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# Advances in Organ and Tissue Xenotransplantation

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## Keywords

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## Abstract

End-stage organ failure can result from various preexisting conditions and occurs in patients of all ages, and organ transplantation remains its only treatment. In recent years, extensive research has been done to explore the possibility of transplanting animal organs into humans, a process referred to as xenotransplantation. Due to their matching organ sizes and other anatomical and physiological similarities with humans, pigs are the preferred organ donor species. Organ rejection due to host immune response and possible interspecies infectious pathogen transmission have been the biggest hurdles to xenotransplantation's success. Use of genetically engineered pigs as tissue and organ donors for xenotransplantation has helped to address these hurdles. Although several preclinical trials have been conducted in nonhuman primates, some barriers still exist and demand further efforts. This review focuses on the recent advances and remaining challenges in organ and tissue xenotransplantation.

## 1. INTRODUCTION

**GM:** genetically (multi)modified

**HAR:** hyperacute rejection

**α-Gal:** galactose-α1,3-galactose

**Neu5Gc:** *N*-glycolylneuraminic acid

**NHP:** nonhuman primate

**NAb:** natural antibody

**GGTA1:** α-1,3-galactosyltransferase

**CMAH:** cytidine monophosphate-*N*-acetylneuraminic acid hydroxylase

**B4GALNT2:** β-1,4-*N*-acetyl-galactosaminyl transferase 2

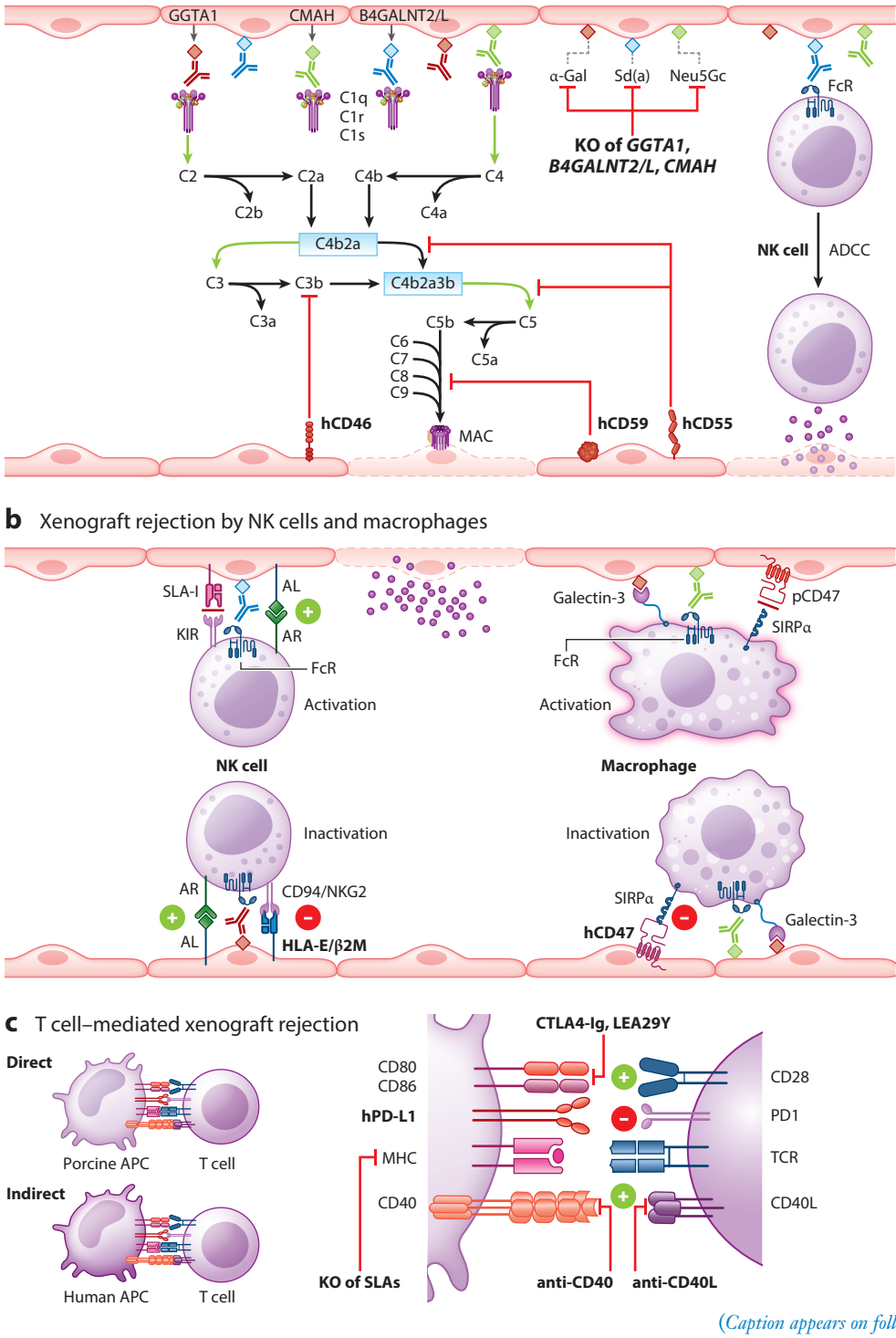
Organ transplantation is the most promising therapeutic option for end-stage organ failure (1). Organ donation for allotransplantation is not sufficient to meet the demand for transplantable organs, and many patients die while waiting for an organ transplant. According to Eurotransplant Statistics, as of February 2023, more than 13,000 patients were on the waiting list for an organ transplant in the Eurotransplant region alone (2). This indicates the insufficiency of human organ donors for allotransplantation and the demand for alternative sources for organs. Substituting human organs with animal organs, referred to as xenotransplantation, is a potential solution to the organ shortage problem. Due to similarities in organ size and physiology, pigs are considered the best candidates for animal-to-human xenotransplantation (3). However, the risk of graft rejection is much higher in xenotransplantation than in allotransplantation due to cross-species molecular incompatibilities (4). Porcine viruses are also of great relevance, and concerns about xenogeneic infections or xenozoonoses have always existed (5). With the advancements in gene editing techniques, multiple genetically modified (GM) pig lines have been generated in recent years to address issues like immune rejection, coagulation dysregulation, and xenozoonoses. This review features immune mechanisms of xenograft rejection, genetic modification of source pigs for xenotransplantation, and the current state of organ and tissue xenotransplantation.

## 2. IMMUNE BARRIERS AND GENE EDITING TO ENHANCE XENOGRAFT SURVIVAL

Host immune response–mediated xenograft rejection is a complex process, and depending on the intensity and type of response initiated, the xenograft can be rejected within minutes to several weeks (6). With few exceptions, independent of the type of tissue or organ transplanted, the molecular interactions and pathways involved in xenograft rejection are similar. In general, interspecies molecular incompatibilities trigger the host's immune response against the xenograft. Studies have shown that host immune response can be alleviated to a greater extent by genetically modifying the donor animals. In this section, we briefly describe the molecular mechanisms of immune rejection and genetic modifications to generate pigs suitable for tissue and organ donation to humans (**Figure 1**, **Table 1**).

