RESEARCH PAPER

# Targeted Immunosuppressive Therapy Protocol to Improve Long-Term Xenograft and Patient Survival in Xenotransplantation

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**ABSTRACT**

Xenotransplantation is the transfer of viable cells, tissues or organs between species and is now being considered as a viable option in the face of the ongoing scarcity of organs donated by humans. However, it is still limited in clinical application because of the strong xenogeneic immune recognition and the side effects caused by traditional broad spectrum immunosuppressive therapy. Thus, more specific immune-modulating approaches and genetically engineered pigs as donors have been the focus of interest. Recipient immunity is the key obstacle to long-term functioning of the graft despite the advances in genome editing and graft engineering. Conventional immunosuppressive agents can postpone rejection, but the nephrotoxic, metabolic, infectious and oncologic risks make long-term benefit difficult to achieve and make it hard to translate to standard clinical practice.

This review will focus on targeted immunosuppressive therapies designed to preserve xenografts and minimize toxicity associated with therapy. It is based on costimulation blockade, donor-organ gene editing, complement and cytokine modulation, and regulatory T-cell-based approaches. Recent preclinical and clinical data are presented that help elucidate the potential for selective immune control to facilitate safe and lasting xenotransplantation.

**Keywords:** Organ shortage, Xenotransplantation, Xenograft survival, Immunosuppressive therapy, Targeted immunosuppression, Graft rejection, Immune tolerance, Xenogeneic immune response, Cytokine inhibition, Costimulatory blockade, Porcine organ transplantation, Immunological barriers

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## Introduction

The demand for transplantable organs remains greater than the supply of organs available and xenotransplantation remains the focus of experimental transplant research. Pigs are considered the most promising donor species because their organ size and physiology can be modified to meet human needs by breeding, genetic engineering and husbandry control [1]. Despite the improvements in donor modification and in the care given perioperatively, immune rejection is the major hurdle. Other approaches, including mechanical support devices and tissue-engineered constructs, can reduce the burden in selected settings but cannot fully replace the biological functions of complex organs [4]. For kidney failure in particular, dialysis provides life support but does not restore normal quality of life or survival for many patients, and a substantial proportion of waitlisted patients die before transplantation [3]. Mechanical devices are also limited by infection, thrombosis, the need for anticoagulation, and risks such as bleeding during pregnancy and childbirth. Organs such as the liver perform metabolic and synthetic functions that present technologies still cannot reproduce completely.

Conventional immunosuppression, based mainly on calcineurin inhibitors (CNIs), corticosteroids, and antiproliferative agents, remains useful for controlling acute rejection but is poorly suited to indefinite, high-intensity use. Long-term treatment can cause renal injury, diabetes and other metabolic effects, cardiovascular complications, opportunistic infection, and malignancy. CNI-associated nephrotoxicity is especially important because recipients of liver, heart, or lung grafts could develop kidney disease in the future and require kidney transplantation. The same toxicities can shorten graft survival, increase post-transplant morbidity, and restrict eligibility for

patients who cannot tolerate aggressive immune suppression. Tacrolimus may worsen renal function and insulin resistance; antiproliferative drugs can suppress bone marrow; corticosteroids contribute to metabolic and infectious complications; and mTOR inhibitors have shown inconsistent effects on xenograft outcomes. These limitations support the need for better-directed immunomodulation that preserves graft function without imposing the full burden of systemic toxicity.

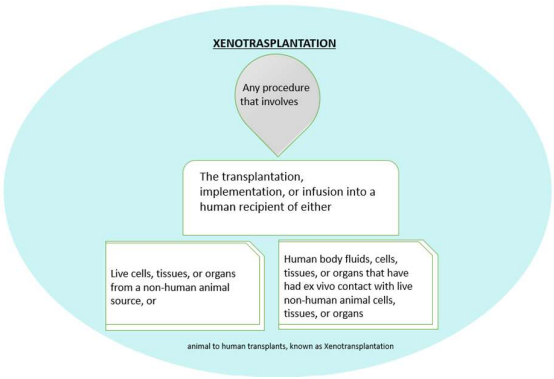



**Figure 1: Xenotransplantation**

It is estimated that over 1.5 million people around the world are waiting for an organ transplant. Some waiting-list burdens reported are approximately 103,000 cases in the USA, approximately 14,000 patients in the Eurotransplant region, nearly 7,000 patients in the United Kingdom, more than 180,000 patients per year awaiting kidney transplantation in India, over 300,000 patients in China, over 14,000 kidney candidates in Japan, and over 25,000 patients in South Korea. Symeou et al. (2025) estimated that about 70% of patients worldwide die without receiving an appropriate organ. Table 1 shows the regional transplant activity, which represents the continuing imbalance between demand and supply [4].

**Table No. 1: Worldwide region-wise total reported transplants in the year 2022 (Symeou et al., 2025)**

| Region | Total | Decease | Living |
|---|---|---|---|
| Americas | 62,153 | 20,946 | 10,527 |
| Europe | 40,337 | 11,649 | 9,816 |
| Western | 29,014 | 6,702 | 8,535 |
| SE Asia | 17,214 | 1,244 | 13,824 |
| Eastern | 8,490 | 1,251 | 5,674 |
| Africa | 286 | 0 | 286 |

In this context, xenotransplantation is a means to increase the donor pool beyond human organs. The process of transplanting living cells, organs or tissues between different species and the transplanted material is known as a xenograft. This is different from allotransplantation (between genetically different members of the same species), isotransplantation (between genetically identical individuals) and autotransplantation (in which tissue is transferred within the same individual). Historically, xenografts were referred to as heterografts and allografts were referred to as homografts, though the term heterograft was sometimes incorrectly used for xenografts [3].

## 2. Biomolecular Pathways of Xenograft Rejection

The primary biological obstacles to clinical xenotransplantation include rapid innate immune recognition of the graft, and adaptive immune responses. Coagulation incompatibility and inflammation further injure and may hasten graft loss. Strategies to prolong xenograft survival thus need to target immune, vascular and inflammatory pathways simultaneously. Therefore, it is important to comprehend these methods before rational prevention or treatment of rejection can be designed [2].

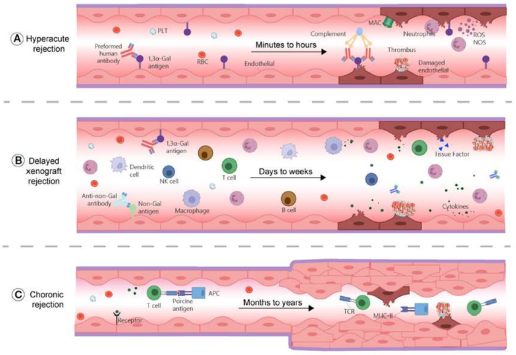
### 2.1 Hyperacute rejection (HAR):

Hyperacute rejection (HAR) occurs when the graft is suddenly rejected within the first day after transplantation, sometimes within a few minutes or hours. It is the binding of preformed human or non-human primate (NHP) antibodies to antigens on the donor organ (Figure 2A). The most widely recognized targets are the galactose-α1,3-galactose (α-Gal) epitopes on glycoproteins and glycolipids that are synthesized by the enzyme α1,3-galactosyltransferase (α1,3GT). Humans, apes, and Old World monkeys do not have functional α1,3GT due to an inactivating mutation, but it is found in many non-primate mammals and New World monkeys. Consequently, these species have a high level of anti-Gal antibodies in circulation, making up a significant proportion of natural antibody activity.

