

Lung xenotransplantation: From the first human milestone to the path of clinical translation

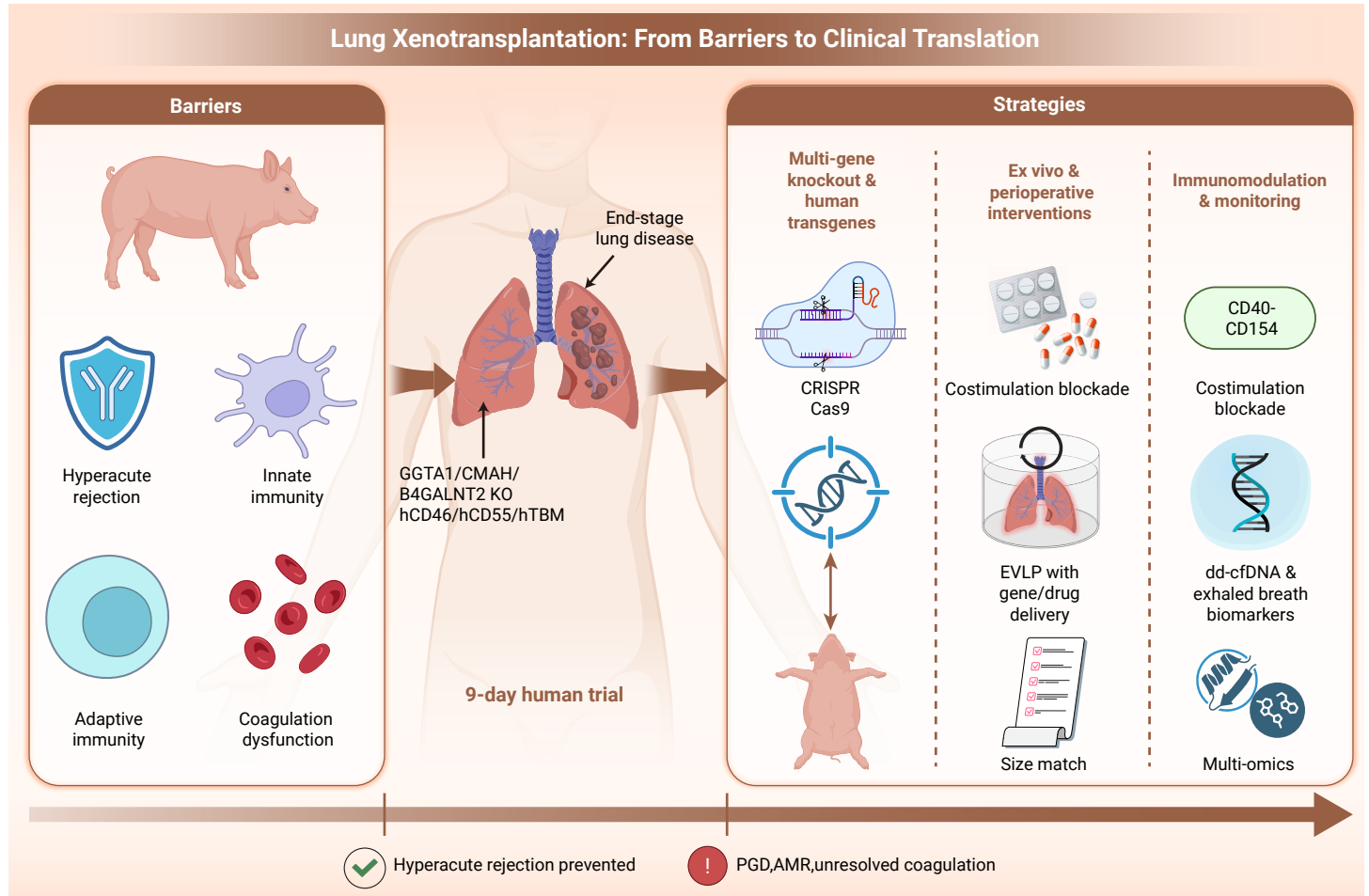
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GRAPHICAL ABSTRACT



PUBLIC SUMMARY

- Lung transplantation faces donor shortage; xenotransplantation using gene-edited pigs offers promise.
- In 2025, a gene-edited pig lung was transplanted into a brain-dead human, preventing hyperacute rejection.
- However, the nine-day observation showed severe primary graft dysfunction and antibody-mediated injury.
- This review analyzes lung xenograft barriers and a shift from passive camouflage to active immune remodeling.
- Clinical translation needs integrated solutions: donor production, biosafety, perfusion, and monitoring.