### 2.1. Hyperacute Rejection

Hyperacute rejection (HAR) of a pig-to-primate xenograft occurs within minutes to hours (<24 h) after perfusion with the recipient's blood (7). Pigs have some cell-surface carbohydrate moieties or xenoantigens, including galactose-α1,3-galactose (α-Gal), *N*-glycolylneuraminic acid (Neu5Gc), and Sda, against which humans and—in part—also nonhuman primates (NHPs) have natural antibodies (NAbs) (8). The enzymes involved in the synthesis of these xenoantigens are α-1,3-galactosyltransferase (GGTA1), cytidine monophosphate-*N*-acetylneuraminic acid hydroxylase (CMAH), and β-1,4-*N*-acetyl-galactosaminyl transferase 2 (B4GALNT2)/B4GALNT2-like (B4GALNT2L), respectively (9). A significant proportion (1–4%) of circulating immunoglobulins in humans and NHPs are anti-α-Gal NAbs, making α-Gal the most critical xenoantigen. Infant primates develop antibodies against the porcine carbohydrate antigens when their gastrointestinal tract is colonized by microorganisms expressing carbohydrate antigens matching those on pig cells (4). Once a porcine tissue or organ is transplanted into a human or NHP, the NAbs interact with the xenoantigens on porcine cells (10, 11), and the immune complexes formed trigger complement activation, via both classical and alternative pathways (11, 12). Complement activation results in the formation of membrane attack complexes, causing xenogeneic cell lysis and HAR of the xenograft (13).




*(Caption appears on following page)*

**Figure 1** (*Figure appears on preceding page*)

Mechanisms of xenograft rejection and strategies to overcome them. (*a*) HAR of pig-to-primate xenografts is triggered by the binding of the recipient's preformed natural antibodies to specific carbohydrate antigens (α-Gal, Neu5Gc, Sda) on the surface of pig cells and subsequent activation of the complement system. In addition, bound antibodies activate NK cells via FcR, causing ADCC by lytic granule release. To overcome HAR, donor pigs are genetically multi-modified to lack GGTA1, CMAH, and B4GALNT2/B4GALNT2L and to express one or more hCRPs (hCD46, hCD55, hCD59) to prevent complement-mediated cell lysis. (*b*) NK cell and macrophage responses. In addition to ADCC, NK cells exhibit direct cytotoxicity to pig cells because SLAs do not effectively bind to primate NK cell inhibitory receptors. Additionally, activating signals, resulting from ALs on pig cells with their corresponding ARs on primate NK cells, may be involved. NK cell activation may be prevented by expressing HLA-E/β2M in transgenic pigs. HLA-E binds the inhibitory NK cell receptor CD94/NKG2. Macrophages are activated by FcRs binding the Fc portion of anti-pig antibodies. In addition, they are activated by galectin-3 binding α-Gal on pig cells. Porcine CD47 does not activate the "don't eat me" signal by binding SIRPα on human macrophages. Therefore, transgenic pigs expressing hCD47 were generated to inhibit macrophage activity against xenogeneic cells. (*c*) Activation of T cells against xenografts may occur directly via porcine APCs or indirectly via human/primate APCs presenting porcine peptides. In addition to the interaction of the peptide-presenting MHC with the TCR, costimulatory signals are required, most importantly CD40-CD40L (CD154), which can be blocked by treatment with anti-CD40 and/or anti-CD40L antibodies to prevent T cell activation. Another costimulatory pathway, CD80/CD86–CD28, can be blocked by treatment with CTLA4-Ig or its affinity-optimized variant LEA29Y. Another strategy involves the coinhibitory pathway PD-1–PD-L1 expressing hPD-L1 in transgenic pigs. Finally, pigs lacking SLAs or expressing SLAs with reduced activating capacity have been produced to reduce T cell activation via the direct pathway. Figure modified with permission from Reference 15. Abbreviations: ADCC, antibody-dependent cellular cytotoxicity; AL, activating NK cell ligand; APC, antigen-presenting cell; AR, activating receptor; B4GALNT, β-1,4-*N*-acetyl-galactosaminyl transferase; CD94, killer cell lectin like receptor D1; CMAH, cytidine monophosphate-*N*-acetylneuraminic acid hydroxylase; FcR, Fc receptor; GGTA1, α-1,3-galactosyltransferase; HAR, hyperacute rejection; hCRP, human complement pathway regulatory protein; HLA, human leukocyte antigen; KIR, killer cell immunoglobulin-like receptor; KO, knockout; MAC, membrane attack complex; MHC, major histocompatibility complex; Neu5Gc, *N*-glycolylneuraminic acid; NK, natural killer; PD-L1, programmed death ligand-1; SIRPα, signal regulatory protein α; SLA, swine leukocyte antigen; TCR, T cell receptor.

**CRP:** complement pathway regulatory protein

**DAF:** decay accelerating factor (CD55)

**KO:** knockout

**DXR:** delayed xenograft rejection

**AHXR:** acute humoral xenograft rejection

**CXR:** cellular xenograft rejection

**SLA-I:** swine leukocyte antigen class I

**SLA-II:** swine leukocyte antigen class II

**HLA:** human leukocyte antigen

Complement pathway regulatory proteins (CRPs), such as membrane cofactor protein (MCP/CD46), decay accelerating factor (DAF/CD55), and membrane inhibitor of reactive lysis (MIRL/CD59), inhibit complement activation by intervening at different steps in the complement cascade (14). However, porcine CRPs are not compatible with the corresponding molecules in primates. Hence, the HAR can be circumvented either by eliminating the α-Gal xenoantigen [*GGTA1*-knockout (KO)] or by the expression of human CRPs (hCD46, hCD55, hCD59). A strong consensus exists about the essentiality of *GGTA1*-KO in organ- or tissue-donor pigs, and transgenic expression of one or more hCRPs synergizes the effect of *GGTA1*-KO and further prolongs the survival of pig-to-primate xenografts (3, 15–17).

## 2.2. Delayed Xenograft Rejection

In addition to HAR, several other layers of humoral and cellular xenograft rejection are mediated by non-α-Gal NAbs, innate immune response, and adaptive immune response.

**2.2.1. Non-α-Gal NAbs.** The xenograft surviving HAR can be rejected through delayed xenograft rejection (DXR), which occurs within days to weeks post transplantation. DXR is considered a syndrome of acute humoral xenograft rejection (AHXR), cellular xenograft rejection (CXR), and coagulation dysregulation (7). Although these terms are also used alternatively, there are some differences in their pathophysiology. The non-α-Gal NAbs (anti-Sda and anti-Neu5Gc NAbs) are key players in AHXR, and its core mechanism is similar to HAR (41). Swine leukocyte antigens class I and II (SLA-I and SLA-II), the porcine orthologs of human leukocyte antigens (HLAs), are the major histocompatibility proteins on porcine cells. In individuals sensitized to HLAs (e.g., after organ allotransplantation, transfusion, or pregnancy), the anti-HLA class II antibodies can interact with the SLA-II molecules and aggravate AHXR (42). AHXR can be alleviated by eliminating Neu5Gc and Sda antigens from porcine cells through *CMAH*-KO and