Anti-Gal antibodies can bind immediately to the endothelial surfaces bearing α-Gal when the pig organ is transplanted to a human or NHP recipient. This binding is able to activate complement, induce deposition of C3b, and cause the formation of membrane attack complex (MAC). Damage to the endothelium then results in vascular leakage, ischemia, necrosis and rapid graft failure. Fibrinoid injury of vessel walls, microvascular thrombosis and recruitment of neutrophils contribute to this process, and reactive oxygen and nitrogen species further amplify tissue injury.

Histologically, HAR is defined by diffuse vascular damage, oedema, platelet- and fibrin-rich thrombi, interstitial haemorrhage and deposition of immunoglobulins and terminal complement products in the graft vessels [2].

**Figure 2: Mechanisms of xenograft rejection.**




Hyperacute rejection is an immediate rejection of the graft, triggered by prior antibodies in the recipient against α-Gal antigens on the graft vessels. Antibody binding leads to activation of complement, formation of membrane attack complex (MAC), injury to endothelial cells and fibrinoid thrombosis with rapid vascular collapse. Reactive oxygen species (ROS) and nitric oxide species (NOS) from neutrophils can further exacerbate the injury.

Delayed xenograft rejection (DXR) typically occurs over days to weeks and involves acute humoral xenograft rejection (AHXR), cellular rejection and coagulation imbalance. AHXR is primarily antibody mediated against both α-Gal and non-Gal targets. The innate and adaptive immune cells, inflammatory mediators and disturbed coagulation processes amplify each other, resulting in deposition of immunoglobulin and fibrin, damage to the endothelium and interstitial haemorrhage.

(C) Chronic rejection occurs over months to years. Xenoantigens are processed by host antigen presenting cells (APCs) and activate T lymphocytes, which maintains inflammation. The characteristic features consist of thrombotic microangiopathy, endothelial cell proliferation in the graft, progressive narrowing of the vessels and interstitial fibrosis. Adapted from [2].

Abbreviations: APC, antigen presenting cell; MAC, membrane attack complex; MHC-II, major histocompatibility complex class II; NK cell, natural killer cell; NOS, nitric oxide species; PLT, platelet; RBC, red blood cell; ROS, reactive oxygen species; TCR, T-cell receptor.

## 2.2 Delayed xenograft rejection (DXR)

Delayed xenograft rejection (DXR) occurs following the avoidance or control of hyperacute rejection and the term used for this phase varies between studies. Mechanistically, this has been called by many authors as acute humoral xenograft rejection (AHXR) and the vascular pattern of injury, acute vascular xenograft rejection (AVXR). Both are typically antibody-mediated injury to graft vessels in which complement plays a variable role. In other reports, DXR also encompasses cellular xenograft rejection (CXR) which can occur without antibody or complement dominance. These entities overlap clinically but differ mechanistically and consistent international definitions are still needed [2].

### 2.2.1 Acute humoral xenograft rejection (AHXR)

Once a xenograft is past AHXR, HAR, or AVXR is the next big immune barrier. This rejection pattern usually occurs within days to weeks after transplantation (Figure 2B) and gradually causes destruction of the graft. Pathology commonly shows focal ischemic injury and disseminated coagulation within blood vessels, reflecting combined antibody-mediated and cellular immune activity, endothelial activation, and strong inflammatory signalling [2].

Non-Gal antigens play a role as well to AHXR. Important examples as N-glycolylneuraminic acid (Neu5Gc) and the SDa glycan antigen (Figure 3). SDa expression is controlled by the β-1,4-N-acetylgalactosaminyltransferase 2 (B4GALNT2) gene; disruption of this gene reduces porcine xenoantigenicity and lowers reactivity with human and NHP non-Gal antibodies. Neu5Gc and SDa are therefore considered major focuses of clinical donor modification. Other antigens, as GABA type A receptor-associated protein-like 1 (Gabarapl1) and cyclooxygenase-2 (COX-2), may also participate in antibody-mediated injury [2].

Recipient $CD8^+$ T cells can respond to swine leukocyte antigen class I (SLA-I), and some anti-human leukocyte antigen (HLA) class II antibodies may cross-react with SLA-II. IgM and IgG binding to non-Gal targets can trigger complement and drive antibody-mediated graft damage. Neutrophils might trigger porcine endothelial cells (pECs), while natural killer (NK) cells and macrophages are also implicated, although their exact roles remain incompletely defined [2].

AHXR is typically associated with interstitial haemorrhage, neutrophil infiltration, necrosis, infarction, thrombosis, and deposition of immunoglobulins, complement, fibrin, and platelets in the graft tissue [2].

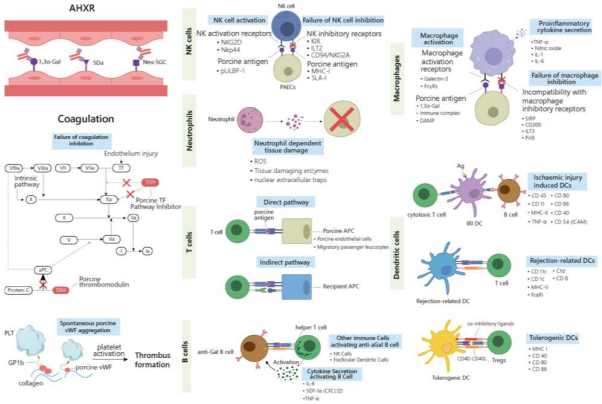



Figure 3: Delayed xenograft rejection as AHXR, cellular xenograft rejection, and coagulation

disturbance. In AHXR, antibodies target α1,3-Gal (α-Gal) and non-Gal antigens such as Neu5Gc and the SDa blood group antigen. Cellular responses are promoted when graft-cell ligands activate recipient NK cells and macrophages, while insufficient inhibitory signalling from graft cells allows cytotoxic and inflammatory responses to continue. Activated neutrophils add injury through proteolytic enzymes, and neutrophil extracellular traps (NETs), reactive oxygen species (ROS). Dendritic cells (DCs) also contribute: ischemia-reperfusion injury (IRI)-associated DCs activate cytotoxic T cells and B cells; rejection-associated DCs promote acute and chronic T-cell responses; and tolerogenic DCs support regulatory T-cell (Treg) development. T lymphocytes reject xenografts through direct recognition by porcine APCs and indirect presentation by host APCs. B cells, stimulated by helper T cells and cytokines, mature into antibody-producing plasma cells.

AHXR and cellular rejection are also linked with coagulation incompatibility. Porcine TFPI does not fully restrain recipient factor Xa activity and therefore provides incomplete inhibition of TF. Porcine TBM is likewise inefficient at supporting recipient protein C activation, and porcine vWF can interact with glycoprotein Ib (GP1b) on recipient platelets. These mismatches favour intravascular thrombosis and graft failure. Adapted from [2].

Abbreviations: AHXR, acute humoral xenograft rejection; Ag, antigen; aPC, activated protein C; DC, dendritic cell; IRI, ischemia–reperfusion injury; NET, neutrophil extracellular traps; TBM, thrombomodulin; TF, tissue factor; Tregs, regulatory T cells; vWF, von Willebrand factor.

Cellular xenograft rejection (acute cellular rejection)

Cellular xenograft rejection (CXR) may occur when immunosuppression is insufficient and usually appears within days to weeks. It reflects coordinated innate and adaptive immune activity involving NK cells, macrophages, neutrophils, dendritic cells, T lymphocytes, and B lymphocytes [2].

