd(a) epitopes as target antigens for xenograft rejection in humans, pigs with inactivated α-1,3-galactosyltransferase (GGTA1),[19] cytidine monophosphate-N-acetylneuraminic acid hydroxylase (CMAH),[20,21] and β-1,4-N-acetyl-galactosaminyl transferase 2 (B4GALNT2)/B4GALNT2-like (B4GALNT2L)[22] genes were generated, resulting in what is commonly referred to as "triple-knock-out (TKO) pigs" (►**Fig. 1**).

However, complement activation can also occur through pathways unrelated to antibody binding, such as ischemia–reperfusion injury. To address this issue, additional human complement pathway regulatory (inhibitory) proteins (CPRPs), namely CD46,[23] CD55,[24] and CD59,[25] have been expressed in pigs by genetic engineering. Organs derived from animals with transgenic expression of one or more human CPRPs show a substantial level of protection against further complement-mediated injury in humans or NHPs. When combined with TKO pigs, these "humanized" porcine organs exhibit significantly reduced cell injury.[26]

Dysregulation of the coagulation pathway represents another facet of the pathobiology associated with XT of pig organs.[27,28] This dysregulation is influenced by several factors, including the previously mentioned immune responses, which promote inflammation and vascular damage, ultimately leading to a procoagulant state in the pig's endothelium. A significant contributing factor to this issue is the molecular incompatibility between coagulation regulators in pigs and those in humans or NHPs, leading to thrombotic microangiopathy even when using clinically approved anticoagulation therapy. Physiologically, thrombomodulin (TBM) on endothelial cells binds thrombin from the circulation, and the TBM–thrombin complex—with the help of an endothelial protein C receptor (EPCR)—activates protein C that has an anticoagulation effect (►**Fig. 2**). After organ xenotransplantation, porcine TBM on the transplant's endothelial cells can bind human or NHP thrombin, but the complex appears not to effectively activate human or NHP protein C. As a consequence, harmful fibrin clots form within the capillary system of the donor organ, finally leading to thrombotic microangiopathy. This can be effectively prevented by using source pigs expressing human TBM on their vascular endothelial cells.[29]

Despite the compatibility of the porcine EPCR in facilitating protein C activation in the human or NHP protein C pathway,