**Table 1 Genetic modifications to generate organ donor pigs compatible with primates**

| Obstacle and countering strategy | Corresponding genetic modification | Reference(s) |
|---|---|---|
| **Hyperacute rejection** | | |
| Deletion of α-Gal epitopes | *GGTA1*-KO (α-1,3-galactosyltransferase knockout) | 18, 19 |
| Transgenic expression of human complement regulatory proteins | hCD46-tg (transgenic human membrane cofactor protein) | 20 |
| | hCD55-tg (transgenic human decay-accelerating factor) | 21 |
| | hCD59-tg (transgenic human membrane inhibitor of reactive lysis) | 22 |
| **Delayed xenograft rejection** | | |
| ***Acute humoral xenograft rejection*** | | |
| Deletion of *non* α-Gal epitopes (Neu5Gc and Sda) | *CMAH*-KO (cytidine monophosphate-*N*-acetylneuraminic acid hydroxylase knockout) | 23 |
| | *B4GALNT2/L*-KO (β-1,4*N*-acetylgalactosaminyltransferase 2/β-1,4*N*-acetylgalactosaminyltransferase 2 like knockout) | 19 |
| ***Innate cellular xenograft rejection*** | | |
| Inhibiting macrophage-mediated xenograft damage | hCD47-tg (transgenic human integrin-associated protein) | 24 |
| | hCD200-tg (transgenic human OX-2 membrane glycoprotein) | 25 |
| Inhibiting NK cell–mediated xenograft damage | hHLA-E-tg (transgenic human leukocyte antigen-E) | 26 |
| | hβ2M-tg (transgenic β-2-microglobulin gene) | 26 |
| | hHLA-G1-tg (transgenic human leukocyte antigen-G1) | 27 |
| Inhibiting neutrophil action | hCD31-tg (transgenic human platelet endothelial cell adhesion molecule-1) | 28 |
| Inhibiting dendritic cell action | hTRAIL-tg (transgenic human TNF-related apoptosis-inducing ligand) | 29 |
| ***Adaptive cellular xenograft rejection*** | | |
| Inhibiting T cell–mediated xenograft damage | hCIITA-DN-tg (transgenic human dominant-negative class II transactivator) | 30 |
| | *β2M*-KO (β-2-microglobulin gene knockout) | 31 |
| | hLEA29Y-tg (transgenic human variant of CTLA4-Ig) | 32 |
| | hPD-L1-tg (transgenic human programmed cell death ligand 1) | 33 |
| **Coagulation dysregulation** | | |
| Coagulation regulation by human coagulation-regulatory gene expression | hTBM-tg (transgenic human thrombomodulin) | 34 |
| | hTFPI-tg (transgenic human tissue factor pathway inhibitor) | 35 |
| | hCD39-tg (transgenic human ectonucleoside triphosphate diphosphohydrolase) | 36 |
| | hEPCR-tg (transgenic human endothelial protein C receptor) | 35 |
| | h*pvWF-tg (replace pig von Willebrand Factor gene region with human orthologs) | 37 |
| | *ASGR1*-KO (asialoglycoprotein receptor 1 knockout) | 38 |
| **Systemic inflammation** | | |
| Inhibiting systemic inflammation and apoptosis | hHO-1-tg (transgenic human heme oxygenase-1) | 39 |
| | hA20-tg [transgenic human TNF α–induced protein 3 (TNFAIP3)] | 39 |
| | shTNFRI-Fc-tg (transgenic soluble human TNF-α receptor inhibitor-Fc) | 40 |

*B4GALNT2/B4GALNT2L*-KO, respectively (41). Additionally, knockout or downregulation of SLAs from the donor pigs also reduces SLA-mediated immunogenicity (7).

**NK:** natural killer

**DC:** dendritic cell

**2.2.2. Macrophages.** CXR, also known as acute cellular rejection, is mediated by both innate immune cells [macrophages, natural killer (NK) cells, neutrophils, and dendritic cells (DCs)] and adaptive immune cells (T cells and B cells) (14). Macrophages infiltrate the xenograft through

**SIRPα:** signal regulatory protein α

**CD47:** integrin-associated protein

**CD200:** OX-2 membrane glycoprotein

**ADCC:** antibody-dependent cellular cytotoxicity

**MHC:** major histocompatibility complex

**β2M:** β2-microglobulin

**NET:** neutrophil extracellular trap

different mechanisms: (*a*) Monocyte chemoattractant protein-1 on the porcine cells attracts them (43); (*b*) immune complexes and α-Gal antigens on the xenograft interact directly with the Fc-gamma receptor and galectin-3 on the macrophages, respectively (44); or (*c*) Toll-like receptors on macrophages interact with damage-associated molecular patterns released by the damaged xenograft (45). The activated macrophages function as xenoantigen-presenting cells and recruit $CD4^+$ and $CD8^+$ T cells into the xenograft, leading to its rejection (44, 46). Additionally, macrophages can also have a direct cytotoxic effect on the xenograft by secreting proinflammatory cytokines (47). In the normal physiological environment, signal regulatory protein α (SIRPα) on macrophages identifies the self-cells by interacting with CD47 and generates a "do not eat me" signal (48). However, there is a cross-species incompatibility between these two regulatory molecules; the porcine CD47 cannot interact with SIRPα on human or NHP macrophages, and the macrophage action on the xenogeneic cells is not inhibited (49). Therefore, transgenic expression of hCD47 in organ donor pigs was used to suppress the phagocytic action of host macrophages (50, 51). Another macrophage-inhibitory signaling pathway is the interaction between CD200 and its receptors (CD200R) on macrophages, which leads to a reduced generation of proinflammatory cytokines by the macrophages (52). Transgenic expression of hCD200 in pig cells can reduce macrophage infiltration as well as macrophage-mediated xenograft rejection (25, 53).

**2.2.3. Natural killer cells.** Human NK cells can lyse the porcine xenograft cells through either antibody-dependent cellular cytotoxicity (ADCC) or direct interaction (54). In ADCC, the Fc region of immune complexes deposited on the xenogeneic cells interacts with CD16 (an Fc receptor) on NK cells. Subsequently, NK cells secrete granules containing perforins and granzymes and cause the lysis of xenogeneic cells (55). The direct interaction of NK cells with their target cells can be through inhibitory and activating receptors. The inhibitory receptors expressed by human NK cells, such as KIR2DL4 (killer cell immunoglobulin-like receptor 2DL4), ILT2 and ILT4 (leukocyte immunoglobulin like receptor B1/B2), and CD94 (killer cell lectin like receptor D1)/NKG2A (killer cell lectin like receptor C1), recognize the human major histocompatibility complex (MHC)-I molecules, such as HLA-G and HLA-E, and inhibit NK cell activation (56). In contrast, porcine MHC-I/SLA-I molecules cannot generate the inhibitory signal (57). NKG2D (killer cell lectin like receptor K1) and CD2 are activating receptors expressed by human NK cells and interact with porcine UL16-binding protein 1 and a CD58 ortholog on porcine cells, respectively. The activated NK cells lead to target cell lysis by secreting lytic particles as well as proinflammatory cytokines (57, 58). The action of NK cells through ADCC can be inhibited by using anti-CD16 antibodies or by removing IgG through immune absorption (59). Because SLAs cannot inhibit the direct cytotoxic effect of human NK cells, transgenic expression of HLA-E/β2M (26) or HLA-G1 (27) in pigs can inhibit human NK cell–mediated cytotoxicity.

**2.2.4. Neutrophils and dendritic cells.** Neutrophils are recruited into the xenograft either independently or in response to complement activation (14). The activated neutrophils undergo NETosis, a type of programmed cell death, and form neutrophil extracellular traps (NETs) (60). NETs generate reactive oxygen species and digestive enzymes to damage the xenograft cells (54, 61). Furthermore, NETs can also activate macrophages and exacerbate macrophage-mediated xenograft damage (62). Another type of cells, most studied with kidney xenotransplantation, are the resident DCs, which are involved in both acute and chronic graft rejection. Once activated by signals such as damage-associated molecular patterns, DCs promote graft rejection (63, 64). The porcine endothelial cells with transgenic expression of hCD31 (platelet endothelial cell adhesion molecule-1) inhibit NETosis and neutrophil-mediated damage to the xenogeneic cells in vitro (28). A potential strategy to suppress xenograft rejection mediated by resident DCs is the

transgenic expression of human tumor necrosis factor (TNF)-related apoptosis-inducing ligand in the organ donor pig (29).