**2.2.2.1 NK cells:**

NK cells contribute to xenograft injury through a balance of activating and inhibitory signals on porcine endothelial cells. They can kill target cells through perforin-granzyme release, FasL/Fas and TRAIL pathways, and antibody-dependent cell-mediated cytotoxicity (ADCC). Activating receptors such as NKG2D and NKp44, together with adhesion and signalling molecules including CD49, CD11a/CD18, CD11b/CD18, and CD99, recognize porcine ligands and promote NK-cell degranulation. Inhibitory receptors such as killer immunoglobulin-like receptors (KIRs), ILT2, and CD94/NKG2A normally restrain NK cells through MHC-I recognition. Because porcine MHC-I molecules do not provide fully compatible inhibitory signals, NK activation may persist. Expression of human HLA-C, HLA-G, or HLA-E on porcine cells can reduce this cytotoxicity [2].

ADCC-mediated NK rejection occurs when antibodies, including anti-α-Gal or anti-Neu5Gc antibodies, coat porcine cells and engage CD16 (FcγRIIIa) on NK cells. NK cells may also support non-Gal antibody formation through interactions with B lymphocytes. Although these effects are well documented in vitro and in animal models, their precise clinical contribution to CXR still requires further pig-to-primate investigation [2].

Because NK cells can damage xenografts, therapies that modulate rather than completely abolish NK-cell activity are being explored. Proposed approaches include blocking activating receptors such as NKG2D and NKp44, limiting NK survival through IL-15 pathway inhibition, and shifting immune tone with regulatory cytokines such as transforming growth factor-β (TGF-β) and interleukin-10 (IL-10). Dampening inflammatory mediators such as interleukin-27 (IL-27) may also reduce ischemia-reperfusion injury and early graft dysfunction.

Together, these genetic and pharmacological strategies show that NK-cell regulation is likely to be an important component of future xenotransplant protocols. But, data from cell culture and small animal studies are not enough; additional pig-to-primate studies are required to establish the timing of NK cell-mediated rejection and how to target them clinically.

2.2.2.2 Macrophages: Macrophages are involved in phagocytosis, antigen presentation, cytokine production and tissue repair, in xenotransplantation they can also contribute to rejection. They recognize injured xenogeneic tissue by means of Toll-like receptors (TLRs) that recognize danger-associated molecular patterns (DAMPs), pathogen-associated molecular patterns (PAMPs), polysaccharides, and polynucleotides. TLR activation is enhanced by interferon-γ (IFN-γ), leading to enhanced macrophage activation, antigen processing and inflammatory damage to the graft [2].

2.2.2.3 Neutrophils: Neutrophils cause damage to xenografts by oxidative, enzymatic and inflammatory processes. Reactive oxygen species (ROS) are produced by activation of NADPH oxidase, and proteolytic enzymes break down extracellular matrix proteins like collagen, elastin and fibronectin and disrupt endothelial junctions. Neutrophil extracellular traps (NETs) also increase inflammation. Neutrophils can also enhance the cytotoxicity of NK-cells and enhance the expression of adhesion molecules on porcine endothelial cells (pECs) and thus exacerbate endothelial activation [2].

**2.2.2.4 Dendritic cells:**

Dendritic cells (DCs) are antigen presenting cells that are able to activate T cells or induce tolerance

depending on their phenotype. In Xenotransplantation, there are three functional groups that are discussed. IRI associated DCs respond to ischemia-reperfusion injury and activate cytotoxic T cells and B cells. Acute and chronic T-cell activation is maintained by rejection associated DCs. Tolerogenic DCs inhibit $CD4^+$ and $CD8^+$ effector responses and promote regulatory T-cell (Treg) activity. These tolerogenic cells typically express less MHC and co-stimulatory molecules (e.g., CD40 and CD80/86) and more inhibitory ligands (e.g., programmed death-ligand 1 [PD-L1]).

DC behaviour is plastic and host-derived DCs can switch from inflammatory to tolerogenic and vice versa depending on the local signals. Several factors have been tried to promote tolerogenic DC development in experiments, including rapamycin, interleukin-10 (IL-10), vitamin D, and low dose granulocyte-macrophage colony-stimulating factor (GM-CSF). Despite this, the specific role of DC subsets in xenograft rejection and tolerance is still a focus of research [2].

**2.2.2.5 T-Cells:**

Activation of T-cells in xenotransplantation can be thought of as a three signal event. Signal 1 is the binding of xenogeneic SLA-I/II molecules on porcine antigen presenting cells (APCs) by the T-cell receptor (TCR) and causing direct damage to the graft endothelium. Co-stimulatory pathways, particularly CD28-CD80/86 and CD40-CD154, provide signal 2 for direct and indirect activation of T-cells. In the indirect pathway, porcine antigens are processed by the recipient APCs and are presented to host T cells. Cytokines like interleukin-2 (IL-2), interleukin-6 (IL-6), and interferon-γ (IFN-γ) provide signal 3 and promote T-cell proliferation, differentiation and effector function. These signals are combined to produce a potent anti-xenograft response.

Targeted immunosuppression is an effort to disrupt these activation steps in a more specific way than conventional, nonselective therapy. Anti-CD40 antibodies, CTLA4-Ig based drugs and strategies that reduce SLA-I expression can reduce T-cell and NK-cell mediated damage and may reduce systemic toxicity. Belatacept is an example of this. It is a fusion protein of CTLA4 which binds to CD80 and CD86 and blocks the co-stimulation of CD28, thereby inhibiting the delivery of the second signal needed for full T-cell activation. This leads to decreased T-cell proliferation, cytokine production, cytotoxic differentiation and T-cell-dependent antibody formation [2].

**2.2.2.2 B-cells:**

A meaningful fraction of natural human antibodies targets α-Gal, including approximately 1% of IgG and 1-4% of IgM. These antibodies arise mainly from splenic B cells, with additional production in lymph nodes and bone marrow, particularly from $Mac1^+B1b$-like populations. Anti-Gal antibody development depends on interactions among B cells, T cells, NK cells, and follicular DCs. After exposure to porcine antigen, B cells proliferate, undergo T-cell-dependent maturation in germinal centres, and generate high-affinity anti-Gal antibodies. Marginal zone B cells can also produce xenoantibodies with NK-cell support, while follicular DCs enhance responses by displaying α-Gal-containing immune complexes.

Because B cells generate the anti-porcine antibodies responsible for AHXR, they are a logical therapeutic target. Peri-transplant anti-CD20 monoclonal antibody therapy can deplete B cells, reduce anti-pig antibody production, limit graft injury, and delay coagulation abnormalities and thrombotic microangiopathy [2].

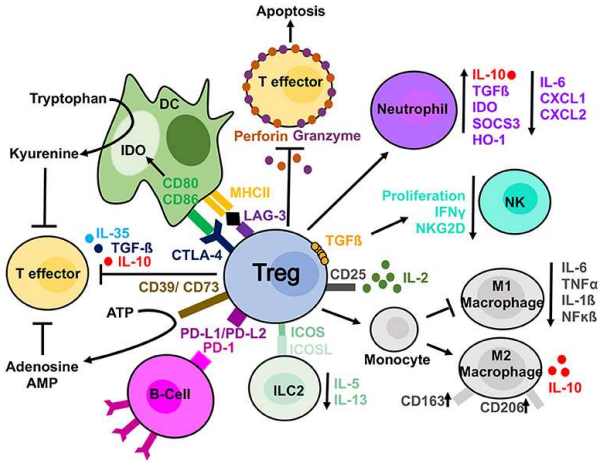



Figure 4: Regulatory T-cell (Treg)-mediated immune suppression. Tregs inhibit immune activation through several direct and indirect mechanisms. They secrete anti-inflammatory cytokines, including interleukin-35 (IL-35), interleukin-10 (IL-10), and transforming growth factor-β (TGF-β), which reduce T-cell activation and inflammatory signalling. They may also induce target-cell apoptosis through perforin and granzyme release, disrupting target-cell membranes.