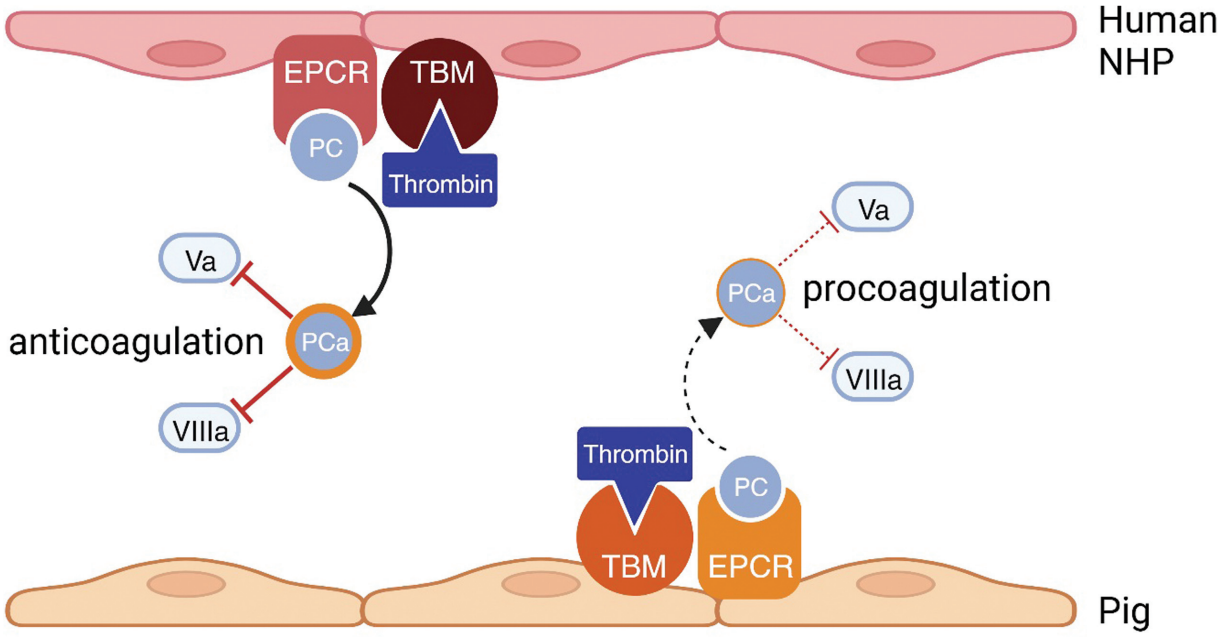

Fig. 2 Activation of protein C by the thrombin–thrombomodulin complex after allogeneic (top) and xenogeneic transplantation (bottom). EPCR, endothelial protein C receptor; PC, protein C; PCa, activated protein C; TBM, thrombomodulin; Va, activated factor V; VIIIa, activated factor VIII. Created with BioRender.com.

transgenic pigs have been developed to express human EPCR.[30] This modification aims to elevate EPCR levels and, consequently, may enhance protective thromboregulation.

Further Prerequisites for Successful Xenotransplantation

Nonischemic Perfusion Technique of the Porcine Donor Heart

For over two decades, preclinical outcomes following orthotopic xenogeneic HTx were inconsistent, with a perioperative mortality rate ranging from 40 to 60%.[31] This unpredictability was attributed to "perioperative cardiac xenograft dysfunction" (PCXD), believed to be linked to ischemia/reperfusion injury.[17,32] Porcine hearts are notably less resistant to ischemia compared to human hearts. Since 2015, PCXD has been consistently prevented through continuous, nonischemic perfusion of grafts with an 8°C hyperoncotic, oxygenated cardioplegic (Steen) solution containing erythrocytes, nutrients, and hormones.[33,34] This perfusion preservation technique was also utilized in the already mentioned first clinical case at the University of Maryland, Baltimore.[11,12]

Development of a Nonnephrotoxic Immunosuppressive Regimen with CD40 or CD154 Costimulation Blockade

Initial pig-to-baboon cardiac XT studies employed conventional immunosuppressive regimens without long-term success. Since 2000, costimulation blockade, initially with anti-CD154 monoclonal antibodies (mAb), has been applied.[35,36] However, due to thrombotic complications in humans, a chimeric anti-CD40 mAb (2C10)-based regimen was introduced instead, contributing to longer cardiac xenograft survivals in baboons.[7,10,37] In the recent Maryland case, a humanized version of the anti-CD40 antibody (KPL-404, Kiniksa Pharmaceuticals, Lexington, MA, United States) was used, along with cortisone, ATG, and rituximab (anti-CD20). Maintenance included tapering down cortisone, mycophenolate mofetil, and/or rapamycin for graft overgrowth control.[11]

Postimplantation Growth Control of the Xenoheart

Pig breeds used for XT experiments, such as German Landrace or Large White, weigh outgrown 200 to 300 kg, resulting in proportionately large hearts of approximately 1 kg, much too big for a human recipient, not to mention a baboon weighing between 15 and 20 kg. While it was previously believed that grafts would adapt to recipient growth regulation, recent findings[7,38] indicate that donor organ growth is genetically regulated: the porcine donor heart behaves as if it is still in a fast-growing pig's body; additionally, elevated afterload in baboon recipients causes concentric myocardial hypertrophy of juvenile porcine grafts. In combination, these intrinsic (donor-specific) and extrinsic (recipient-specific) factors led to extensive cardiac overgrowth and the development of dynamic outflow tract obstruction in preclinical experiments.[38] This "overgrowth" phenomenon was also observed after xenogeneic kidney transplantation experiments.[39,40] Strategies to prevent cardiac overgrowth in a preclinical setting include lowering blood pressure, early discontinuation of cortisone, and treatment with sirolimus, a ubiquitous growth inhibitor.

In the future, smaller donor animal breeds, such as Auckland Island pigs from New Zealand, with a weight range of 70 to 90 kg, may be preferred for clinical applications, and consequently, a small porcine endogenous retrovirus-C

Fig. 3 Auckland Island pigs in the Center for Innovative Medical Models (CiMM; www.lmu.de/cimm/) at LMU Munich.

(PERV-C) free herd near Munich, within the experimental LMU-farm, has been established (►**Fig.3**).

