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# Xenotransplantation An Emerging Future Of Organ Transplantation.

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*Abstract:* Xenotransplantation is the process of transplanting, grafting, or transferring organs, tissues, or cells from one species to another. In the context of medical procedures, it most commonly refers to the transplantation of animal organs or tissues into humans. It holds promise for addressing the scarcity of human organs. Rooted in mythology and history, this practice has evolved with significant advancements in genetic engineering and immunosuppression. Despite potential benefits, rejection and physiological barriers pose challenges. Often pig's are preferred animal donors and are genetically modified to enhance compatibility. Xenotransplantation also utilizes immunomodulation strategies aim to minimize rejection risks. While xenotransplantation offers a solution to organ shortages, ethical concerns and uncertainties remain. This review explores the rich history, current advancements, and the delicate balance between the advantages and disadvantages of xenotransplantation, shedding light on its potential impact on the future of organ transplantation.

***Keywords* – Xenotransplantation, xenograft, Genetic engineering, nonhuman primates, xenosis, hyperacute Rejection, immunosuppression.**

## ABBREVIATIONS

XTx : Xenotransplantation
NHP : Non-Human Primate
HIV : Human Immunodeficiency Virus
AIDS : Acquired Immunodeficiency Syndrome
HLA : Human Leukocyte Antigen
GTKO : Galactosyltransferase gene-knockout
NK : Natural killer
PERV : Porcine Endogenous Retrovirus
GGTA1 : Alpha-1,3-galactosyltransferase
MHC : Major Histocompactibility Complex
XNA : Xenoreactive Natural Antibodies

## INTRODUCTION

Xenotransplantation, derived from the Greek words "xenos" meaning foreign or strange, it involves the transplantation of organs or tissues from one species to another, with a primary focus on utilizing porcine organs for transplantation into humans. This innovative medical approach holds promise as a potential solution to the critical shortage of organs available for transplantation, addressing the growing demand for life-saving procedures.[1]

The term "xenotransplantation" emerged in the late 20$^{th}$ century, marking a new chapter in the history of organ transplantation. While the concept of cross-species transplantation has ancient roots, modern

xenotransplantation gained prominence with scientific advancements and breakthroughs in genetic engineering.[2]

Dr. Keith Reemtsma is often regarded as a pioneer in the field, having conducted early experiments in the 1960s involving the transplantation of chimpanzee kidneys into humans. However, it is the contemporary efforts of researchers like Dr. David K.C. Cooper that have brought xenotransplantation to the forefront of medical research.[3]

The critical shortage of organs for transplantation has become a global healthcare challenge. According to the World Health Organization (WHO), millions of people suffer from end-stage organ failure worldwide, and the demand for transplantation far exceeds the available supply of human organs. XTx emerges as a promising avenue to bridge this gap and offer a viable alternative to conventional organ donation.[4]

Statistics highlights the severity of the organ shortage crisis. In major geopolitical regions, including North America, Europe, and Asia, the demand for organs for transplantation is consistently high. In the United States alone, approximately 110,000 individuals are on the organ transplant waiting list, with an average of 20 people dying each day while waiting for a suitable donor organ. Similar trends are observed in Europe and Asia, emphasizing the urgency for innovative solutions like xenotransplantation.[5]

When two distinct species are transplanted, this is referred to as xenotransplantation. Even though there have been numerous attempts at clinical xenotransplantation on humans since the nineteenth century, the activity primarily takes place at the scientific level using pig-to-non-human primate (NHP) models. Concordant xenotransplantation occurs when two closely related species (such as a mouse to rat or non-human primate to human) or discordant XTx occurs when unrelated species (such as a pig to human).[6]

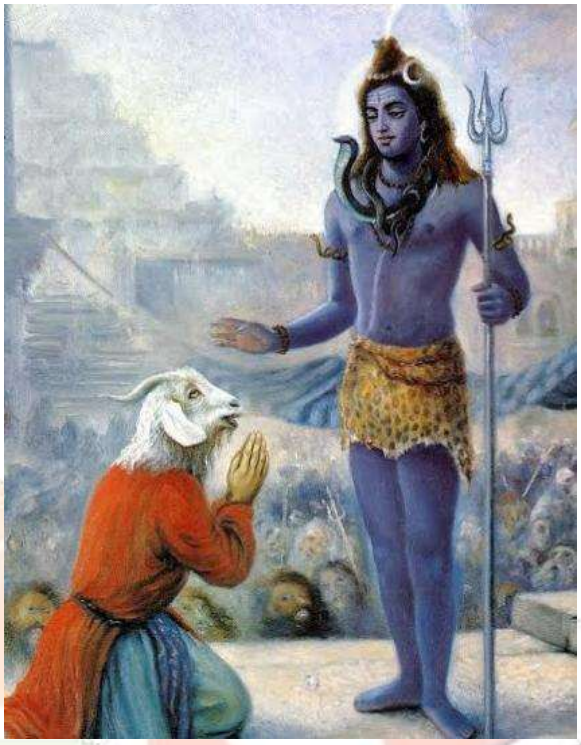
The procedure of xenotransplantation is experimental. Only the rarest and most severe circumstances are permitted. Clinical trials haven't started yet because of FDA restrictions. To determine whether this treatment is actually safe and successful, these trials will be crucial.[7]