**APC:** antigen-presenting cell

**PD-L1:** programmed death ligand-1

**TBM:** thrombomodulin (CD141)

**EPCR:** endothelial protein C receptor (CD201)

**2.2.5. Adaptive immune cells.** T cells are the key players in CXR, and both $CD4^+$ and $CD8^+$ T cells infiltrate the xenogeneic tissue (65). T cells interact with the xenograft antigens via direct and indirect pathways (66). In the direct pathway, the host's $CD4^+$ and $CD8^+$ T cell receptors interact with intact SLA-II and SLA-I, respectively, on the porcine antigen-presenting cells (APCs, such as endothelial cells and resident DCs) (67). In the indirect pathway, the peptides of xenogeneic origin are presented to the host's $CD4^+$ and $CD8^+$ T cells by the host's APCs (67). Hence, SLA removal can suppress T cell responses against the pig-to-primate xenografts. Transgenic expression of hCIITA-DN (human dominant-negative class II transactivator) reduces SLA-II expression on porcine cells, whereas *β2M*-KO pigs lack functional assembly of SLA-I, and these genetic modifications alleviate T cell–mediated xenograft rejection (31, 59). Because SLAs are also involved in protective immune responses in pigs, *β2M*-KO can negatively affect the animals' viability (68). A recently reported mutation that results in reduced expression, rather than complete knockout, of β2M and SLA-I can be useful to avoid the potential effects of a complete *β2M*-KO (69).

In addition to the MHC–T cell receptor interaction, T cell activation depends on costimulatory signals (59). The most studied costimulatory pathways include the interaction between CD80/CD86 on xenogeneic APCs and CD28 on the host's T cells and the interaction between CD40 on xenogeneic cells and CD154 (CD40L) on the host's T cells (70). These pathways can be blocked by specific antibodies or synthetic proteins. For instance, blocking the CD80/CD86-CD28 costimulatory axis with soluble CTLA4-Ig or by transgenic expression of CTLA4-Ig in donor pigs modulates T cell–mediated xenograft rejection (71). Moreover, transgenic expression of LEA29Y, a potent derivative of human CTLA4-Ig, in pigs also blocks the host's T cell costimulation (32). Similarly, the CD40-CD154 costimulatory signal can be blocked by either anti-CD154 or anti-CD40 monoclonal antibodies (59). Another target is the coinhibitory pathway involving the interaction between PD-1 on $CD4^+$ and $CD8^+$ T cells and its ligand PD-L1 on the target cells, which supports graft survival by blocking T cell proliferation and activation (70). Cells and tissues derived from hPD-L1 transgenic pigs have an activated coinhibitory PD-1–PD-L1 axis, which makes the xenograft less immunogenic and reduces human $CD4^+$ T cell proliferation (33). Moreover, the activated T cells aggravate NK cell- and macrophage-mediated cytotoxicity and instigate B cell activation and antibody production (41). B cell depletion or alleviation of B cell function by anti-CD40, anti-CD20, or anti-CD83 antibodies prolongs xenograft survival (59, 72).

## 2.3. Coagulation Dysregulation

Both AHXR and CXR of organ xenografts are accompanied by coagulation dysregulation, which becomes more evident if the xenograft has survived HAR and DXR (50). Endothelium injury in the xenograft during DXR exposes tissue factor and collagen, making it procoagulant and facilitating immune cell infiltration (73). Tissue factor instigates thrombin generation and conversion of fibrinogen to fibrin, whereas collagen stimulates platelet aggregation and activation (73). Thrombosis caused by fibrin deposition and platelet aggregation results in thrombotic microangiopathy and ischemic injury in the xenograft and negatively impacts its survival (7). In the normal physiological environment, thrombomodulin (TBM) expressed on the vascular endothelial cells binds thrombin, blocks its procoagulant actions, and leads to the generation of activated protein C. Thereby the endothelial protein C receptor (EPCR) enhances the generation of activated protein C, which inhibits further thrombin generation, hence blocking thrombotic microangiopathy (74). In pig-to-primate xenografts, the porcine endothelial surface molecules are partially incompatible

**vWF:** von Willebrand factor

**CD39:** ectonucleoside triphosphate diphosphohydrolase 1

**TFPI:** tissue factor pathway inhibitor

**CD73:** 5′-nucleotidase ecto

**ASGR1:** asialoglycoprotein receptor 1

**PCMV:** porcine cytomegalovirus

**PERV:** porcine endogenous retrovirus

with the soluble molecules in the recipient's blood, leading to coagulation dysregulation (74). In addition, the porcine von Willebrand factor (vWF) on the vascular endothelial cells can activate platelets and cause coagulation dysfunction (75). Transgenic expression of one or more human coagulation-related proteins (hTBM, hCD39, hEPCR, hTFPI, hCD73) in the donor pigs can extend xenograft survival (59, 76). Additionally, replacing porcine vWF with human vWF (37) or knockout of asialoglycoprotein receptor 1 (ASGR1) from the donor pigs (38) can also alleviate platelet sequestration and resultant coagulopathies.

### 2.4. Chronic Rejection

Chronic xenograft rejection occurs several months to years post transplantation. In organ xenografts thereof, thrombotic microangiopathy is its major histopathological characteristic (77). Although understanding of the chronic rejection mechanisms is limited because only a few experimental xenografts have achieved long-term survival, the molecular incompatibilities between porcine and NHP/human coagulation factors may play a vital role in its pathophysiology (77). $CD4^+$ T cells are thought to be the key players in chronic xenograft rejection, and pig kidney xenografts transplanted into NHPs with $CD4^+$ T cell depletion achieved long-term survival of >1 year (78). Moreover, transgenic expression of hTBM in the pig xenograft and the recipient's treatment with anti-CD40 monoclonal antibodies help avoid coagulation-related problems and thrombotic microangiopathy in the xenograft (79).

### 2.5. Suppression of Systemic Inflammation

Pig-to-NHP xenotransplantation is accompanied by systemic inflammation. C-reactive protein, a marker of inflammation, remained higher for several months after pig-to-baboon organ transplantation, suggesting a persistent systemic inflammation (80). Moreover, several other inflammation indicators are also increased in the recipient post transplantation (80). Transgenic expression of human anti-inflammatory proteins (hHO-1 or hA20) in organ donor pigs might reduce systemic inflammation in the xenograft recipients (39). Similarly, TNF-α-mediated inflammation and immune reaction can be suppressed by transgenic expression of soluble human TNF-α receptor inhibitor-Fc (shTNFRI-FC) in the donor pigs (40).