Identifying "Low-Risk" Donor–Recipient Combinations for Clinical Xenotransplantation

The level of histocompatibility between donor and recipient is an important parameter determining the risk for rejection in the course after allo- and xenotransplantation. High titers of antibodies to donor antigens in a prospective recipient are associated with an enhanced risk for antibody-mediated rejection. The existence of antidonor antibodies is usually demonstrated in vitro by incubating the serum of a prospective recipient with cells from a prospective donor (cross-matching). Antibody binding to donor cells can be visualized by flow cytometry or by antibody-induced complement activation resulting in cytotoxicity.[41,42] An assessment of the level of anti-pig antibodies by previous cross-match studies has been performed in recent pig-to-human heart and kidney xenotransplantations in deceased human recipients.[13,43] Incompatibility between donor and recipient is not only the reason for the deleterious effects of antibodies, but in addition, it also influences the intensity of T cell responses against a transplant. Thus, high numbers of human leukocyte antigen (HLA) class-I and/or class-II mismatches between donor and recipient have been associated with a poorer outcome in the long-term course after kidney and heart allotransplantation.[44–46]

Preformed IgM and IgG antibodies directed against the three carbohydrate antigens on porcine cells mentioned above are present in all individuals.[47–49] Binding of these antibodies to their targets is the key event to induce hyperacute rejection of xenografts. With the generation of TKO pigs,[22] it could be revealed that 30% of patients have very low or no IgM and IgG binding to TKO peripheral blood mononuclear cells.[50] Based on these findings, it was recommended to use pigs as donors for initial clinical studies where the TKO platform is combined with additional genetic modifications.[51,52]

Nevertheless, the question arises whether a low-risk organ can also be provided for those 70% of recipients having a positive cross-match with TKO cells.[50] A possible solution for this problem was provided by the characterization of the specificity of anti-TKO antibodies. These studies revealed that some of the residual antibody binding to TKO cells is mediated by anti-HLA antibodies which cross-react on porcine MHC molecules (SLA, swine leucocyte antigen[50,53,54]). The existence of antibodies in human serum with reactivity to porcine SLA is also supported by recent data characterizing the antibody repertoire against TKO cells.[55] To define the level of anti-SLA antibodies in prospective recipients of xenografts, flow cytometry cross-match could be performed using genetically engineered cells expressing individual SLA-I orSLA-II antigens.[56,57] Based on the observed reactivity patterns (e.g., dominance of anti-SLA antibodies) organ-source pigs with genetic modifications (e.g. SLA-I knockout) could be selected to avoid damaging effects of anti-SLA antibodies.[58] Organs from pigs expressing neither SLA-I nor SLA-II[59] may be of further advantage for recipients with antibodies against a broad spectrum of different SLA alleles.

Detailed characterization of some anti-SLA-I and -SLA-II antibodies revealed that single-amino acid epitopes are responsible for antibody cross-reactivity with HLA and SLA. This observation could be of great relevance for clinical XT because we also found that mutation of the amino acid eliminated antibody binding.[53,57] It has been discussed that