Due to organ failure the kidney is the most transplanted organ worldwide followed by the liver and then heart. In 2021, there were a total of around 144,302 organ transplants worldwide.[4] With concerning 42 donors per million persons, the United States had the highest rate of deceased organ donors within a few OECD member countries in 2021. For those who are experiencing organ disfunction and failure and advanced sickness, donating an organ to such individuals is a crucial life-saving technique[8]

Organ failure is a complex phenomenon, arises from a multiple factors affecting various organs. Chronic diseases play a pivotal role, with the liver susceptible to conditions such as fatty liver disease and hepatitis C, while the kidneys may be impacted by glomerulonephritis, polycystic kidney disease, hypertension, and diabetes. The heart, a vital organ, faces threats from Coronary Artery Disease (CAD) and Congenital Heart Disease (CHD). Inflammatory bowel diseases like Crohn’s Disease and chronic intestinal motility disorders contribute to the intricate web of causes. Traumatic injuries can also lead to organ failure. Toxic injuries, often resulting from exposure to harmful substances, present another risk factor, alongside the detrimental effects of chronic drug and alcohol consumption. Environmental toxins further compound the issue, emphasizing the intricate interplay of external factors. Bacterial infections, a common concern, can compromise organ function. Finally, the loss of blood or oxygen, essential for sustaining organ health, emerges as a critical factor. Understanding these multifaceted causes is crucial for addressing and preventing organ failure, and researchers continually works into these intricate connections to safeguard human health. [9]

## MYTHOLOGY

Hinduism highlights the mythological traces of Xenotransplantation and that for millions of years back, people have been fascinated by the idea of merging the distinctive features of different species of animals.[10] Hindu mythology states that Lord Ganesha, who is also known as the God of Transplantation, was given the elephant's head after his father, Lord Shiva accidentally decapitated it.[11](Fig.1) Likewise Father-in-law of Lord Shiva, Daksha was beheaded by the incensed Lord Shiva, whose head was then thrown into the yajna .Later, after diminishing all of his rage, Lord Shiva gives Daksh a goat's head transplant. [12](Fig.2)



[Fig.1 Lord Ganesha ; Fig. 2 Lord Shiva (right) and Prajapati Daksh (left)]

The mythological stories goes on, including the Egyptian god Anubis (a man with a canine head) and the Greek sphinx, whom had the head of a woman, the body of a lion, and the wings of a bird.[13]
The International Xenotransplantation Association and its official scientific journal, Xenotransplantation have chosen the lamassu as their mythological representative, while the transplantation of cells and organs between members of the same species is symbolized by the chimera.[14]

## HISTORICAL BACKGROUND

### 1.Kidney/Renal Xenotransplantation

The kidney is the most demanding organ among organ transplants.[15] In 1902, Emerich Ullman transplanted a pig's kidney into the arm of a woman.[16],[17] In 1905, the French surgeon M. Princeteau transplanted rabbit kidney slices into a toddler suffering from chronic kidney disease. The youngster passed away after 16 days of xenotransplantation due to pulmonary complications.[18],[19] Next year after this, in 1906, the heterotopic transplantation of a pig kidney was also attempted by Jaboulay from a pig donor into the elbow of a 48-year-old woman, but eventually it failed due to thrombosis. The second kidney xenotransplant performed by Jaboulays was performed on April 9, 1906, which was almost three months later. Jaboulay notes that the kidney of a goat was smaller and had better-quality vessels than that of a pig, but he does not provide a clear explanation for his decision to go from a pig to a goat.[18],[20],[21]
In 1909, a 21-year-old woman had received a macaque kidney transplanted into her thigh by the Ernst Unger of Germany resulted into varying outcomes with the survival period of 32-hours, her autopsy report confirmed the venous thrombosis.[15],[22]
In 1963, two xenotransplantation of kidney were performed in the United States. That year Dr. Starzl transplanted the kidneys of baboons in six patients at Denver,while at Tulane University of Louisiana Keith Reemtsma transplanted the kidneys of thirteen chimpanzees into patients whom survived for nine months.[23],[24]
On 25 September 2021 World's first genetically modified pig kidney transplanted into two brain-dead human recipients was performed by Dr. Robert Montgomery in the NYU Langone transplant institute.[25]