## 3. VIRUS SAFETY OF XENOTRANSPLANTATION

Cross-species transmission of potentially zoonotic viruses from pigs to humans is a potential threat to bringing xenotransplantation into the clinic (81). Preclinical trials have shown that porcine cytomegalovirus (PCMV)/porcine roseolovirus and porcine circovirus 3 can be transmitted into the host and result in reduced xenograft survival time (82). However, PCMV/roseolovirus and many other porcine pathogens can be eliminated from a breeding herd via early weaning of piglets (83). Of major interest in xenotransplantation are the porcine endogenous retroviruses (PERVs) (reviewed in 5). PERVs have three subtypes: PERV-A, PERV-B, and PERV-C. There can be more than 60 PERV provirus integration sites in pigs; hence, they are vertically transmitted from parents to offspring and cannot be eliminated by conventional methods (81, 84). PERV-A and -B are found in all porcine species, whereas PERV-C is found in most, but not all, pigs (85, 86). Recombination between PERV-A and PERV-C can generate PERV-AC viruses with a higher replication rate and a greater efficiency to infect human cells (87). Hence, use of PERV-C-free animals reduces the risk of PERV infection (82). Although PERVs can infect human cells in vitro (88), clinical trials of xenotransplantation have never reported an in vivo infection of human cells by PERVs (82). There is thus a prevalent opinion that generating PERV-free animals (see below) might not be critical for xenotransplantation success.

Different strategies can be adapted to minimize the risk of PERV transmission, such as use of pig breeds with low or no PERV expression, use of antiretroviral drugs, development of vaccines against PERVs, use of PERV-specific small interfering RNAs, and use of donor pigs with the knockout of PERV genes (82). Moreover, PERV expression differs between different pig breeds as well as between different animals of the same breed, and animals with a low virus titer are ideal for xenotransplantation (82). For instance, Guizhou miniature pigs have a lower PERV copy number and should be preferred over the other miniature pig breeds (89). Another interesting pig breed is the Auckland Island pig, an inbred population free from a wide range of porcine pathogens (82). In clinical trials of Auckland Island islet xenotransplantation, there was no PERV transmission to human recipients, making them preferable tissue and organ donors for xenotransplantation (82).

**tg:** transgenic

Eliminating PERVs through gene editing is a complex process due to multiple provirus integration sites in pigs (82). Using CRISPR-Cas9-based genome editing, Yang and colleagues (90, 91) successfully generated pigs with a knockout of all PERV copies. Zheng et al. (92) used a highly efficient cytosine base editor tool to mutate all PERV copies in porcine cells in vitro. The off-target effects of cytosine base editors are minimal, and this approach can be used to generate PERV-free donor pigs for xenotransplantation (92). In a recent study, a heart from a genetically multi-modified pig was transplanted into a 57-year-old recipient, and xenograft failure was observed on day 49 (93). Polymerase chain reaction testing showed no presence of PERVs in the recipient. Of concern was the detection of PCMV in the patient on day 20, which appeared to increase over time. Despite mixed opinions about PCMV's role in xenograft failure in this study (93), the detection of PCMV in the recipient's plasma reiterates the need to choose virus-free porcine donors for organ xenotransplantation.

## 4. PIG ORGANS FOR XENOTRANSPLANTATION

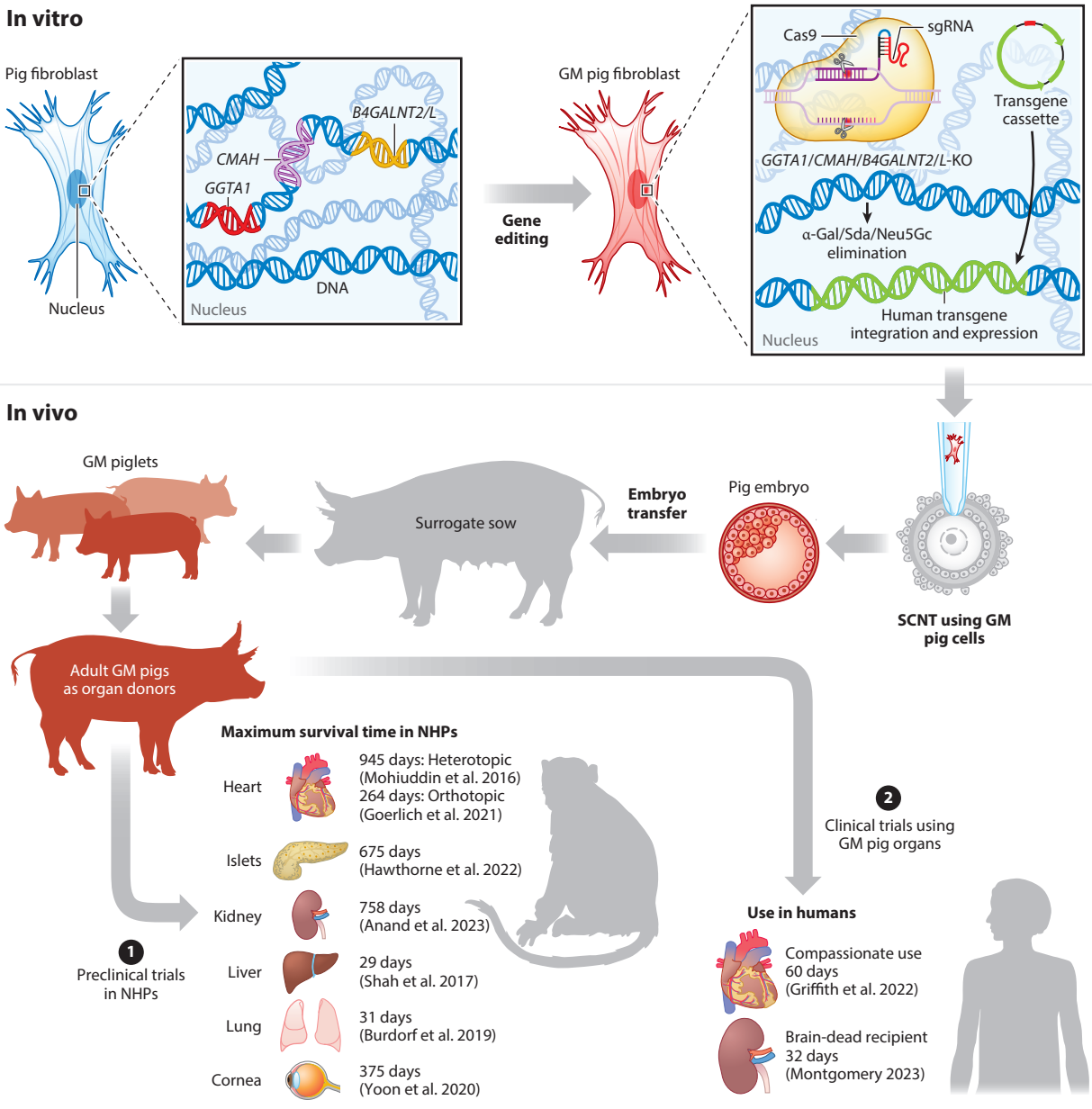
The shortage of donors and the nonavailability of transplantable tissues and organs for human allotransplantation are global issues. Extensive work has been done to explore the use of GM pigs as organ donors for pig-to-human xenotransplantation. Significant progress has been made to address different barriers in xenotransplantation, and organs from GM pigs have been used in preclinical and clinical trials (**Figure 2**). In this section, we briefly describe the progress in the xenotransplantation of different porcine cells, tissues, and organs.