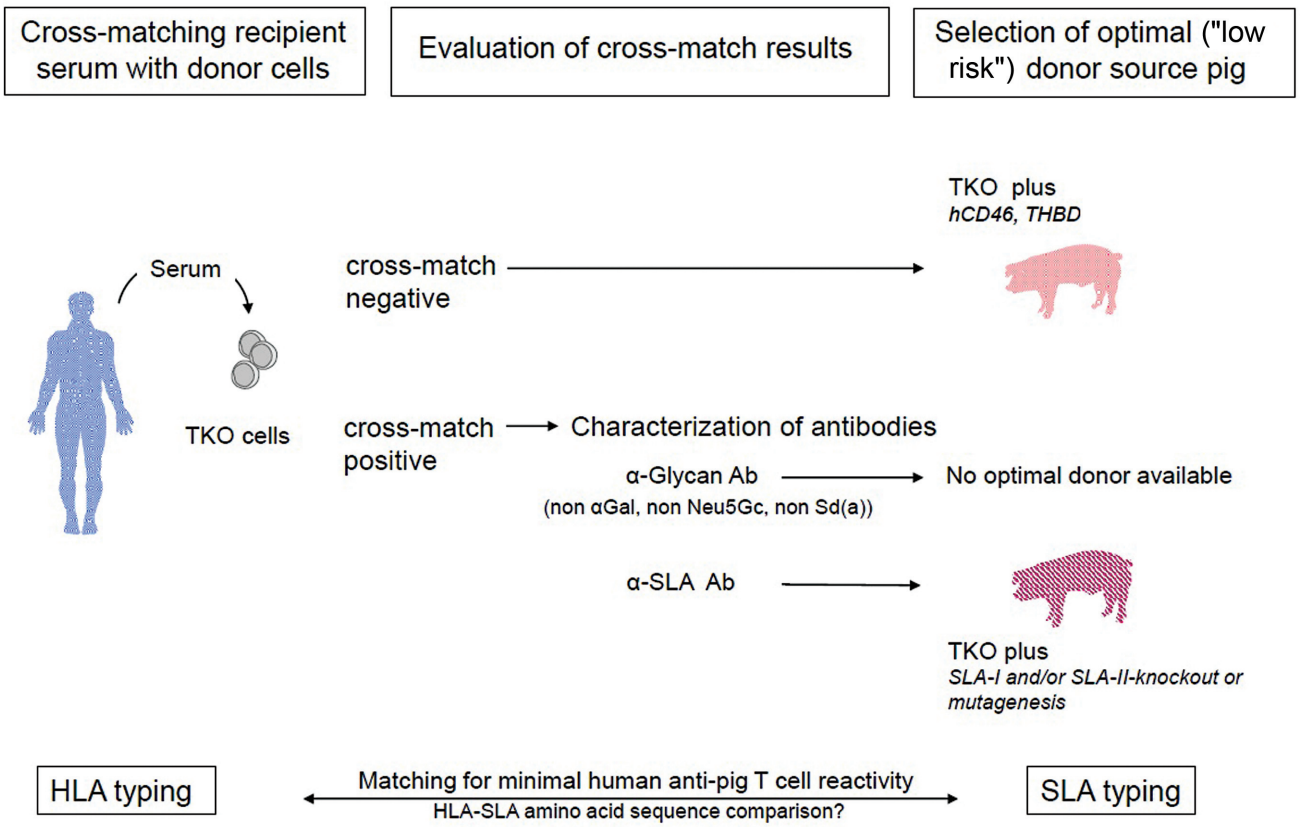

Fig. 4 Flowchart to achieve optimal donor–recipient-combinations for clinical xenotransplantation. Cross-matching of sera from potential recipients should be performed by using cells from TKO pigs lacking αGal, Neu5Gc, and Sd(a). Negative cross-match: Organs from TKO donor pigs combined with additional genetic modifications will be used as previously explained. In case of a positive cross-match, further characterization of antipig antibodies will be required. Recipients expressing anti-SLA antibodies may be transplanted with organs from SLA-I/II knockout pigs or pigs expressing mutated SLA to avoid antibody binding (both on "TKO plus" platform). Cross-matching may be complemented by HLA–SLA matching to identify donor–recipient combinations with low level T cell reactivity.[94]

SLA-I/II mutated xenografts may be sufficient to prevent anti-SLA antibody binding[60] instead of using grafts with complete absence of SLA. For individuals who have antibodies directed to other carbohydrates than αGal, Neu5Gc, and Sd(a), there is currently no genetically engineered pig available to avoid the binding of such antibodies. Thus, it would be safer to exclude these patients from initial studies (►**Fig. 4**).

Microbiological Safety

XT may be associated with the transmission of porcine microorganisms, for example, viruses, bacteria, fungi, and parasites.[61] Whereas bacteria, fungi, and parasites can be easily eliminated from the donor pigs, the situation with viruses is more complicated but can be solved.

It is important to remember in this context, that during allotransplantation, human viruses such as human immunodeficiency virus 1, human cytomegalovirus, rabies virus, and others have been transmitted to the recipient due to lack of time and methods of detection. In contradistinction, pigs as donor animals can be screened for viruses carefully long before surgery, and consequently, XT will be safer compared with allotransplantation.

Whereas the total number of viruses in pigs—their virome—is high,[62] the actual number of viruses able to infect humans and, ultimately cause diseases in humans, is still unknown. Diseases induced by animal viruses in humans after XT are called xenozoonoses.[63] The risks of infections should be negligible if state-of-the-art knowledge is applied.