Throughout the procedure and subsequent observation period, no signs of rejection were detected. Genetically modified kidney xenografts from pigs remained viable and functioning in brain-dead human recipients for 54 hours, without signs of hyperacute rejection.[26] Followed by less than two months after the first XTx surgery, On 22 November 2021, NYU Langone Health and transplant institute has performed its second successful investigational xenotransplantation procedure using a genetically engineered pig kidney. [27]

**2. Heart/Cardiac Xenotransplantation**

James Hardy carried out the first cardiac xenotransplantation in 1964 at the University of Mississippi using a chimpanzee heart. The heart was too small and it failed within just a few hours.[28],[29] In 1977 Christiaan Barnard used the hearts of baboon and chimpanzee for the temporary back-up pumps during the heart failure surgery of two patients. [30] On 26 October 1984, Leonard L Bailey and his heart transplantation team were transplanted a baboon heart into a prematurely born baby girl, Stephanie Fae born with hypoplastic left heart syndrome, a severe heart defect.[31]

In Sonapur, Assam, India, December 1996, Dr. Dhaniram Baruah carried out the first human heart, lung, and kidney transplant from a non-genetically modified pig. The news was released in January 1997 that the Purno Saikia, a 32-year-old man with terminally illness, was the receiver; he passed away soon after the procedure due to multiple infections. [32],[33],[34]

On 7 January 2022, Dr. Bartley Griffith and team at the University of Maryland Medical Center on special dispensation successfully transplanted a genetically modified pig heart into a 57-year-old patient David Bennett who was suffering with end-stage heart failure. The patient survived for around two months after the procedure and died due to unknown reason.[35]

After this successful transplant soon on 20 September 2023, Dr. Bartley Griffith at the University of Maryland Medical Center (UMMC) A 58-year-old patient Lawrence Faucette with terminal heart disease became the second patient in the world to receive the transplant of a genetically-modified pig heart. The U.S. FDA was granted emergency approval for this surgery through its single patient investigational new drug (IND) "compassionate use" policy. [36] Mr. Faucette had made significant progress after his surgery however later the transplanted heart intiated the signs of rejections and after 6 weeks of the procedure unfortunately Mr. Faucette succumbed on 30 October 2023.[37]

**3. Liver Xenotransplantation**

Between the period of 1969 and 1974, Dr. Starzl had performed attempts of world's first-ever chimpanzee liver xenotransplantation into the three children. Starzl's first liver transplant attempt was performed in 1963 on a three-year-old kid with biliary atresia and the subsequent next two attempts are all failed due to significant bleeding and technical issues, despite the fact that this liver transplant from chimpanzee to human was not successful. Further in 1967, Starzl successfully conducted the first liver transplant on a kid suffering from hepatoblastoma; however, the youngster passed away after the 18 months of survival due to recurrent metastatic disease. Continuing his work further in year 1992 and 1993 Starzl performed two more liver XTx from baboon into patients. [38],[39],[40],[41]

**4. Corneal Xenotransplantation**

The concept of keratoplasty has been credited to Franz Reisinger. In 1824 he proposed employing an optically clear animal cornea in place of an obstructive human cornea. So, later Richard Kissam performed the first successful human corneal xenotransplantation in 1838 using a porcine graft. Considering that this was the first human transplant, in general, the report is extremely significant. This took place almost 50 years prior to the first renal and corneal allotransplants in 1905. After that, unconfirmed reports of corneal xenotransplantation using fish and gibbon into the humans were made up until the early 1970s. Among them, 50% of the 10 recipients of gibbon-to-human corneal xenotransplantation exhibited a survival rate of more than five months. [42][43][44]

**5. Cell/Tissue Xenotransplantation**

Numerous studies of tissue xenotransplant clinical trials have been proposed or are being conducted to treat diseases such as Parkinson's or HIV/AIDS, respectively.When compared with organ xenotransplants, cell and tissue xenotransplants, like pancreatic islet cells or specific type of nerve cell xenotransplant may experience a less immunological reaction. Islet xenotransplantation has been encouraged by considering its numerous advantages, increased prevalence of type-1 diabetes and the resemblances between pig and human insulin. In 1993, a group led by Carl Groth in Sweden made the first attempt at pig islet (porcine fetal pancreas) transplantation for diabetic patients. No clinical advantages was observed, despite the fact that

some blood samples from patients contained porcine C-peptide, which indicates that some islets were survived. [45]

**6. Blood Transfusion/ Xenotransfusion**

The process of incorporating blood from one species into the veins of another species is a type of xenotransplantation also known as xenotransfusion. In 17th century the first documented case of xenotransfusion, or the transfusion of blood from animals into humans, was recorded in 1667, a 15-year-old boy with high fever was received blood from a Lamb by an attempt of French physician Jean-Baptiste Denis and surgeon Paul Emmerez and were resulted as successful recovery of the boy. Together, Denis and Emmerez carried out a number of xenotransfusions on lambs and calves, with varying levels of success. However, In 1670 xenotransfusions were banned for a many of years by the both the French and English parliaments. [46][47]