High CD25 expression enables Tregs to capture interleukin-2 (IL-2) from the local environment, reducing the cytokine supply available to effector T cells. The same reduction in IL-2 availability can restrict NK-cell proliferation and function. Tregs can also suppress NK cells directly through membrane-bound TGF-β-dependent contact.

Tregs influence B cells through PD-L1/PD-1 signalling and regulate dendritic cells (DCs) through CTLA-4 and lymphocyte activation gene-3 (LAG-3). CTLA-4 reduces CD80/CD86 co-stimulation on antigen-presenting cells and supports activity of indoleamine 2,3-dioxygenase (IDO), which favours tolerance. CD39 expression on Tregs converts extracellular ATP to adenosine monophosphate (AMP) and adenosine, helping suppress effector T-cell proliferation.

Tregs also shape innate immune responses. They can push monocyte differentiation toward anti-inflammatory M2 macrophages and away from pro-inflammatory M1 macrophages. Comparable suppressive effects have been described in neutrophils and in type 2 innate lymphoid cells (ILC2s), where Tregs reduce cytokine secretion.

**Chronic rejection**

Chronic rejection typically appears from months to several years following transplantation and resembles several chronic patterns seen in allotransplantation. It is commonly associated with thrombotic microangiopathy, gradual vessel narrowing, endothelial proliferation, and interstitial fibrosis, all of which can eventually impair graft function (Figure 2C). Because only a limited number of xenografts have survived long term, the mechanisms of chronic xenograft rejection remain less defined, but persistent low-grade immune activation is considered an important contributor.

Available evidence suggests that chronic rejection is multifactorial, involving both non-immune and immune pathways. Incompatibility between pigs and non-human primates (NHPs) coagulation systems is thought to be one mechanism that promotes progressive vascular injury and long-term graft deterioration [2].

Mohiuddin et al. showed that gene-edited pig hearts expressing "GTKO.hCD46.hTBM", used with anti-CD40 monoclonal antibody therapy, survived for as long as 236 days in baboons. The findings indicate that human thrombomodulin (hTBM) expression and CD40-directed immunosuppression can work together to extend survival and reduce thrombotic microangiopathy and related coagulation injury.

Another approach used transgenic activity of human CD39, a vascular ectonucleotidase that transforms ATP and ADP to AMP and, subsequently, to adenosine. Because adenosine has antithrombotic and anti-inflammatory effects, CD39 expression reduced thrombotic events in cardiac grafts and doubled survival in mice, from about a three-day period in the wild-type group of animals to about six days in transgenic recipients.

The prevention of xenotransplantation rejection

Since 2009, genomic alteration of donor pigs has advanced rapidly to improve compatibility with human recipients. Transcription activator-like effector nucleases (TALENs), Zinc finger nucleases, and especially CRISPR/Cas9 have made it easier to create pigs with multiple targeted edits. The following section provides an overview of the most frequently used immunosuppressive drugs in xenotransplantation, their mode of action and their clinical or preclinical significance (Figure 5).

The mainstay of rejection prevention and treatment is immunosuppressive drugs. Conventional agents like cyclophosphamide, tacrolimus and corticosteroids can be used at high enough doses to delay graft loss. Such protocols have been used to achieve extended graft survival in concordant NHP kidney and liver xenotransplant models. In 2000, however, a major paradigm change was achieved in xenotransplantation research with the introduction of costimulation blockade, which ultimately proved more effective than many of the traditional drug combinations [2].

**3.1 Glucocorticoids**

Glucocorticoids (GCs) are a class of steroid hormones which have been employed in transplantation for decades as induction and maintenance immunosuppressants. They help to decrease acute rejection by suppressing immune-cell activity and inflammation. GC-containing treatment protocols have been part of many experimental protocols in pig-to-primate xenotransplantation. In one islet xenotransplantation model, GC treatment was able to normalize diabetic and glycaemic parameters within 4 days in all monkeys, indicating a possible reversal of diabetes mellitus. The longest reported graft survival of 78 days has been reported in a renal xenotransplantation model using GCs in combination with other immunosuppressive drugs [2].

**3.2 Calcineurin inhibitors**

The main calcineurin inhibitors (CNIs) are tacrolimus and cyclosporine. Both block the dephosphorylation of nuclear factor of activated T cells (NFAT) by blocking calcineurin. If NFAT is unable to enter the nucleus, calcineurin-dependent gene transcription is decreased. This inhibits activation and maturation of T-cells and decreases lymphokine production, particularly interleukin-2 (IL-2).

**3.2.1 Cyclosporin**

Cyclosporin is a cyclic polypeptide of 11 amino acids, the majority of which are hydrophobic. It has been introduced in the early 1980s and has revolutionized transplantation practice by decreasing the incidence of acute graft rejection. The drug forms a drug-immunophilin complex that binds to and inhibits calcineurin. This inhibits the dephosphorylation and nuclear translocation of NFAT, which decreases the activation of T-cells, the production of IL-2 and the clonal expansion of T-cells.

Cyclosporin has been shown to be beneficial in Xenotransplantation models. Steroid-treated cardiac xenografts with cyclosporin (Cx) had a long survival period of up to 77 days, with no hyperacute rejection or cyclosporin-associated malignancy. Cyclosporin after transplantation has been linked to liver xenotransplant survival of 91 and 1076 days in baboon-to-monkey liver xenotransplantation studies [2].

**3.2.2 Tacrolimus**

Tacrolimus is a 23-membered macrolide lactone first isolated from Streptomyces tsukubaensis in 1987. It

binds FK506-binding protein (FKBP), forming a complex that competitively inhibits calcineurin. Through this action, tacrolimus blocks NFAT nuclear translocation and decreases transcription of cytokine-related genes, including TNF-α, IL-2, IL-3, IL-4, CD40L, IFN-γ, and GM-CSF. The overall effect is suppression of T-cell activation and proliferation.

After first receiving clinical approval for liver transplantation in 1994, tacrolimus became a core drug in solid-organ transplant protocols. It is used during induction and maintenance, frequently with glucocorticoids that are tapered over time. Tacrolimus lowers acute rejection rates and improves rejection-free graft survival, and pig-to-rat islet xenotransplantation experiments have also shown substantial immunosuppressive activity [2].

### 3.2 Antiproliferative agents

#### 3.2.1 Cyclophosphamide

Cyclophosphamide (CYC) is used in transplantation to prevent graft rejection and graft-versus-host disease. As a nitrogen mustard derivative, it alkylates DNA in a non-cell-cycle-specific manner and can affect many cell types. Its active metabolites crosslink DNA and RNA, interfere with protein synthesis, induce programmed cell death, and inhibit cell proliferation.

CYC suppresses immunity mainly by depleting mature host T cells that are proliferative and donor-reactive. It can also reduce regulatory T-cell populations, lower production of T-cell growth factors such as type I interferons, and condition host immune cells to support donor-cell engraftment. In xenotransplantation studies, CYC-containing regimens have shown benefit; in pig-to-rhesus corneal transplantation, intravenous CYC followed by porcine bone marrow cell infusion reduced inflammatory-cell infiltration.