Lung xenotransplantation: From the first human milestone to the path of clinical translation

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Lung transplantation remains the only curative treatment for end-stage lung diseases, yet a critical shortage of donor organs persists. Xenotransplantation using genetically modified pigs has emerged as a promising solution, with recent breakthroughs in cardiac and renal xenotransplantation demonstrating clinical feasibility. However, the unique anatomical and physiological characteristics of the lung pose distinct challenges that have delayed progress in pulmonary xenotransplantation. The first reported transplantation of a gene-edited pig lung into a brain-dead human recipient in 2025 represents a pivotal milestone, successfully preventing hyperacute rejection through triple-gene knockout combined with transgenic expression of human complement regulatory proteins and thrombomodulin. Yet the nine-day observation window revealed persistent challenges, including severe primary graft dysfunction and active antibody-mediated injury, highlighting that current genetic modifications remain inadequate for achieving long-term graft survival. This review systematically analyzes the layered immunological and physiological barriers to lung xenotransplantation and evaluates evolving intervention strategies that reflect a paradigm shift from passive molecular camouflage toward active immunological remodeling. Looking beyond biological barriers, successful clinical translation requires integrated solutions spanning scalable donor production systems, surgical adaptation, rigorous biosafety frameworks, ex vivo perfusion platforms, and non-invasive monitoring technologies. Ultimately, the success of lung xenotransplantation will depend not on isolated technological breakthroughs but on the systematic integration of genetic engineering, immune modulation, and translational infrastructure, offering new hope to patients with end-stage lung disease.

INTRODUCTION

End-stage lung diseases, including idiopathic pulmonary fibrosis, chronic obstructive pulmonary disease, and pulmonary arterial hypertension, pose a severe threat to human life and health. For these conditions, lung transplantation remains the only curative treatment capable of significantly extending survival and improving quality of life. However, a critical global shortage of donor lungs persists as one of the most significant crises in modern medicine.¹ IPF patients, with their rapid functional decline, pose a narrow window for recipient evaluation.² COPD patients, who frequently harbor chronic airway infections, present heightened infectious risks during intensified immunosuppression.³ PAH patients often depend on ECMO bridging and are complicated by right ventricular dysfunction.⁴ They face greater perioperative hemodynamic challenges and may be particularly susceptible to xenograft coagulation dysregulation. These disease-specific considerations must inform clinical trial design as the field moves toward human translation.⁵

Despite advances in donor management and the increasing adoption of ex vivo lung perfusion (EVLV) to rehabilitate marginal grafts, donor lung use for transplantation has been limited to approximately 20% of existing donors,

which is the lowest reported utilization for solid organ transplants with many organs refused for donor quality.⁶ The total organ preservation time is constrained to a median of approximately 5.3 hours, far more restrictive than for other solid organs.⁷ ECMO can serve as a temporizing bridge for select patients awaiting lung transplantation, but approximately one quarter of patients placed on ECMO with intent to transplant may not survive to transplantation,⁸ and ECMO bridging is associated with an increased risk of post-transplant mortality compared with non-bridged recipients.⁹ The COVID-19 pandemic has simultaneously expanded the population of patients with irreversible end-stage lung failure and further strained an already depleted donor pool,¹⁰ lending new urgency to the search for alternative organ sources. In this context, xenotransplantation could provide a theoretically unlimited, genetically optimized, and on-demand source of lungs. It therefore represents a uniquely compelling investigational pathway and a potential definitive alternative for patients who would otherwise succumb on the waitlist.

Xenotransplantation, defined as the transplantation of organs across species, has been envisioned for decades as a potential solution.^{11,12} Pigs are considered the most promising donor source due to their comparable organ size, physiological function, reproductive characteristics, and relatively distant phylogenetic relationship from humans, which may reduce the risk of certain zoonoses. Breakthroughs in gene-editing technologies, particularly the CRISPR-Cas9 system, have enabled precise genetic modifications of donor pigs.^{13,14} By knocking out key antigenic genes such as GGT1 and CMAH,¹⁵ which trigger hyperacute rejection, and simultaneously introducing human protective transgenes like the complement regulatory proteins CD46 and CD55,^{16,17} early immunological barriers to cross-species transplantation can be overcome (The overall workflow of lung xenotransplantation is illustrated in Figure 1).

In the fields of cardiac and renal xenotransplantation, organs from such genetically modified pigs have achieved functional survival for months to years in non-human primate models,¹⁸ and early-phase human clinical trials have been successfully initiated. This marks a turning point where xenotransplantation is transitioning from the laboratory toward clinical application. Nevertheless, the lung's structure and physiology create unique barriers for xenotransplantation. The alveolar surface is constantly exposed to airborne pathogens, sustaining a highly active innate immune system.¹⁹ Resident alveolar macrophages readily attack the xenograft.²⁰ Moreover, the lung's vast endothelial surface amplifies the consequences of molecular incompatibilities.²¹ Porcine coagulation and complement regulators function poorly in the human context, leaving the graft endothelium vulnerable to activation, platelet sequestration, and microvascular thrombosis. These innate and endothelial insults are compounded by ischemia-reperfusion injury during cold preservation and reperfusion. Together, they trigger a vicious cycle of vascular leak, alveolar flooding, and progressive gas exchange failure, which are the hallmarks of primary graft dysfunction and the leading cause of pulmonary xenograft failure.