## HISTORICAL ATTEMPTS OF XENOTRANSPLANTATION

| Sr. no. | Years | Surgeon | Transplant | Donor | References |
|---|---|---|---|---|---|
| 1. | 1667 | Jean-Baptiste Denis | Blood | Lamb | [46][47] |
| 2. | 1838 | Richard kissam | Cornea | Pig | [43][44] |
| 3. | 1902 | Emerich Ullman | Kidney | Pig | [18][19] |
| 4. | 1905 | M. Princeteu | Kidney | Rabbit | [19][20] |
| 5. | 1906 | Jaboulay | Kidney | Pig | [18][19][21] |
| 6. | 1906 | Jaboulay | Kidney | Goat | [18][21] |
| 7. | 1909 | Ernst Unger | Kidney | Macaque (Monkey) | [10][22] |
| 8. | 1963 | Dr. Thomas Starzl. | Kidney | Baboon | [23][24] |
| 9. | 1963 | Keith Reemtsma | Kidney | Chimpanzee | [23][24] |
| 10. | 1964 | James Hardy | Heart | Chimpanzee | [28][29] |
| 11. | 1969-1974 | Dr. Thomas Starzl | Liver | Chimpanzee | [38][39] |
| 12. | 1977 | Christiaan Barnard | Heart | Chimpanzee | [30] |
| 13. | 1984 | Leonard L Bailey | Heart | Baboon | [31] |
| 14. | 1992-1993 | Dr. Thomas Starzl | Liver | Baboon | [40][41] |
| 15. | 1993 | Carl Groth | Islet cells | Pig | [45] |
| 16. | 1996 | Dr. Dhaniram Baruah | Heart, Lungs and Kidneys | Pig | [32] |
| 17. | 2021(SEP) | Dr. Robert Montgomery | Kidney | Pig | [25] |
| 18. | 2021(NOV) | Dr. Robert Montgomery | Kidney | Pig | [27] |
| 19. | 2022 | Dr. Bartley Griffith | Heart | Pig | [35] |
| 20. | 2023 | Dr. Bartley Griffith | Heart | Pig | [36] |

## DONORS FOR XENOTRANSPLANTATION

### 1. Pigs (Sus scrofa domesticus)

Pigs are often preferred in xenotransplantation due to physiological and anatomical similarities with humans, making them suitable for organ transplantation. Pigs have similar organ sizes and metabolic rates, reducing the risk of rejection. Additionally, the breeding and maintenance of pigs are relatively cost-effective. Certainly! Pigs (Sus scrofa domesticus) have been a primary focus in xenotransplantation research for several reasons, and their potential use as donors has been a subject of extensive study. Pigs share many physiological similarities with humans, including organ size, metabolic rates, and other anatomical features. This reduces the risk of rejection and improves the chances of successful transplantation. Pigs are readily available, and their reproductive cycle is relatively short, allowing for efficient breeding and maintenance of a sustainable donor population. This factor contributes to the cost-effectiveness of using pigs as xenotransplant donors. Advances in genetic engineering have enabled researchers to modify pig organs to make them more compatible with the human immune system. For example, efforts have been made to eliminate certain pig antigens that could trigger a strong immune response in humans. Pigs have been at the forefront of xenotransplantation research, with various preclinical studies showing promising results. Researchers have achieved improved survival rates in pig-to-primate xenotransplants, demonstrating the potential viability of pig organs for human transplantation. Despite the advantages, challenges remain. Immune rejection, the risk of transmission of porcine viruses to humans, and ethical considerations are significant challenges that researchers are actively addressing. Ethical concerns related to using animals for organ transplantation are an ongoing part of the discussion. Striking a balance between the potential benefits of xenotransplantation and ethical considerations remains a critical aspect of the research. Numerous research initiatives and clinical trials are ongoing to further explore the safety and efficacy of pig xenotransplants. These studies aim to address remaining challenges and gather essential data for potential future clinical applications. [48][49]

### 2. Non-Human Primates (e.g. Baboons, Rhesus Monkeys)

Non-human primates share a closer genetic similarity with humans, but ethical concerns, limited availability, and risk of disease transmission have led to a preference for other animals. Non-human primates are considered as a last resort when other options are not feasible. [50]

### 3. Sheep (Ovis aries)

Sheep have been explored for xenotransplantation due to their physiological compatibility and organ size. However, they are less commonly considered compared to pigs. The use of sheep as xenotransplant donors is still an area of ongoing research. [51]