Cyclophosphamide can be given continuously or as intermittent pulses by oral or intravenous routes, with reported dosing generally ranging from 10 to 40 mg/kg [2].

3.2.2 Mammalian target of rapamycin inhibitors

The mammalian target of rapamycin (mTOR) pathway is involved in the regulation of cell growth, proliferation and metabolism. Rapamycin forms a complex with the 12-kDa FK506-binding protein (FKBP12) that is a gain-of-function inhibitor of mTOR complex 1 (mTORC1). This leads to immunosuppressive and antiproliferative effects, partly due to the decrease in the activity of S6K1, a serine/threonine kinase that is activated downstream of multiple signalling pathways. A reported rapamycin regimen was 0.2 mg/kg for the first 3 days after transplant and then alternate day dosing until day 14 [2].

#### 3.2.3 Leflunomide

Leflunomide is an inhibitor of dihydro-orotate dehydrogenase, an enzyme that is rate-limiting for pyrimidine synthesis. By disrupting pyrimidine availability, it interferes with progression from S phase to G2 phase and reduces cell proliferation. Although xenotransplantation data remain limited, leflunomide has been reported to reduce rat-to-mouse cardiac xenograft rejection by suppressing NF-κB signalling and adaptive immune responses [2].

#### 3.3.4 Mycophenolate mofetil

MMF is a semisynthetic morpholinoethyl ester of mycophenolic acid. It limits T- and B-cell proliferation by selectively inhibiting the purine synthesis pathway required for lymphocyte replication. MMF is often included in maintenance regimens with other agents and has been administered intravenously at 20 mg/kg twice daily in experimental protocols. In cardiac xenotransplantation studies, MMF-containing therapy has been associated with substantial survival gains, including reported graft survival of up to 945 days.

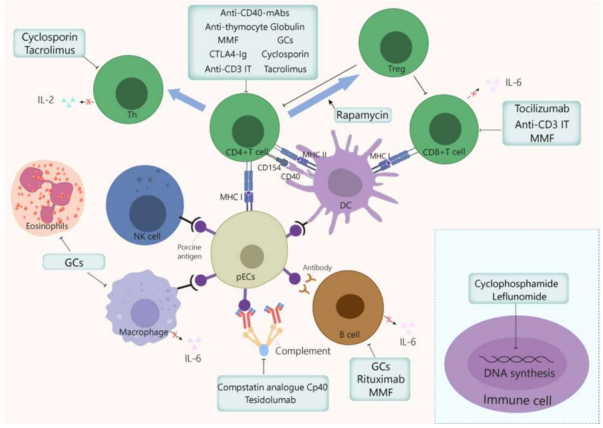



**Figure 5:**

Mechanisms of action of immunosuppressive agents used in xenotransplantation. Glucocorticoids (GCs) bind cytoplasmic GC receptors and suppress macrophages, T lymphocytes, eosinophils, and, to a lesser degree, B lymphocytes. Cyclosporin binds cyclophilin, and the resulting drug-immunophilin complex inhibits calcineurin, reducing T-helper (Th) cell activation, interleukin-2 (IL-2) production, and clonal T-cell expansion. Tacrolimus binds FKBP and similarly blocks transcriptional pathways required for pro-inflammatory cytokine synthesis.

Cyclophosphamide alkylates DNA and promotes cell death across several cell populations. Leflunomide blocks pyrimidine biosynthesis and arrests the cell cycle during S phase. MMF limits T- and B-lymphocyte replication by targeting the purine synthesis pathway. Polyclonal anti-thymocyte globulins mainly deplete T cells, although other immune cells with shared surface antigens may also be affected.

Monoclonal antibodies provide pathway-specific immune control by targeting cytokine systems, such as the IL-6 receptor (IL-6Rα), or surface markers such as CD3 and CD20. Tocilizumab blocks IL-6 receptor signalling and reduces inflammatory responses as well as $CD8^+$ T-cell and B-cell differentiation. Anti-CD3 immunotoxins transiently deplete $CD3^+$ T cells in blood and lymph nodes. Rituximab removes $CD20^+$ B cells. Rapamycin suppresses T-cell proliferation through S6K1 and phosphoinositide 3-kinase (PI3K)-related pathways. CTLA-4Ig and anti-CD40 monoclonal antibodies interrupt CD80/86:CD28 and CD154:CD40 co-stimulation, respectively. Cp40 and tesidolumab target complement components C3 and C5 to reduce complement-driven injury. Adapted from [2].

Abbreviations: FKBP, FK506-binding protein; IT, immunotoxin; GCs, glucocorticoids; mAb, monoclonal antibody; pECs, porcine endothelial cells MMF, mycophenolate mofetil;.

3.4 Monoclonal or polyclonal antibodies

MAbs are used in transplant research and clinical immunosuppression because they can target defined immune-cell populations or pathways. Some agents act on $CD3^+$ T lymphocytes, a central population in solid-organ rejection. In primate studies, transient T-cell depletion with anti-CD3 immunotoxins (ITs) produced stable renal tolerance without irradiation or long-term maintenance immunosuppression. Peripheral-blood and lymph-node T-cell populations were reduced to nearly 1% of baseline. Protocols using anti-CD3 IT at 100 mg/kg two hours before transplantation and again the day after transplantation have shown promise, although further optimization is necessary.

Recent mAb development has increasingly focused on cytokine pathways that connect inflammation, coagulation, and tissue injury. Interleukin-6 (IL-6) supports $CD8^+$ T-cell and B-cell differentiation, systemic inflammatory responses, and endothelial-cell survival after xenotransplantation. Zhao et al. cuggested that IL-6 may also contribute to coagulation and inflammatory complications, while Ezzelarab et al. reported that IL-6 pathway blockade with agents such as tocilizumab can reduce body-wide inflammation in xenograft recipients and may help control coagulation dysregulation.

Tocilizumab targets human IL-6 receptor alpha (IL-6Rα) and reduces downstream STAT3 signalling. In pig-to-NHP models, it has been reported to delay revascularization of transplanted xeno-islets. Case reports also suggest that tocilizumab combined with other immunosuppressants can support pig kidney graft survival for up to 136 days. However, Zhang et al. found increased serum IL-6 in baboons given tocilizumab before xenotransplantation, raising concern that excess IL-6 could interact with porcine IL-6 receptors and activate pig-derived cells. Its overall role in xenotransplantation therefore remains uncertain and requires additional study.

Rituximab is a chimeric anti-CD20 antibody that depletes B lymphocytes. Beyond its established role in post-transplant lymphoproliferative disorders, it has been evaluated for acute rejection, where it may reduce progression toward chronic antibody-mediated rejection. It likely has effects on B-cell regulation of T-cell responses and long-term changes in plasma-cell differentiation.

Polyclonal anti-thymocyte globulins (ATGs) are made by immunizing animals (typically rabbits) with human lymphoid cells (peripheral T cells, thymocytes, or B lymphoblasts) and purifying the resulting immunoglobulins. ATGs are directed mainly against T lymphocytes, but can also recognize antigens on B cells, monocytes, and neutrophils. They kill lymphocytes by activation-induced apoptosis and complement-mediated cytolysis. In xenotransplantation models, ATGs have improved neonatal porcine xeno-islet engraftment and extended pig kidney graft survival when combined with other agents [2].