### 4.1. Cardiac Xenotransplantation

Approximately 1,022 patients were waiting for a heart transplant in the Eurotransplant region as of February 2023, and the global figure is much higher (2). Recently, we published a comprehensive review on the use of GM pigs as donors in cardiac xenotransplantation (15). The factors essential to achieve long-term xeno-heart survival include protection of the xenograft against the host's immune response, the development of a clinically acceptable immunosuppressive regimen, nonischemic preservation of the xenograft before implantation, and control of postimplantation xenograft growth (15). Eliminating porcine xenoantigens (α-Gal, Neu5Gc, Sda) and transgenic expression of human complement and coagulation regulatory factors in the donor pigs significantly alleviates the host's immune response (94). In 2014, a GM pig heart [*GGTA1*-KO/hCD46-transgenic (tg)/hTBM-tg] was heterotopically transplanted into an immunosuppressed baboon, and long-term survival of >200 days was achieved (79). Later, using a modified immunosuppression regimen in the recipients, the same group reported survival of a GM pig heart for up to 2.5 years in baboons (34). In 2018, Längin et al. (95) reported the first successful series of life-supporting orthotopic transplantations of GM pig hearts (*GGTA1*-KO/hCD46-tg/hTBM-tg) into baboons, with a maximum survival time of 195 days. Importantly, postimplantation heart growth

**GHR:** growth hormone receptor

was controlled by reducing the recipient's blood pressure to the porcine level, tapering cortisone treatment, and using the sirolimus prodrug temsirolimus to lessen myocardial hypertrophy (95). A genetic intervention to reduce donor pig size involves inactivating the growth hormone receptor gene (*GHR*) (96). Goerlich et al. (97) first tested this concept for cardiac xenotransplantation in NHPs. Using orthotopic transplantation of GM pig heart (*GGTA1*-KO/*B4GALNT2*-KO/*GHR*-KO/hCD46-tg/hTBM-tg/hEPCR-tg/hCD47-tg) into a baboon, they achieved survival of up to 264 days (97).




(*Caption appears on following page*)

**Figure 2** (*Figure appears on preceding page*)

Workflow of xenotransplantation from the lab to the clinic. Using modern gene editing tools like CRISPR-Cas9-based gene editing, the porcine fibroblast cells are GM to eliminate xenoantigens and to express human transgenes. Fibroblasts with the desired set of genetic modifications are used to generate GM pigs through SCNT. Organs from GM pigs are transplanted into NHPs in preclinical trials. Heterotopic abdominal heart xenotransplantation is an experimental model where the porcine aorta is anastomosed to the recipient abdominal aorta, and the porcine pulmonary artery to the recipient inferior vena cava. The transplanted heart is perfused via the coronary arteries, and the coronary venous blood leaves the heart through the pulmonary artery trunk. The heart beats but does not support the recipient circulation. This model is used mainly to evaluate the efficacy of immunosuppressive regimens and new combinations of genetic modifications. Orthotopic heart xenotransplantation is the replacement of the recipients' heart by the organ of a source pig. After consistent success in preclinical trials under clinically acceptable conditions, a GM pig organ goes into human clinical trials. Abbreviations: B4GALNT, β-1,4-*N*-acetyl-galactosaminyl transferase; CMAH, cytidine monophosphate-*N*-acetylneuraminic acid hydroxylase; GGTA1, α-1,3-galactosyltransferase; GM, genetically modified; KO, knockout; Neu5Gc, *N*-glycolylneuraminic acid; NHP, nonhuman primate; SCNT, somatic cell nuclear transfer; sgRNA, single guide RNA.

A standard practice in clinical heart allotransplantation is to preserve the heart using cold storage on ice. A relatively new development is the nonischemic preservation of the heart by its ex vivo perfusion with an 8°C oxygenated cardioplegic solution containing nutrients, hormones, and erythrocytes (98). Längin et al. (99) compared the effect of ischemic (on-ice) preservation versus nonischemic perfusion preservation on GM pig hearts (*GGTA1*-KO/hCD46-tg/hTBM-tg) by transplanting them into baboons. Nonischemic conditions prevented early graft failure compared to storage under ischemic conditions (99). A recent breakthrough in the field of cardiac xenotransplantation is the story of David Bennett (93). A 57-year-old man, David Bennet, with nonischemic cardiomyopathy and excluded from the allotransplantation category, received a GM pig heart with 10 genetic modifications (*GGTA1*-KO/*B4GALNT2*-KO/*CMAH*-KO/*GHR*-KO/hCD46-tg/hDAF-tg/hTBM-tg/hEPCR-tg/hCD47-tg/hHO1-tg). The heart was preserved under nonischemic conditions, and the recipient was administered an immunosuppressive regimen. The xenograft functioned normally up to day 49 post transplantation, followed by sudden myocardial thickening and xenograft failure, and the patient survived up to day 60. The authors reported different possible mechanisms resulting in xenograft failure, including antibody-mediated, complement-dependent cytotoxicity and PCMV transmitted with the xenoheart (93). Using similar porcine donors with 10 genetic modifications, Moazami et al. (100) recently transplanted porcine hearts into brain-dead human recipients and observed the cardiac function over a period of 66 h. Although the cardiac function declined over time due to size mismatch, there were no signs of cellular or antibody-mediated rejection or zoonosis (100).

## 4.2. Islet Xenotransplantation

Compared to using exogenous insulin, β-cell replacement is considered a superior therapeutic option for type 1 diabetes mellitus patients in a labile metabolic condition (101). More than 400 patients are waiting for pancreatic islet transplantation in the Eurotransplant region alone (2). Porcine insulin is active in humans (102); hence, pig islets have been long considered as an alternative to human islet allotransplantation (reviewed in 8, 103). Pig islets can be isolated from the embryonic, fetal, neonatal, or adult pancreas. Adult porcine islets (APIs) have the advantage of a lower α-Gal expression and higher insulin content and immediate graft function upon transplantation compared to neonatal porcine islets (NPIs) isolated from neonates or fetuses (104). However, APIs are more difficult to isolate, and the long-term maintenance of donor pigs under designated pathogen-free conditions is expensive. In contrast, NPIs are easier to isolate but are immature and require an in vitro functional maturation. Many groups prefer the use of NPIs from the 1–5-day-old pancreas and have developed concepts to improve their functional maturation in vitro (105–108).

**HO1:** haeme oxygenase 1

**API:** adult porcine islet

**NPI:** neonatal porcine islet

**IBMIR:** instant blood-mediated inflammatory reaction

**WT:** wild type

**VEP:** vascularized endocrine pancreas

In clinical allotransplantation, islets are transplanted mainly into the portal vein, and more than 50% of islets may be lost soon after transplantation due to the instant blood-mediated inflammatory reaction (IBMIR) (109). IBMIR is an innate immune response triggered by direct contact between islets and the recipient's blood and is characterized by coagulation and complement activation, the release of proinflammatory cytokines and chemokines, leukocyte infiltration, and platelet activation (10, 109). Although IBMIR and CXR challenge both alloislets and xenoislets, the immune reaction is more severe for xenoislets (10). The longest survival of porcine xenoislets in NHPs (>900 days) was achieved using wild-type (WT) islets; however, the recipients were under a clinically unacceptable immunosuppressive regimen (110). Additionally, a substantially high quantity of WT porcine xenoislets is required to maintain long-term normoglycemia in diabetic NHPs compared to allogeneic islet transplantation, possibly due to the quick loss of WT xenoislets by IBMIR (110). These issues can be addressed to a greater extent by using xenogeneic islets from GM pigs. APIs with transgenic expression of hCD46 survived for >396 days in cynomolgus monkeys (111). Deletion of α-Gal antigens alleviates IBMIR, and *GGTA1*-KO NPIs survived for up to 249 days in the immunosuppressed diabetic rhesus monkeys (112). Hawthorne et al. (113) transplanted NPIs from GM pigs (*GGTA1*-KO/hCD55-tg/hCD59-tg/hHT-tg) into baboons, which provoked minimal IBMIR but were rejected after one month due to CXR. Bottino et al. (114) transplanted APIs from GM pigs with different genetic modifications [*GGTA1*-KO/hCD46-tg/hTFPI-tg/CTLA4-Ig (4-GM), or *GGTA1*-KO/hCD46-tg/hCD39-tg/hTFPI-tg/CTLA4-Ig (5-GM), or hCD46-tg] into diabetic cynomolgus monkeys. APIs from both 4- and 5-GM pigs avoided the early islet loss due to IBMIR and survived for >90 days. Using a dual-transplant model in rhesus monkeys, Kirk and colleagues (115, 116) demonstrated the beneficial impact of hCRPs such as hCD46-tg in addition to *GGTA1*-KO in alleviating IBMIR and platelet aggregation 24 h post NPI transplantation. Recently, Hawthorne et al. (117) reported that NPIs from GM pigs (*GGTA1*-KO/hCD55-tg/hCD59-tg) survived for a maximum of 675 days and cured diabetes in diabetic baboons under judicious immunosuppression. A variable survival time of xenoislets in the preclinical trials suggests that an optimal combination of genetic modifications and clinically acceptable immunosuppression regimens is yet to be found. Although not yet tested in NHPs, expression of the T cell costimulation blocking molecule LEA29Y in pig islets is a promising concept to prevent their T cell–mediated rejection. Proof of concept was shown in short- and long-term transplantation studies in diabetic mice with a humanized immune system (118, 119).