## BARRIERS OF XENOTRANSPLANTATION

### 1. Immunological Barrier

#### 1.1 Hyperacute Rejections

The rejection of an organ graft within 24 hours of reperfusion is referred to as hyperacute rejection, and it is certainly the most severe and proactive immunological reaction due to the signals the organ's destruction and loss of graft function in a matter of hours. Within minutes of a newly transplanted organ getting perfused, hyperacute rejection initiates in, characterized by bleeding into the graft and the occurrence of platelet thrombi. Transplanted organs among distinct species are especially susceptible to hyperacute rejection. This kind of rejection happens minutes or hours after the xenograft blood circulation has been restored, and it is mediated by the recipient’s xenoreactive natural antibodies (XNAs). XNAs attach to the xenoantigens in the xenograft and trigger the recipient’s classical complement system, which causes interstitial bleeding, edema, and thrombosis in the xenograft before it eventually necrotizes and becomes inactive in a matter of minutes or hours. [52][53]

#### 1.2 Acute Vascular Rejections

After xenotransplantation, acute vascular rejection generally appears two to three days later. A whole mechanism for this reaction is still unknown, though. The interaction of the recipient’s macrophages, platelets, or xenoantibodies with the xenograft may be the reason for this rejection. When XNAs and xenograft endothelial cells are combined, the xenograft endothelium cells and their receptor for macrophages become activated. This leads to the induction of particular proteins such as blood coagulation factors, endothelial

adhesion molecules, and cytokines. These elements may result in xenograft loss or inactivation by thrombosis, inflammation, cellulose precipitation, or widespread blood coagulation. [54][55]

### 1.3 Acute Cellular Rejections

The humoral immune system plays a major role in hyperacute rejection and acute vascular rejection, while cell immunity is the predominant factor in acute cellular rejection. T lymphocytes and NK cells play a major role in acute cellular rejection. After xenotransplantation, recipient CD4+ T cells receive xenoantigen epitopes by antigen-presenting cells via xenogeneic MHC class II molecules. The recipient's MHC class I molecules can also carry the graft's xenoantigen to CD8+ T lymphocytes. A cascade of immunological rejection responses is triggered by the generation of interleukin-2 (IL-2) and interferon-γ (IFN-γ) brought on by the growth of CD4+ T cells and CD8+ T cells. [56]

### 1.4 Chronic Rejections

Currently, there is no compelling explanation for the chronic rejection associated with immunological diseases. After xenotransplantation, chronic rejection typically develops months or years later. It mostly happens as a result of humoral immunity, which is mediated by complement and XNAs. The circulatory system's poor reaction to XNAs leads to perivascular inflammation along with harm to the xenograft's vascular endothelium. This is characterized by the growth of vascular smooth muscle cells, which obstruct blood vessels, and eventually outcomes in arteriosclerosis and xenograft loss.[57]

## 2. Infection Barriers

The Porcine herpes virus (PERV) transmission to humans is a serious risk, especially for recipients of xenografts. Preclinical and clinical research has not revealed any evidence of PERV transmission to humans; however, methods of preventing transmission have been developed, such as vaccinations, antiretroviral therapy, low-virus-producing pigs, and drugs that interfere with RNA. Porcine virus detection is performed on transgenic 10-gene altered pigs every three months. Whereas cesarean delivery and careful donor animal separation can avoid the transmission of suid beta herpes virus 2 from mother to swine/porcine offspring, as genetic alterations cannot stop this from taking place.[58]

One of the significant barriers in xenotransplantation is the potential transmission of porcine endogenous retroviruses (PERVs) to human recipients. PERVs are integrated into the genome of pigs and have the potential to be activated and transmitted during xenotransplantation, posing a concern for the safety of the procedure. PERVs are part of the pig genome, and their sequences have been found in all pig breeds. These retroviruses can be transmitted horizontally or vertically and are categorized into three groups: PERV-A, PERV-B, and PERV-C.[59] During xenotransplantation, there is a risk that PERVs present in the pig's cells could be activated and transmitted to human recipients, leading to potential infections.[60]

In vitro studies have demonstrated that PERVs can infect human cells, raising concerns about the potential for cross-species transmission in vivo.[61] There are concerns that PERVs could adapt to human cells and become more infectious. As a precaution, pre-transplant screening of donor pigs for the presence of PERVs is essential.[62] PERVs have demonstrated the ability to integrate into the DNA of human cells, making it crucial to assess the risk of PERV transmission and the potential long-term effects.[63]

## 3. Physiological Barrier

It arises from the inherent differences in physiology between humans and pigs. Despite genetic modifications to make pig organs more compatible with human recipients, certain functional disparities may persist, potentially affecting the success and long-term viability of xenografts.