3.5 Blockade of costimulatory signals

3.5.1 Blockade of CD80/86:CD28 costimulatory pathway by CTLA4Ig

Full T-cell activation requires co-stimulation, most notably through CD28 on T cells interacting with CD80/86 on antigen-presenting cells (APCs). Cytotoxic T-lymphocyte-associated protein 4 (CTLA4) competes for CD80/86 binding and thereby dampens T-cell activation. This biology led to CTLA4-Ig fusion proteins, in which CTLA4 is linked to the heavy chain of human IgG, as a strategy to reduce graft rejection.

Belatacept, a later CTLA4-Ig molecule, showed stronger affinity for CD80/86 in preclinical primate kidney transplantation and improved control of adaptive immune responses. It also reduced anti-graft humoral responses in studies involving intracerebral transplantation of mesencephalic porcine xenografts into primates.

In vivo work indicates that CTLA4-Ig can suppress T-cell-dependent immunity and extend xenograft and allograft survival. Levisetti et al. reported prolonged survival in two of five CTLA4-Ig-treated monkeys, with reduced humoral responses in all treated animals. Buerck et al. later developed transgenic pigs expressing the CTLA4-Ig analogue LEA29Y and found that transplanted INSLEA29Y-transgenic porcine neonatal islet-like cell clusters preserved β-cell function and avoided rapid T-cell-mediated rejection during a 30-day observation period.

Even so, the durability of CTLA4-Ig-based protection in xenotransplantation remains unresolved. In a pig-to-baboon model, blocking the CD28-B7 pathway

with human CTLA4-Ig failed to prevent rejection, illustrating that this approach may not be sufficient across donor organs, models, or rejection settings [2].

3.5.2 Targeting of CD154:CD40 costimulatory signal with anti-CD40mAb

CD154 on activated T lymphocytes interacts with CD40 on APCs and increases CD80/86 expression, strengthening co-stimulatory signalling. Because platelets also express CD154, anti-CD154 strategies have been associated with thrombotic risk in primate studies. For this reason, attention has shifted toward CD40 itself. Several anti-CD40 mAbs are under investigation, and CD40/CD154 pathway blockade has prolonged graft survival and reduced rejection in preclinical models. The fully humanized anti-CD40 antibody iscalimab is one example of a candidate with transplantation relevance.

Anti-CD40 mAbs have shown encouraging results in xenotransplantation experiments. The anti-CD40 antibody 2C10R4, combined with tacrolimus, extended islet graft survival in pig-to-NHP models. In pig-to-mouse islet transplantation, short-term MR-1 anti-CD40 therapy with anti-LFA-1 mAb improved neonatal porcine islet survival, and MR-1 alone could prolong survival while recruiting $CD4^+$ Tregs to grafts and lymphoid tissues. These findings are consistent with observations that lower $CD4^+$ Treg levels correlate with higher rejection risk in pig-to-NHP cardiac xenotransplantation [2].

**3.6 Complement inhibition**

Complement activation contributes to several phases of xenograft rejection, so complement-directed treatment is an important part of many proposed protocols. Strategies have included complement depletion or inhibition to limit tissue injury. Cobra venom factor can prolong graft survival in experimental allotransplantation but has a short-lived effect. C1-esterase inhibitor has shown benefit in NHP studies and is considered a more practical alternative to cobra venom factor.

Cp40, a compstatin analogue that inhibits complement component C3, is a promising newer option. It reduces leukocyte adhesion, limits neutrophil attachment to porcine endothelial cells (pECs), and suppresses activation of both pECs and leukocytes. Cp40 also lowers endothelial adhesion molecules including E-selectin, ICAM-2, ICAM-1, and VCAM-1, as well as the neutrophil integrin CD11b. Schmitz et al. reported improved median allograft survival in NHPs, with about half of treated primates retaining normal renal function at the end of therapy. In another study, Tibetan macaques with liver xenografts and immunosuppression using Cp40 did not suffer from severe coagulation disorders or from significant immune rejection.

Other complement inhibitors are being tested. Anti-C5 monoclonal antibody (tesidolumab) has been demonstrated to have promise in decreasing early antibody-mediated rejection and prolonging renal xenograft survival.

3.7 Genetic engineering strategies

Multi-transgenic pigs with deletion of major xenoantigens (e.g. GGTA1 (α-Gal), CMAH (Neu5Gc), and β4GalNT2) have been produced using CRISPR-Cas9 editing. These changes minimize binding of existing recipient antibodies. Further improvement of graft compatibility can be achieved by introducing human genes for immunoregulatory and complement-regulatory proteins. Donor genetic engineering in combination with pharmacological humoral blockade could be used to decrease antibody-mediated xenograft rejection by complementary mechanisms [2].

**Additional Strategies**

Adjunctive strategies have also been tested to improve graft survival while limiting systemic toxicity. Depletion of immune effector cells and induction of tolerance with total lymphoid irradiation (TLI), especially in combination with cyclosporine, can prolong graft survival in experimental models.

Another strategy designed to achieve antigen-specific tolerance via the gastrointestinal immune system is oral exposure to xenoantigens. Oral tolerance strategies could induce immune unresponsiveness to donor antigens and be useful to suppress cellular and antibody-mediated rejection.

Local drug delivery may help achieve immunosuppressive levels at the graft site and limit systemic exposure. Another idea, called phase-directed therapy, is the idea that treatment should be tailored to the type of rejection, hyperacute, acute, or chronic. These approaches, in combination, complement genetic, cellular and pharmacological interventions and could contribute to a safer long-term graft function in xenotransplantation.

## ADVANCED IMMUNOSUPPRESSION PROTOCOLS FOR XENOTRANSPLANT TRIALS

In the 1970s, the advent of cyclosporine dramatically increased graft survival and brought clinical allotransplantation back into the picture. It proved to be a success and revealed the importance of strong immune control in transplantation. Cyclosporine also demonstrated a chronic issue: higher levels of immunosuppression can lead to opportunistic infection and drug toxicity, particularly with long-term use.

The survival benefit seen with CD40/CD154 blockade led to the next step of increasing treatment to include targeting of other immune pathways in xenotransplantation. Several of the resulting protocols include multiple drugs that have similar and often redundant mechanisms. These regimens have resulted

in long xenograft survival in some animal studies, but also in serious infections and frequent euthanasia of recipients, which may be evident in the censored survival curves, albeit not always highlighted in the text.

Very deep immunosuppression can be achieved by repeated doses of rituximab to suppress post-xenotransplant humoral responses. It offers valuable proof of principle, but is unlikely to be a safe routine clinical approach as B-cell depletion is likely to result in severe or irreversible infection in the recipient. The concern of infectious risk is further boosted by the reported involvement of porcine cytomegalovirus (CMV) in the death of the first clinical pig-heart xenotransplant recipient. Other more selective agents, such as proteasome inhibitors and anti-CD38 antibodies, may provide better humoral control, but have not been studied sufficiently in xenotransplantation.

Thrombotic microangiopathy (TMA) and interstitial haemorrhage are also very common in xenografts besides antibody-mediated rejection (AMR). It remains unclear if these lesions are distinct causes of xenograft failure, as similar pathology is seen in other transplant settings such as ABO incompatible allotransplantation. They can also be formed downstream of AMR, instead of being individual rejection mechanisms. Thus, it is still unclear whether TMA and interstitial haemorrhage alone can cause graft loss when antibody-mediated injury is absent [13].