First preclinical trials in nonhuman primates, and first clinical trials transplanting pig tissues into more than 200 human recipients, demonstrated that the number of xenozoonotic viruses was low.[64] At present, the hepatitis E virus genotype 3 is most important. It is transmitted to humans by eating undercooked pork or by contact with pigs. In immunosuppressed individuals, chronic infections are induced, preexisting liver diseases are aggravated.[65,66] A herpes virus type, the porcine cytomegalovirus (PCMV), is another possibly dangerous microorganism. PCMV is actually a porcine roseolovirus (PRV) related to the human herpesviruses 6 and 7.[67] Of note PCMV/PRV is not closely related to the human cytomegalovirus, which causes major pulmonary complications when transmitted during allotransplantation.[68] Until recently PCMV/PRV was shown to be harmful only for NHPs: transmission of the virus to baboons and rhesus monkeys significantly reduced the survival time of the transplant.[69] However, when a GM pig heart was transplanted into the

first patient in Baltimore, PCMV/PRV was transmitted and obviously contributed to his death.[11] Although there is no evidence that PCMV/PRV infects NHP and human cells, consumptive coagulopathy and multiorgan failure were observed in the infected transplanted baboons and the patient. The levels of interleukin-6, tumor necrosis factor α, tissue plasminogen activator, and plasminogen activator inhibitor 1 were significantly increased when compared to noninfected baboons.[69] The virus obviously interacts directly with the recipient's immune system and endothelial cells. Therefore, a major lesson learned from the study in Baltimore is that viral safety is pivotal for the success of XT and that testing should be done with assays of the highest quality and following an optimal strategy.[70]

Since there are no antivirals or vaccines available, a preventive strategy was developed by the Munich group: both viruses, PCMV/PRV[71,72] and HEV have been eliminated from the pig facility in Munich by applying "early weaning," which means, the piglets did not drink milk from their mother which may transmit the viruses during that time via its snout.

This strategy cannot be used to eliminate the risk of PERVs, which are integrated in the genome of all pigs[73]: PERV-A and -B are present in all pigs, but they are able to infect human cells only in vitro (under experimental conditions), PERV-C infects only porcine cells and is indeed not present in all pigs: in Munich imported Auckland-Island pigs were selected and were PERV-C free. Why is the absence of PERV-C so important? PERV-A and -C can recombine and the resulting recombinants can infect human cells.[74–76] Until now PERV transmission has never been observed neither in preclinical nor clinical XT studies.[77]

Ethical Considerations

As a novel treatment strategy, XT raises several ethical issues[78–81] which require thorough scrutiny before entering a first clinical trial. The ethical assessment should proceed in a transparent and structured manner.[82] ►**Table 2** shows relevant criteria for the ethical evaluation and its justifications. While it is beyond the scope of this report to give a full assessment of all criteria, we highlight how the most important ethical concerns can be addressed appropriately.

First of all, the heart XT recipients must have a benefit with sufficient certainty. Due to the persistent shortage of human donor organs, patients with terminal heart failure are in high need of an allograft. Some even die on the waiting list or experience detrimental side effects. In contradistinction, the risk of hyper-acute/humoral rejection of a cardiac xenograft could be reduced significantly due to multiple genetic modifications of the donor pigs.[7,18] In 2000, the Xenotransplantation Advisory Committee of the International Society of Heart and Lung Transplantation set up criteria, when the first clinical trial should be considered.[83] The required preclinical results have been met: consistent survival of two-third of the life-supporting porcine heart replacements in NHPs, in good health for up to a minimum of 3 months (has recently been extended for 6 months, or in single case longer).[7,8,10,84]

Taken together so far, a heart XT can be expected to have a rather large benefit with sufficient certainty for patients with terminal heart failure, given the highly unmet need for human donor hearts. And, the higher quality of xenografts compared to an average allograft from a brain-dead donor is an additional benefit, also the elective planning of the XT.

On the contrary, the risk of *potential harm* of the XT, especially the risk of xenogeneic infections, could substantially be reduced over the last years[61]: the donor pigs are screened with highly sensitive methods to prevent transmissions of xenogeneic viruses. A transmission of PERVs has never been observed, neither in preclinical nor in clinical studies.[61,77] If sufficiently sensitive tests are used, the risk of transmission of other viruses, like PCMV, can also be controlled sufficiently.[85] With appropriate sensitive screening for xenogeneic infections, the potential harm for third parties, hospital staff, and close relatives does not appear to represent an obstacle from an ethical perspective.