Another strategy to survive IBMIR and CXR is to encapsulate islets in a biocompatible material permeable to nutrients, oxygen, and hormones but impermeable to immune cells and large immunoglobulins (reviewed in 120). Although safer, the encapsulated porcine islets are not efficient to induce insulin independence in diabetic recipients (77, 121–123). In clinical trials of encapsulated NPIs, the insulin requirement of the recipients changed only minimally (121–123). Co-transplantation of free (124) or encapsulated (125) islets along with adipose tissue–derived mesenchymal stem cells increased islet engraftment and revascularization and reduced early islet loss due to IBMIR. Recently, Citro et al. (105) bioengineered a xenogeneic vascularized endocrine pancreas (VEP) containing the extracellular matrix, an endocrine compartment of NPIs, and a vascular compartment made by endothelial cells. NPIs were matured in VEP in vitro and did not require an in vivo maturation period. VEPs performed immediately upon transplantation and for more than 18 weeks, and the recipient diabetic mice returned to normoglycemia within 1 day after transplantation (105). Although significant progress has been made, most strategies are yet to meet the criteria of obtaining sustainable and consistent diabetes management for >6 months in preclinical trials before they can be introduced in human clinical trials.

### 4.3. Kidney Xenotransplantation

The number of patients waiting for a kidney transplant is strikingly high, and more than 10,000 patients are on the waiting list in the Eurotransplant region alone (2). The first animal-to-human kidney xenotransplantation was performed in 1905 using a rabbit as a donor, and the patient survived for 16 days (126). Since then, different animal-to-human kidney xenotransplantation trials have been performed, which remained largely unsuccessful due to xenograft rejection (reviewed in 127). Other than the host's immune reaction, the causes of kidney xenograft rejection include hypovolemia syndrome, erythropoietin function–associated anemia, and rapid postimplantation growth of the porcine kidney xenograft into primates (128). However, significant progress in kidney xenotransplantation has been made using GM pigs as organ donors. In 2015, kidneys from GM pigs (*GGTA1*-KO/hCD46-tg/hCD55-tg/hTBM-tg/hEPCR-tg/hCD39-tg) were transplanted into an immunosuppressed baboon (129). Even though hTBM and hCD39 expression in the kidney was insufficient, the xenograft survived for 136 days. The xenograft increased in size postimplantation; however, the reported cause of the recipient's death was a septic shock (129). Later, the same group transplanted kidneys from GM pigs with 6 genetic modifications (6-GM; *GGTA1*-KO/hCD46-tg/hCD55-tg/hEPCR-tg/hTFPI-tg/hCD47-tg) or 3 genetic modifications (3-GM; *GGTA1*-KO/hCD46-tg/hTBM-tg) into immunosuppressed baboons (128). The 6-GM kidney xenografts showed long-term survival of up to >8 months without any signs of HAR or DXR, and the studies were terminated due to peritonitis and pneumocystis pneumonia. On the other hand, the 3-GM kidney xenografts developed consumptive coagulopathies and were terminated on day 12 post transplantation (128). Adams et al. (130) transplanted *GGTA1*-KO/*B4GALNT2*-KO porcine kidneys into chemically immunosuppressed rhesus monkeys and achieved a variable graft survival from a minimum of 5 days to a maximum of 435 days. A long-term functioning of a life-sustaining xenograft (499 days) was reported by Kim et al. (78), who transplanted GM pig kidneys (*GGTA1-KO*/hCD55-tg) into rhesus macaques. Noticeably, the recipient had a pretransplant depletion of $CD4^+$ T cells and a low titer of antipig antibodies (78). Similarly, Ma et al. (131) transplanted kidneys from GM pigs (*GGTA1*-KO/*B4GALNT2*-KO/*CMAH*-KO/hCD46-tg/hCD55-tg/hCD59-tg/hHLA-E-tg/hβ2M-tg/hCD47-tg) into cynomolgus monkeys and achieved a long-term survival of up to 316 days. Markedly, prolonged depletion of $CD4^+$ T cells or screening of recipients for low anti-pig antibody titers was not required for long-term xenograft survival in this study (131). Recently, Firl et al. (132) reported another long-term life-sustaining survival of kidney xenografts in NHPs (>648 days) by transplanting Yucatan minipig kidneys with 10 genetic modifications (*GGTA1*-KO/*B4GALNT2*-KO/*CMAH*-KO/hCD46-tg/hCD55-tg/hEPCR-tg/hTBM-tg/HMOX1-tg/TNFAIP3-tg/hCD47-tg) into cynomolgus macaques. The xenografts did not disrupt the host's renin-angiotensin-aldosterone-system and had minimal growth post transplantation (132). Most recently, the longest life-supporting survival of humanized porcine renal xenograft in NHPs (758 days) was achieved by transplanting porcine kidneys with 69 genomic edits (inactivation of *GGTA1*, *CMAH*, and *B4GALNT2*; transgenic expression of hCD46, hCD55, hTBM, hEPCR, hCD47, hA20, and hHO1; inactivation of multiple PERV integrants) into cynomolgus monkeys (133).

In 2022, Montgomery et al. (134) connected *GGTA1*-KO porcine kidneys heterotopically to the circulation of two different brain-dead humans and perfused them for 54 h. The porcine kidneys started urine production soon after being connected to the recipients' circulation, and there was a significant reduction in the recipients' blood creatinine levels, indicating the physiological functioning of kidneys. Moreover, there were no signs of HAR, coagulation dysregulation, complement activation, or systemic inflammation (134). Porrett et al. (135) orthotopically transplanted kidneys from a genetically multi-modified pig (*GGTA1*-KO/*B4GALNT2*-KO/*CMAH*-KO/*GHR*-KO/hCD46-tg/hCD55-tg/hTBM-tg/hEPCR-tg/hCD47-tg/hHO1-tg) into a brain-dead human

recipient. The study was terminated at 74 h post transplantation, and there was no retroviral transmission from the porcine kidney xenograft to the recipient. Although HAR was not detected, the kidney xenograft suffered thrombotic microangiopathy during the 74 h of the experiment. Moreover, the kidneys produced variable urine volumes, but creatinine clearance was not achieved (135). Most recently, Montgomery et al. (136) transplanted a pig kidney with only one genetic modification (*GGTA1*-KO) into a brain-dead human receipient. The transplanted pig kidney started producing urine immediately after transplant and continued functioning for 32 days without any signs of rejection, the longest reported functioning and survival of a gene-edited pig kidney in a human to date (136). Due to the use of brain-dead recipients, heterotopic transplantation, and short study periods, these studies may not be seen as predictive for clinical trials but are notable progress toward the therapeutic application of kidney xenotransplantation.