### 3.1 Cardiovascular System

Pigs have a higher heart rate and smaller coronary arteries compared to humans. This difference may affect blood flow dynamics and predispose xenografts to ischemic events.[64]

### 3.2 Blood Pressure Regulation

The regulation of blood pressure and response to vasoactive agents can differ between species, leading to potential challenges in maintaining hemodynamic stability post-transplantation.[65]

### 3.3 Coagulation System
Variations in the coagulation cascade, platelet function, and fibrinolysis may contribute to thrombotic events or bleeding complications in xenografts.[66]

### 3.4 Metabolism and Physiology
Differences in metabolic rates, temperature regulation, and overall physiological processes may impact the overall function and health of the xenograft.[67]

### 3.5 Renal Function
Distinct renal physiology and handling of waste products may influence the performance of xenotransplanted kidneys, affecting overall graft function.[68]

### 3.6 Immunological Challenges
Despite genetic modifications, immunological differences may persist, leading to ongoing immune responses against xenografts, affecting their function and survival.[69]

## 4. Social and Ethical Barriers
The moral challenges surrounding animal genetic modification is represented by Bernard E. Rollin's Frankenstein's monster. To ensure ethical, societal, and legal acceptance of pig-to-human heart XTx, the current clinical application framework may need to be modified. Cultural and religious diversity, equal organ distribution, and organ availability by purchase are among the issues. Animal rights, welfare, equal distribution of resources, and teaching physicians and patients about informed consent are just a few examples of ethical issues. Scientific advancements must be accompanied by thoughtful consideration and examination of these issues.[70]

## 5. Healthcare and Resource Based Barriers
Xenotransplantation, the process of transplanting organs or tissues from one species to another, holds great promise in addressing the shortage of human donor organs. However, several healthcare and resource-based barriers exist, encompassing financial constraints, availability issues, and manual aspects.

### 5.1. Financial Barriers
Xenotransplantation involves significant costs related to research, development, and implementation. The need for specialized facilities, genetic engineering, and ongoing monitoring contributes to the financial burden. Additionally, the potential for long-term medical care post-transplantation can strain healthcare budgets.[71] These financial barriers can be overcome by increase in funding for xenotransplantation research from government, private organizations, and philanthropic sources also conducting collaboration between research institutions, biotech companies, and healthcare providers to share costs and resources.

### 5.2. Availability Barriers
The availability of suitable donor animals and organs for xenotransplantation remains a challenge. Breeding and maintaining a stable supply of genetically modified animals suitable for transplantation require careful planning and resources.[72] Such availability barriers can be overcome by developing sustainable breeding programs for genetically modified donor animals.Implement strict regulatory frameworks to ensure ethical and responsible breeding practices.

### 5.3. Manual Aspects Barriers
The manual aspects involve the technical challenges in performing xenotransplantation surgeries, including the need for highly skilled and specialized healthcare professionals. Training and educating medical staff in xenotransplantation procedures pose logistical hurdles.[73] Establishing specialized training programs for surgeons and healthcare teams involved in xenotransplantation and fostering international collaboration to share expertise and best practices can be helpful to overcome these mannual aspect problems.

For the advancement of xenotransplantation, addressing these healthcare and resource based barriers requires a holistic approach involving increased funding, ethical breeding practices, and comprehensive training programs and collaboration between researchers, healthcare providers, and policymakers is essential for overcoming these challenges.

## GENETIC MODIFICATION TO OVERCOME IMMUNOLOGICAL REJECTIONS

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### 1. For Hyperacute Rejection

Over a century of research, various methods have been developed to prevent hyperacute rejection in xenotransplantation.[74][75] The most essential method is to reduce or eliminate the expression of galactose-α1,3-galactose (α-Gal), the main xenoantigen recognized by XNAs.[76] The first clone pigs with GGTA1 gene deficiency were seen as a milestone in the field. GTKO pigs have been used in xenotransplantation research, resulting in prolonged survival times and reduced hyperacute rejection frequency. [77][78][79]

Two other non-Gal epitopes, N-glycolylneuraminic acid (Neu5Gc) and the SDa blood group, present additional barriers to xenotransplantation. Inhibiting the complement response, such as using human complement regulatory proteins (hCRPs), has also been shown to reduce hyperacute rejection. Other methods include transducing enzymes that compete with α-1,3-galactosyltransferase, specific siRNA, and plasmapheresis.[80]81]

### 2. For Acute Vascular Rejection

The approach for the management of acute vascular rejection involves the inhibition of endothelial and macrophage activity, an increase of anticoagulant expression on the surfaces of pig organs, and the inhibition of NF-kB signaling. Cytokines released by macrophages and endothelial cells promote coagulation development, thrombosis, and inflammation. Porcine endothelium cells can be shielded from natural killer cells (NK cells) by human leukocyte antigen G (HLA-G), leading to HLA-G transgenic pigs an appropriate donor for xenotransplantation. While the A20 protein are able to regulate NF-kB expression, inhibiting nuclear transfer and activation as well as endothelial cell activation, genetically modified anticoagulants may inhibit platelet aggregation and activation.[82][83]