Due to the multiple genetic modifications, XT patients may need less aggressive immunosuppressive (even nonnephrotoxic) treatment and may therefore suffer less side-effects. Negative psychological effects of XT cannot be excluded completely, but appear rather unlikely: potential xenograft recipients are more concerned with the benefit–risk ratio than the source of the graft.[86] Nevertheless, XT patients should receive appropriate psychological support.[81]

Table 2 Criteria for the ethical evaluation of clinical xenotransplantation with their justification

Evaluation criteria	Ethical justification
Expected patient benefit of XT	Principle of beneficence
Potential harm of XT for patient	Principle of nonmaleficence
Promotion and respect of patient autonomy	Principle respect for autonomy
Potential harm for third-parties	Principle of nonmaleficence
Fair access to XT	Principle of justice
Efficiency of XT	Principle of utility maximization
Burden for animals as organ source	Animal welfare

Abbreviation: XT, xenotransplantation.

Given the novel aspects of the treatment strategy, promoting and respecting patient autonomy must play an important role in the first XT clinical trials. Patients should especially be informed about the expected benefits and risks of a heart XT compared to allotransplantation. While some experts suggest that patients who do not have access to an allotransplant should primarily be selected for a first XT trial,[81,83] participation should also be considered for patients who are on the transplant waiting list and therefore have the (later) option to receive a human allograft in case a xenograft fails (bridge-to-allotransplantation[84,86,87]). These patients would ultimately have a real choice between waiting for an allograft and receiving a xenograft—which could foster their autonomous decision about participating in a first-in-human XT trial.

Overall, heart XT seems to have a considerable expected benefit for terminal heart failure patients, while the potential risks appear comparatively low. While not all uncertainties can be eliminated in preclinical studies, first-in-human XT pivotal (pilot) trials seem to be justified according to the expected benefit–harm ratio. However, the benefits and risks of such a regulated study (in contradistinction to the unregulated compassionate use case of the two Baltimore cases[11,12]) must be documented thoroughly.[88] The risk of the transmission of xenogeneic infections seems to be manageable.

Regulatory Aspects

In the European Union (EU), guidelines and ordinances on advanced therapy medicinal products (ATMP), pharmacovigilance, and clinical trials form a regulatory framework for XT. The framework adequately protects the fundamental rights of both animals as donors and humans as recipients of organs, tissues, and cells. Furthermore, in the 27 EU member states, national laws may be implemented, such as the German AMG (Arzneimittelgesetz, Medicinal Products Act).

The ATMP regulation on XT displays some limitations in regard to animal organs, which are not explicitly mentioned, even though they are (in this case) derived from GM animals. The definition of somatic cell therapeutics, as well that of tissue-engineered products of animal origin, is based on tissues or cells; however, it excludes organs. Naturally, organs derived from GM 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 includes:

(1) designation of the EMA to grant marketing authorization for XT products within the EU
(2) requirement for xenograft traceability from creation through clinical use and ultimate disposition, and
(3) hospital exemption for medicinal products that are not routinely prepared.

In the EU, regulatory pathways to yield marketing authorizations for medicinal products, including those under ATMP regulation, are based on data that cover product quality, nonclinical assessment (i.e., preclinical trials), as well as clinical trials. 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. The application is checked 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 documents are expected to show consistent data on the quality, safety, and efficacy of the particular product. Beforehand, EMA and NCA offer scientific recommendations on the classification of ATMP. Concerning the state-of-the-art of research, appropriate regulations will be adopted.

In the United States of America, the Food and Drug Administration sets the hallmarks for the regulation of medical and other products. There, the Center for Biologics Evaluation and Research (CBER) regulates biological products for human use under applicable federal laws, including the Public Health Service Act and the Federal Food, Drug and Cosmetic Act. CBER is responsible for ensuring the safety and effectiveness of biologics, including XT products. The Center for Veterinary Medicine (CVM) is responsible for assessing GMs in the source pigs.

CVM and CBER collaborate on their assessments of animals used for xenotransplantation. Submission of an Investigational New Drug application is required for the approval of clinical trials; preclinical experimental data must be submitted which demonstrate the safety and effectiveness of the GM porcine hearts for its intended human use.[89]

What Experimental Results Would Justify a Formal Clinical Trial?

In 2000, the Xenotransplantation Advisory Committee of the International Society for Heart and Lung Transplantation recommended that consistent survival of NHPs supported by orthotopic porcine heart transplants for 3 months would be sufficient to warrant a clinical trial.[83] However, advancements in the field have raised the bar for evidence, with some suggesting that consistent survival of up to 6 months without irreversible rejection or infection would be more appropriate for initiating clinical trials in carefully selected patients.[7,10,84] Extending survival durations to nine or even 12 months with one or two recipients would provide further assurance. It is imperative that clinical trials involve teams with expertise in both clinical orthotopic HTx and the preclinical pig-to-NHP model.