Gene editing and improved immunosuppressive design have moved xenotransplantation closer to clinical use, but rejection control remains the key challenge. An ideal regimen would prolong graft survival while limiting harm to the recipient. Current strategies include removal of preformed donor-specific antibodies by plasmapheresis, suppression of T- and B-cell responses, complement inhibition, anticoagulation, and anti-inflammatory treatment to reduce immune-cell trafficking into the graft. In parallel, transgenic pigs have been engineered to reduce humoral, cellular, coagulation-mediated, and complement-mediated injury. Drug compatibility is also important; some combinations, such as calcineurin inhibitors with certain co-stimulatory blockade strategies, may produce adverse effects. Intravenous immunoglobulin, although useful in other rejection settings, may carry xenoantigen-reactive antibodies and therefore may not be appropriate for all clinical xenotransplantation trials.

Most pig-to-NHP protocols under evaluation focus on co-stimulatory pathways, particularly CD40-CD154 and CD28/CTLA4-CD80/86 interactions, because these signals are essential for T-cell activation. Early anti-CD154 mAbs suppressed T-cell responses in pig-to-NHP models, but clinical development was limited by thrombotic complications. Anti-CD40 mAbs later offered a way to inhibit the same pathway without the same degree of platelet-related risk. In 2016, Mohiuddin et al. reported cardiac xenograft survival of 945 days in GTKO.hCD46.hTBM pig-to-NHP models treated with anti-CD40 mAb. These antibodies also affect B-cell activity by interrupting co-stimulation. In the University of Maryland pig-to-human heart transplant, anti-CD40 mAb was combined with rituximab and rabbit anti-thymocyte globulin (RATG). The recipient survived 61 days and died from non-cardiac causes; importantly, the modified pig heart avoided hyperacute rejection and functioned for about two months, making it a major step in life-supporting porcine organ transplantation.

NK cells are another important rejection pathway beyond T- and B-cell immunity. Donor modifications such as GalT knockout (GalT-KO) and expression of HLA-E with human β2-microglobulin may reduce NK-cell cytotoxicity. Regulatory T cells (Tregs) are also being studied as a tolerance-promoting therapy. Recipient-derived, xenoantigen-specific Tregs can suppress effector T cells and support donor-specific tolerance. In pig-to-NHP cardiac transplantation, lower peripheral Treg numbers were linked with rejection in 2018. Wu et al. showed that Tregs help maintain donor-specific tolerance in rodent neonatal porcine islet xenotransplantation, while Huang et al. reported that ex vivo-expanded baboon $CD39^{+}$ Tregs prevented porcine islet rejection for more than 100 days in primatized NOD-SCID IL-2r$\gamma^{-/-}$ mice.

Other regulatory immune populations may also support tolerance. Regulatory B cells (Bregs) can suppress effector T cells, promote Treg activation, and reduce antigen presentation by dendritic cells and macrophages. Tolerogenic DCs contribute to central and peripheral tolerance through clonal deletion, Treg induction, and suppression of memory T-cell responses. Madelon et al. found that co-transplanting autologous IL-10-treated murine tolerogenic DCs improved rat islet xenograft survival in diabetic mice, and later studies showed that NHP-derived tolerogenic DCs could induce porcine-specific Treg populations.

Overall, combining genetically modified donor pigs with more selective immunosuppression has brought pig-to-human transplantation closer to clinical translation. Further clarification of NK-cell, dendritic-cell, and other innate immune pathways will be necessary to design safer and more effective protocols.

## Global Regulatory Framework of Xenotransplantation

The regulation of xenotransplantation varies from country to country and this is important because animal infections may spread across countries. Therefore, there is a need for good oversight to safeguard the recipients, health care workers, close contacts and the general public. The United States and Europe have issued detailed guidance and some

countries, such as Canada, have been more conservative in approving human trials until risks of transmission are more clearly understood. National restrictions are not sufficient, however, as infectious risks are not contained at the borders.

### 5.1 Regulatory Variations Across Countries

The regulatory frameworks for xenotransplantation are different depending on public-health agendas, scientific capabilities and social acceptance. Guidance in the United States and Europe covers donor-animal health, infectious-disease testing, trial management, ethics review, risk management, and follow-up. Canada has maintained a low level of clinical activity until zoonotic risks can be better understood. In less well-regulated countries, regulation can be uneven, raising public-health concerns. Patients, clinicians and pathogens are not bound by international borders and harmonized international standards are needed for safe and ethical clinical development.

### 5.2 Xenotourism and Public Health Risks

Xenotourism is when patients go to countries that have less strict regulations to undergo xenotransplantation. It poses safety issues as post-transplant infection monitoring, complication reporting and follow-up may be disjointed or lacking. This compromises national and international protection measures and may lead to a greater risk of zoonotic diseases emerging. Cross-border tracking, communication and reporting are necessary for effective control, to ensure patient outcomes and public-health risks are monitored in a consistent way.

### 5.3 Need for International Standardization

Clinical xenotransplantation should only be performed where there is sufficient regulatory systems, infrastructure, trained staff, donor-animal oversight, clinical trial governance and follow up capacity. There is a need for international cooperation to harmonise standards, monitor adverse events and minimise risks associated with xenotourism. As the field progresses towards broader clinical testing, standardized protocols can help ensure ethical practice, help to make trials more comparable, and help to maintain public trust.

### 5.4 International Efforts in Xenotransplantation Regulation

International governance is necessary to reduce public-health risk and maintain ethical standards in xenotransplantation. The World Health Organization (WHO) has contributed several key initiatives:

2001 Guidance: The WHO recommended international cooperation in xenotransplantation surveillance, including monitoring for possible cross-species infections and adverse outcomes.

2004 World Health Assembly (WHA) Resolution 57.18: This resolution called on more than 100 Member States to harmonize regulation, establish accountability and traceability, require ethics review, and support international collaboration in xenotransplantation research.

2008 Changsha Communiqué: This communiqué set out principles for the WHO, Member States, and investigators, emphasizing biosafety, informed consent, safe clinical trial design, and long-term recipient monitoring.

Together, these initiatives show the importance of coordinated oversight as xenotransplantation develops. Continued international collaboration is especially important for managing xenotourism, surveillance gaps, and cross-border infectious risk.

### 5.5 Regulatory Agencies:

The clinical translation of xenotransplantation depends on effective regulatory supervision. Important authorities include:

The U.S. Food and Drug Administration (FDA) is the regulatory body for clinical xenotransplantation in the United States, and it has provided guidance regarding source animals, donor screening, manufacturing, preclinical evidence, and clinical safety monitoring. It aims to provide scientific quality, ethical review and biosafety for trials through its framework.

The European Medicines Agency (EMA) 2009 Guideline on Xenogeneic Cell-Based Medicinal Products covers sourcing of donor animals, quality control, preclinical testing, clinical development and marketing authorization. The guidance focuses on the need for high scientific and ethical standards for xenotransplant products.

**New Zealand:**

New Zealand was the first country to authorize a clinical xenotransplantation trial. Approval was granted by the New Zealand Minister of Health and the national ethics committee, and the trial was also registered with the United States National Institutes of Health (NIH) [23].

Preclinical work established the therapeutic rationale, supported efficacy, and enabled development of designated pathogen-free (DPF) pig herds. From July 2009 to March 2011, 14 patients with unstable type I diabetes that remained poorly controlled despite maximal therapy were enrolled. They received porcine pancreatic β-islet cells encapsulated in alginate microcapsules, which were designed to immunoisolate the cells from the host immune system. After intraperitoneal transplantation, systemic immunosuppression was not required. About 60% of recipients had detectable circulating porcine insulin one year later, although baseline exogenous insulin needs and haemoglobin A1c did not fall significantly. No porcine endogenous retrovirus (PERV) transmission or other xenotransplantation-related infection was detected [23].