### 3. For Acute Cellular Rejection

To decrease in acute cellular rejection by the means of multigenic alterations can suppress the T cell response. Pigs modified with GTKO/hCRPs have the ability to reduce cytokine responses and T cell proliferation. The Fas/FasL apoptosis pathway is the mechanism by which human CD8+ T lymphocytes, particularly CD8+ CTL cells, mediate cytotoxic effects. Pig islet cells that overexpress human FasL and Fas may become resistant to cytotoxic effects by interfering with apoptotic pathways. In pigs, C-TA knockdown can lower the number of MHC class II molecules.[84][85]

### 4. For Chronic Rejection

Transducing novel anti-inflammatory genes into GTKO/hCRPs pigs, such as TFPI (tissue factor inhibitor), ENTPD1 (CD39), and hHO-1 (human heme oxidase), may prevent chronic rejection. Of course, before xenotransplantation is used in clinical applications, it still needs to be enhanced in order to overcome chronic rejection.Enhancing the function of a transplanted organ is a major concern in addition to minimizing or preventing the immune system's attack. According to reports, p53 deletion or BCL2 overexpression may prevent cell apoptosis. This may offer a novel strategy for preventing xenograft cell death or extending the survival of xenograft cells.[86][87]

## STRATEGIES TO OVERCOME RISK OF RETROVIRUS TRANSMISSION

### 1. Genetic Modification

Genetic modification of pigs to reduce or eliminate PERV expression has been explored as a potential solution. The use of CRISPR/Cas9 technology has been employed to inactivate PERV genes.[88]

### 2. PERV Neutralization

Developing strategies to neutralize PERVs or inhibit their ability to infect human cells is under investigation. Identifying methods to prevent PERV transmission remains a focus of research.[89]

### 3. Risk Assessment Protocols

Implementing comprehensive risk assessment protocols, including pre-transplant screening and monitoring post-transplantation, to mitigate the risk of PERV transmission.[90]

**4. Regulatory Oversight**

Regulatory bodies play a crucial role in evaluating the safety of xenotransplantation procedures and ensuring that measures are in place to address the potential transmission of retroviruses.[91]