Selection of the First Patients

Selection of the initial patients for clinical trials of cardiac XT requires meticulous consideration to justify the inherent risks and ensure highly favorable outcomes. Potential candidates may include individuals in intensive care units who are unsuitable for mechanical circulatory support. This category encompasses patients with conditions like hypertrophic cardiomyopathy, prior mechanical or biological valve replacements, and postinfarction ventricular septal defects. These high-risk patients often experience increasing instability due

Table 3 Potential indications for the initial clinical trials of pig heart transplantation

1.	Relative or absolute contraindications to mechanical circulatory support, e.g. (a) restrictive or hypertrophic cardiomyopathy (b) presence of a dysfunctional mechanical valve prosthesis or degenerated bioprosthesis (c) atrial or ventricular septal defects
2.	High titres of broad panel-reactive anti-HLA antibodies (high PRA) that do not cross-react with swine leukocyte antigens (SLA) of the donor animal (see also chapter on "low-risk" donor-recipient combinations)
3.	Chronic rejection after cardiac allotransplantation
4.	Heart transplantation after successful carcinoma treatment
5.	• Hypoplastic left heart syndrome (particularly with reduced ejection fraction of the systemic right ventricle and/or severe tricuspid regurgitation) • Other single ventricle patients with AV-valve regurgitation • Pulmonary atresia with intact ventricular septum and right ventricular-dependent coronary circulation • Unstable neonatal Ebstein • Failed initial palliation (after Norwood or Glenn procedure) • Cardiomyopathies with biventricular heart failure

Abbreviations: AV, atrioventricular; HLA, human leukocyte antigen; PRA, panel reactive antibody.
Source: Based on[93]

to their reliance on inotropic medications and the presence of arrhythmias. It is imperative to assess the potential reversibility of secondary liver and kidney damage and the treatability of pulmonary hypertension in these cases[37] (see ►**Table 3** for further details).

Neonates and infants with complex congenital heart diseases may benefit most from cardiac XT due to the lack of donors and the difficulties and poor outcomes of mechanical circulatory support in this age group.

Although there has been some progress in the field of mechanical circulatory support in patients with complex congenital heart disease like hypoplastic left heart syndrome or other forms of single ventricle physiology (e.g., pulmonary atresia with intact ventricular septum and right ventricular-dependent coronary circulation due to sinusoids),[90,91] mortality after ventricular assist device (VAD) implantation as a bridge to transplant is still high (30% at 6 months).[92]

Therefore, we think that children with congenital heart disease not amenable to biventricular repair and with a high risk for palliative procedures or poor outcomes after VAD therapy would be candidates for cardiac XT as a bridge to allotransplantation. The readily available xenograft would overcome the high waiting list mortality in this age group (►**Table 3**).

An advantage in the pediatric population will be the immature immune system of the neonate in combination with the thymectomy at the time of heart transplant. This environment would be ideal to induce immunological tolerance.

Anticipating the Future of Cardiac Xenotransplantation in the Next 5 to 10 Years

It is crucial to acknowledge that allografts will always be the preferred choice for individuals with advanced/terminal myocardial disease. However, due to the long waiting lists for donor hearts, we estimate that pig heart xenografts will be in clinical practice within the next 2 to 3 years. Initially, this might occur as a bridge to allotransplantation on an individual compassionate basis but ideally as part of a formal clinical trial. We foresee the approval of trials for both infant and adult patients. With successful long-term outcomes, cardiac XT may eventually become an accepted form of destination therapy.

We firmly believe that the field of XT will witness significant advancements in the next decade, surpassing those in mechanical assist devices, stem cell technology, and regenerative medicine.

Key Messages

1. Significant progress in the field of xenotransplantation has been made and allowed for the first xenotransplantation of pig hearts into two patients in the United States (compassionate use), who died after 60 and 40 days, respectively.
2. Nevertheless, in preclinical studies extended survival with clinically acceptable immunosuppression has been achieved, organ overgrowth could be controlled, appropriate donor-recipient matching is now established.
3. Microbiological safety is no longer a prohibitive concern.
4. Ethical considerations allow for a cautious start of clinical trials.
5. Regulation and surveillance on a national and European level have been established.
6. The German Heart Transplant Centers agreed to cooperatively select the first patients for a first clinical trial as soon as suitable donor pigs become available.

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Conflict of Interest
None declared.

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