#### 5.5.2 United States

The Food and Drug Administration (FDA) of United States oversees xenotransplantation products that contain live organs, tissues, or cells from nonhuman animals. This oversight also applies to encapsulated products and to materials in which nonhuman live organs, tissues, or cells have ex vivo contact with human body fluids, tissues, cells, or organs before administration to human recipients. Clinical investigation of such products requires an appropriate investigational application and approval under applicable regulations, including 21 CFR Part 312 [24].

### 5.5.3 European Union

- EMA: The 2009 Guideline on Xenogeneic Cell-Based Medicinal Products addresses donor sourcing, testing, quality control, clinical development, and marketing authorization.
- Both regulatory systems emphasize strong ethical, scientific, and biosafety standards.
- Pig-derived medical products may be controlled as MPs or MDs, depending on donor source, whether the donor is gene-edited or wild type (wt), whether viable cells are present, and how the product is intended to act in the recipient (Figure 6) [25].
- In the European Union, animal-origin products seeking authorization may fall under the medicinal-product framework of Directive 2001/83/EC and Regulation (EC) No. 726/2004, with Regulation (EC) No. 1394/2007 applying specifically to advanced therapy medicinal products (ATMPs). Alternatively, some products are assessed as medical devices under Regulation (EU) 2017/745. Xenogeneic cell-based products are further guided by the Committee for Medicinal Products for Human Use (CHMP) safety recommendations evaluation and risk assessment [25].

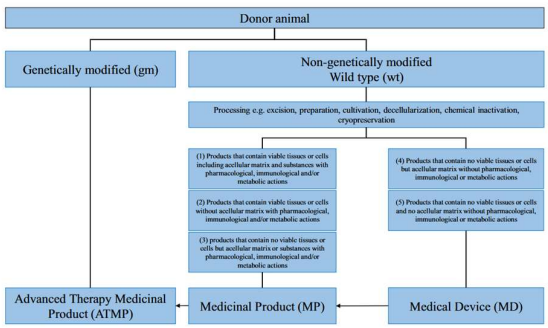



Figure 6: Classification of genetically modified (gm) and non-genetically modified, or wild-type (wt), xenotransplant products. Combination products containing both medicinal-product (MP) and medical-device (MD) components are classified as advanced therapy medicinal products (ATMPs), as shown by the arrows. Adapted from [25].

**Future prospects:**

Daniel Eisenson and colleagues reported evidence that conventional FDA-approved immunosuppressive drugs can sustain long-term solid-organ xenograft function in pig-to-NHP transplantation. In their study, xenograft function was maintained for 285 days using a calcineurin inhibitor (CNI)-based regimen combined with CD28/CTLA4 costimulation blockade, making it one of the longest life-supporting pig-to-baboon survival results reported. The authors linked this outcome to close therapeutic drug monitoring, careful CNI dose titration, and the addition of CD28/CTLA4 blockade. Although the finding needs replication, it helps bridge the gap between pig-to-NHP xenotransplantation data and allotransplantation practice, where CNI-based therapy remains standard. The results may be especially relevant for recipients of 10GE xenografts, in which human complement-regulatory, anticoagulant, and anti-inflammatory transgenes may reduce dependence on CD40/CD154 blockade [3].

As of February 2025, the US FDA had approved two clinical trials of genetically engineered pig kidneys in human recipients. The Massachusetts General Hospital (MGH) study was designed to enroll up to three cases with last-stage kidney disease, and the first transplant had already been performed. A second FDA-approved trial sponsored by a U.S. biotechnology company planned to enroll as many as 50 patients. That larger study was intended to assess both safety and effectiveness of xenogeneic kidney transplantation as a response to the continuing shortage of donor organs [13].

The 2025 Towana case also provided important clinical lessons after more than 130 days of pig-kidney function. It showed that xenotransplantation may remain feasible when immunosuppression is balanced carefully, but it also highlighted the danger of reducing immune suppression to manage unrelated infection. The medical team noted that rejection might have been associated with lowering the immunosuppressive regimen used to manage an infection not associated with the pig kidney. [11].

Tim Andrews, a 67-year-old recipient of the EGEN-2784 genetically engineered pig kidney at Massachusetts General Hospital on January 25, 2025, was reported to have survived more than seven months after transplantation. This established him as the longest-surviving recipient of a genetically engineered pig-derived organ at the time of the cited report.

Before transplantation he had been dependent on dialysis for more than two years, and afterward he was reported to be living without dialysis support [26].

Clinical follow-up in these recipients will be critical to understanding the timing of rejection, the clinical presentation of rejection, and what graft and recipient characteristics predict rejection. This information can be used to optimize immunosuppressive treatment, genetic modifications in the donor, and additional therapies. Strategies to reduce rejection, enhance graft survival and progress towards durable clinical use of xenotransplantation will be informed by careful interpretation of these cases.

**Conclusion:**

Although Xenotransplantation has been suggested as a means of alleviating the organ shortage for a long time, previous experiments have been unsuccessful in sustaining animal-organ graft function for more than a few months.Recent progress has changed the field. Researchers now understand pig-organ immune barriers in greater detail, gene-editing tools have become more precise and scalable, and newer immunosuppressive agents allow more targeted immune control. These advances provide a stronger platform for addressing the limitations that previously prevented durable xenograft outcomes.

Evidence from allotransplantation suggests that anti-CD20 therapy integrated with MMF-based desensitization can support ABO-incompatible kidney transplantation without removal of the spleen. This raises the possibility that similar, clinically familiar strategies could be tested cautiously in xenotransplantation within targeted immunosuppressive protocols. Such an approach may help control immune barriers while avoiding some highly experimental or excessively intensive interventions. It also contrasts with the field's current emphasis on CD40/CD154 blockade and supports evaluation of whether approved drug combinations can provide comparable rejection protection.

The International Xenotransplantation Association (IXA) has issued recommendations for xenotransplantation protocols. Its guidance supports anti-CD40-based regimens used with complement inhibition as an important strategy in clinical trials. Adoption of these approaches may provide a more consistent framework for evaluating xenotransplantation safety and efficacy.

Immunosuppressive agents vary in dose, route, and timing. Among evaluated combinations, regimens as ATG, anti-CD20 monoclonal antibody, MMF, anti-CD154 monoclonal antibody, tacrolimus (FK506), and glucocorticoids (GCs) have been associated with some of the longest reported xenograft survival outcomes.

The Tim Andrews kidney xenotransplantation case represents an important milestone for the field. A patient remaining free from dialysis for 271 days after receiving a genetically engineered pig kidney demonstrates the potential value of coordinated work among academic teams, biotechnology partners, clinicians, and regulators.

Taken together, recent advances suggest that xenotransplantation is moving from a largely experimental discipline toward a translational phase. Carefully designed clinical trials and targeted immunosuppressive strategies will determine whether it can become a reliable component of future organ-replacement therapy.

Further progress in targeted biologics, immunomodulatory agents, and tolerance-promoting therapies will be essential for preventing rejection in xenotransplant recipients. If ongoing and future trials continue to show sustained graft function with acceptable safety, xenotransplantation may become a practical response to the global limited availability of donor organs.

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