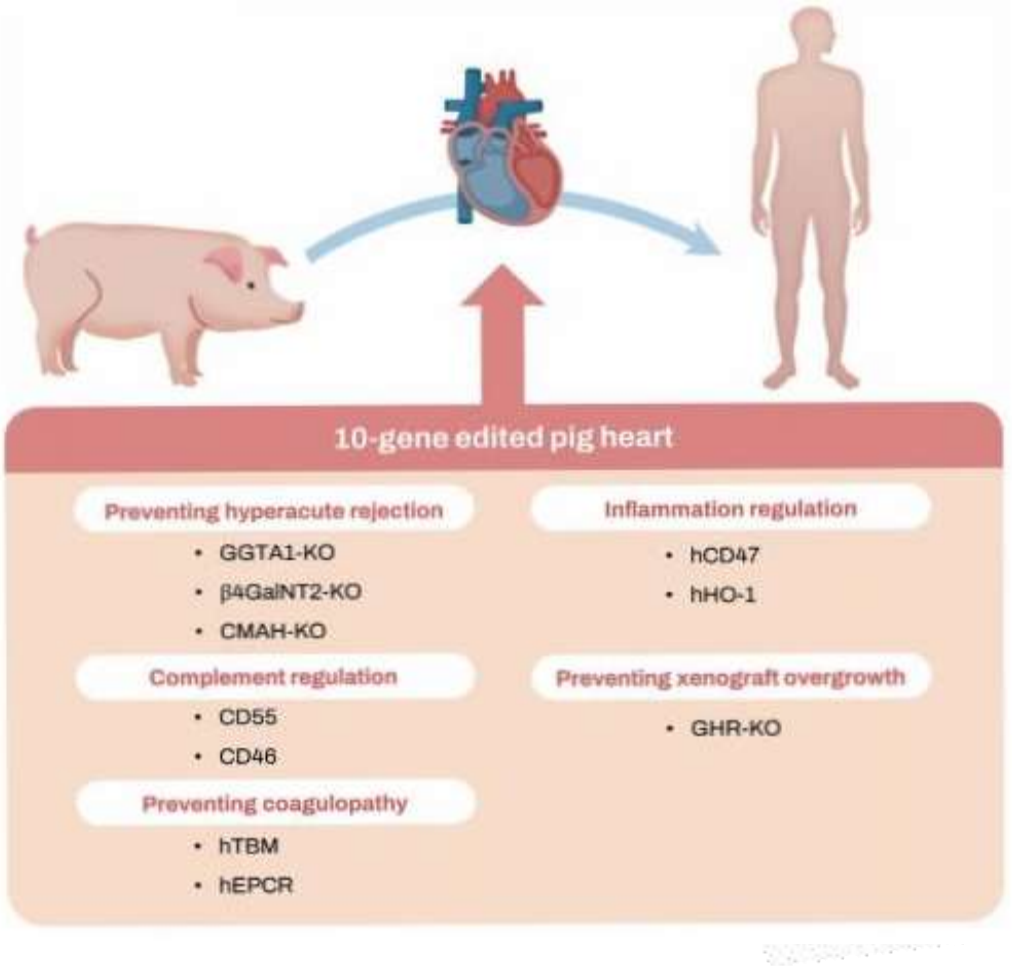
## STRATEGIES TO OVERCOME PHYSIOLOGICAL MISMATCH

**1. Genetic Modifications**

Continued efforts to identify and modify genes associated with physiological differences to enhance compatibility between pig organs and human recipients.[92]

**2. Precision Gene Editing**

Advancements in precision gene editing technologies, such as CRISPR/Cas9, to tailor the genetic makeup of pigs for improved physiological compatibility.[93]




**3. Functional Compatibility Studies**

In-depth studies to understand the functional aspects of xenografts and how they interact with the recipient's physiological systems.[94]

**4. Monitoring and Surveillance**

Implementation of robust monitoring and surveillance protocols post-transplantation to identify and address any functional mismatches promptly.[95]

## IMMUNOSUPPRESSION

Research studies on porcine-to-NHP islet cell transplantation have shown that conventional immunosuppression regimens may not fully overcome cytotoxicity and cytokine production from T cell activation and innate cytotoxic cells. While, longer graft surviving has been showed through maintaining the immunosuppression regimens in pig-to-baboon kidney transplantations which showed the anti-CD40mAb regimens provided longer graft survival. such as Tacrolimus with Rapamycin or CTLA4-Ig. It is possible but still, that human xenografts won't experience graft failure in the identical manner just as NHPs. However, little is get known about the mechanisms of graft failure in NHPs, which may not directly apply to xenograft

transplantation in humans. Additional pharmacologic interventions may be necessary to improve xenograft survival in humans and it's still unclear if traditional immunosuppressive regimes for xenografts are beneficial for long-term rejection-free survival.[96]

## ADVANTAGES OF XENOTRANSPLANTATION

**1.Potential Solution to Organ Shortage**
Xenotransplantation could address the shortage of human organs for transplantation by using organs from animals, potentially saving numerous lives.[97]

**2. Rapid Availability of Organs**
Animals can be bred and raised for xenotransplantation, providing a more readily available source of organs compared to waiting for human donors.[98]

**3. Reduced Dependence on Human Donors**
Xenotransplantation reduces dependence on human donors, potentially alleviating the ethical concerns and limitations associated with obtaining consent for organ d[99]

**4. Customization of Organs**
Genetic engineering in xenotransplantation allows for the customization of organs, addressing issues related to compatibility and reducing the risk of rejection.[100]

**5. Disease Resistance**
Some animals chosen for xenotransplantation may be selected for their resistance to certain diseases, potentially reducing the risk of disease transmission to the recipient.[2]

## DISADVANTAGES OF XENOTRANSPLANTATION

**1. Risk of Zoonotic Infections**
The transmission of infectious agents from animals to humans (zoonoses) poses a significant concern, potentially leading to new diseases.[101]

**2. Immunological Challenges**
Despite genetic modifications, immunological incompatibility remains a significant hurdle, leading to the risk of hyperacute rejection and the need for intense immunosuppressive therapy.[102]

**3. Ethical Concerns**
Ethical issues surrounding the use of animals for organ transplantation, including questions about animal welfare, exploitation, and the blurring of species boundaries.[103]

**4. Long-Term Viability and Functionality**
Questions remain regarding the long-term viability and functionality of xenotransplanted organs, including the potential for chronic rejection and the durability of the transplanted organ.[104]

**5. Public Perception and Acceptance**
Public perception and acceptance of xenotransplantation may pose challenges, with concerns about the "unnatural" use of animals for organ transplantation.[105]

## CONCLUSION

In conclusion, xenotransplantation holds promise for solving the shortage of human organs. Despite facing challenges like organ rejection and ethical concerns, ongoing research in genetic engineering offers hope for safer transplants. Currently it's in an experimental stage but careful regulatory and ethical evaluations will guide its progress. The future impact of xenotransplantation hinges on a delicate balance between reshaping organ transplantation and upholding ethical standards. Collaboration among researchers, policymakers, and the public is crucial in navigating this uncharted territory.

Embracing the potential of xenotransplantation requires caution, compassion, and a commitment to everyone involved. While the journey ahead holds the promise of overcoming organ shortages and saving lives across the world, it also underscores the resilience of scientific inquiry in addressing a critical healthcare challenge.

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