### 4.4. Liver and Hepatocyte Xenotransplantation

Liver allotransplantation is a life-saving treatment for liver failure. As is the case for all other organs, several thousand patients across the globe are on the waiting list for a liver transplant, and pig-to-human liver transplantation is a possible solution for the shortage of human donors. However, the progress in liver xenotransplantation is limited due to the rapid onset of thrombotic microangiopathy and disturbed thromboregulation post transplantation, probably because the liver produces the most coagulation factors (reviewed in 75). In addition, porcine liver sinusoidal endothelial cells and Kupffer cells sequester human platelets, leading to severe thrombocytopenia (137). Porcine liver sinusoidal endothelial cells recognize and bind galactose β1–4 *N*-acetyl glucosamine residues on human platelets via ASGR1 (38). Porcine Kupffer cells can phagocytose the human platelets through species-incompatible SIRPα-CD47, CD40-CD18, and CD40-CD40L axes (75). Use of GM porcine donors can address these issues. *ASGR1*-KO pig livers displayed reduced human platelet phagocytosis in ex vivo perfusion experiments (138). Cimeno et al. (139) used GM pig liver (*GGTA1*-KO/hCD46-tg/hEPCR-tg/hTBM-tg/hCD47-tg/hHO-1-tg) and perfused it with human blood ex vivo. Moreover, the donor pigs were pretreated to deplete pig vWF and Kupffer cells in the liver. The ex vivo perfused livers were functional for a longer duration and had reduced coagulation dysregulation (139). Ekser et al. (140) orthotopically transplanted livers from *GGTA1*-KO ($n = 2$) or *GGTA1*-KO/hCD46-tg ($n = 8$) pigs into baboons under clinically acceptable immunosuppression. Most of the xenografts failed between 1 and 7 days post transplantation, mainly due to profound thrombocytopenia, which developed within an hour after reperfusion (140, 141). Shah et al. (142) orthotopically transplanted *GGTA1*-KO pig liver into an immunosuppressed baboon and achieved a survival time of >25 days. Additionally, the recipient was continuously administered exogenous human coagulation factors (II, VII, IX, X, or VIIa) to avoid coagulation dysregulation (142). The same group performed another 4 pig-to-baboon liver xenotransplantation experiments with a modified immunosuppressive regimen and achieved a survival time of 29 days (143), which is the maximum survival time of a liver xenograft so far. An alternative to pig liver xenotransplantation is pig hepatocyte xenotransplantation (reviewed in 144), which is less invasive and more convenient for using encapsulation and gene editing strategies. However, in recent years, no significant progress has been reported in hepatocyte xenotransplantation.

### 4.5. Lung Xenotransplantation

Lung transplantation is highly challenging due to the larger vascular endothelium and a considerable quantity of native immune cells such as macrophages, lymphocytes, and DCs (reviewed in 145). When perfused with human or NHP blood, pig vWF on vascular endothelial cells can activate and aggregate platelets and cause severe coagulation dysfunction (145). Moreover, the resident immune cells in porcine xenograft are activated due to hSIRPα-pCD47 incompatibilities,

leading to xenograft damage and immune rejection (75). Nguyen et al. (146) showed that the lung xenografts from *GGTA1*-KO pigs were protected from HAR in baboons but functioned for only 3.5 h due to coagulopathy. Watanabe et al. (147) transplanted lungs from *GGTA1*-KO, *GGTA1*-KO/hCD47-tg/hCD55-tg, or *GGTA1*-KO/hCD55-tg/hTBM-tg/hEPCR-tg pigs into baboons. The recipient of *GGTA1*-KO/hCD47-tg/hCD55-tg lung xenograft exhibited the longest survival time of up to 10 days (147). Similarly, lung xenografts from GM pigs with 7 gene edits (*GGTA1*-KO/*B4GALNT2*-KO/hCD46-tg/hEPCR-tg/hTBM-tg/hHO-1-tg/hCD47-tg) were used in life-supporting pig-to-baboon lung xenotransplantation, and a survival time of up to 31 days was achieved (148). Nevertheless, the limited survival time of lung xenografts to date suggests the need for additional genetic and/or drug strategies in lung xenotransplantation.

### 4.6. Cornea Xenotransplantation

More than 33.6 million people have blindness globally, and corneal blindness is one of its major causes (149). A corneal transplant, referred to as keratoplasty, cures corneal blindness, but there is a huge shortage of donors for corneal allotransplantation. To function properly in humans, the corneal xenograft should have minimal immunogenicity and similar functional, optical, and biomechanical properties to that of a human cornea. The optical and biological properties of pig cornea are compatible with humans; hence, pig-to-human corneal xenotransplantation can compensate for the shortage of corneal allografts (reviewed in 150, 151). A major advantage in corneal xenotransplantation is that cornea is immune privileged and may not trigger a strong immune response like other xenografts (150). Moreover, pig cornea has a weak expression of α-Gal, which further reduces its immunogenicity compared to other xenogeneic tissues and organs (152). Clinical trials using decellularized porcine cornea have shown promising results in multiple studies (reviewed in 151); however, the US Food and Drug Administration regards decellularized porcine cornea as a medical device and not as a xenotransplant. Considerable progress has been made in porcine corneal xenotransplantation as well; however, it is still in preclinical trials. WT porcine corneal xenografts survived for 935 days in NHPs under potent immunosuppression (153). Recently, Yoon et al. (154) transplanted full-thickness cornea from *GGTA1*-KO pigs into immunosuppressed rhesus macaques and achieved a long-term survival of >375 days. Additional genetic modifications might increase corneal xenograft survival as well as reduce the requirement for the recipient's immunosuppression.

## 5. CONCLUSION

Pig-to-human xenotransplantation could close the gap between the demand and availability of organs for allotransplantation. Through efficient and precise genetic modifications of the source pigs, major hurdles to successful xenotransplantation, in particular rejection mechanisms and coagulation dysregulation, have been overcome. Consequently, spectacular progress has been made in preclinical xenotransplantation studies of porcine hearts and kidneys in NHPs during the last few years. Survival of a terminally ill patient with a compassionate porcine cardiac xenotransplant further supports the feasibility of this strategy. Xenotransplantation of other organs like the liver and lungs is more challenging, and consistent efforts are being made to address the underlying issues of xenograft rejection or failure. Tissue transplants, like the pancreatic islet, are very promising because they become vascularized by the recipient's circulation and can be immune-protected by the local expression of potent immunomodulatory molecules. The remaining steps for entering regular clinical xenotransplantation trials include (*a*) the development and harmonization of procedures for improved microbiological/virologic safety screening of source pigs and their maintenance under designated pathogen-free conditions and (*b*) medically and ethically

acceptable patient selection criteria for the first clinical pilot studies. Based on the recent progress in the field of organ and tissue xenotransplantation, these can be expected within the next few years.

## DISCLOSURE STATEMENT

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