The first reported case of short-term functional survival of a gene-edited

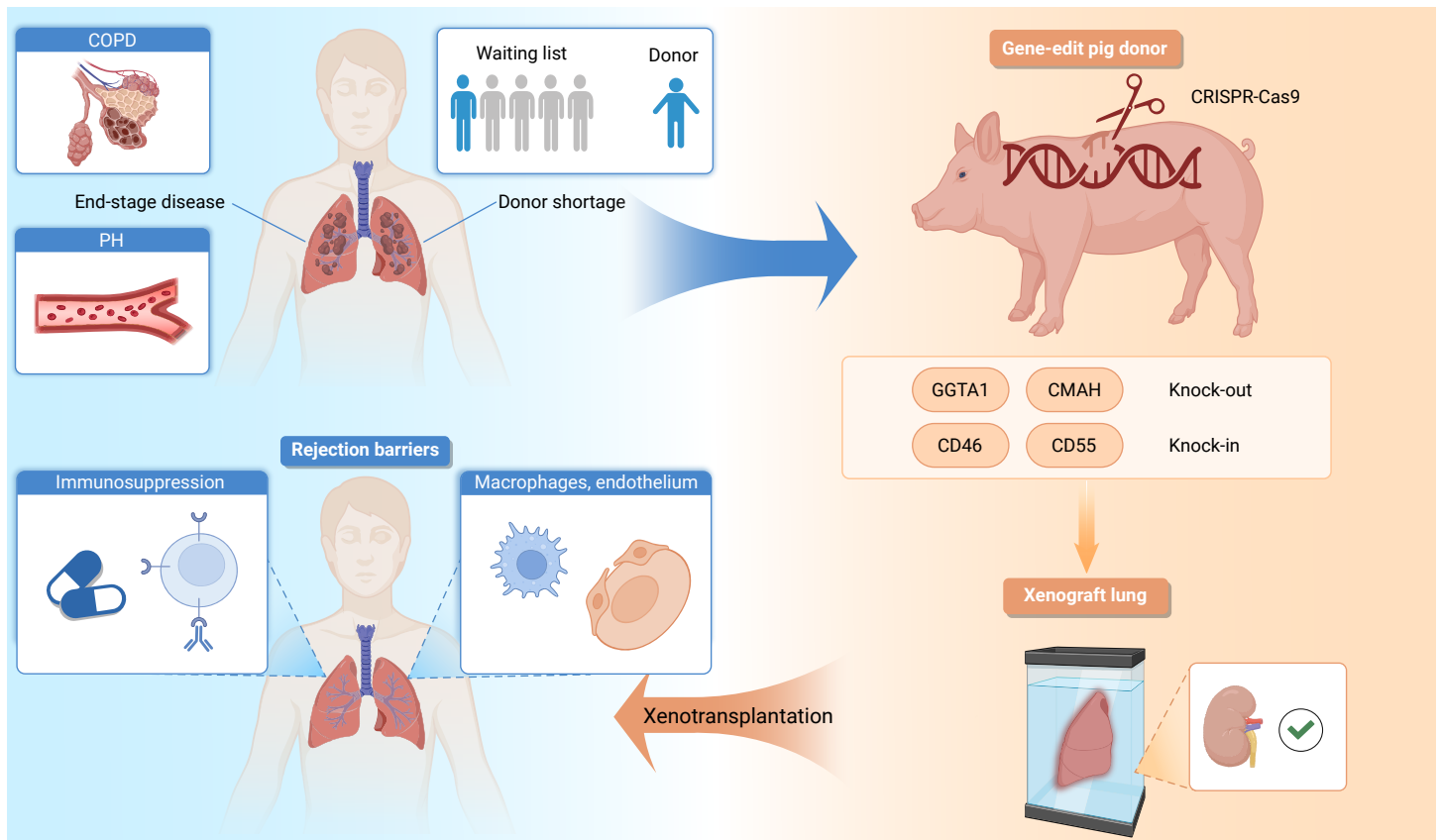


Figure 1. Schematic diagram of the lung xenotransplantation process.

pig lung in a human brain-dead recipient represents a milestone.²² This review systematically summarizes recent advances in lung xenotransplantation and provides an in-depth analysis of its unique immunological and physiological barriers. It also evaluates current gene editing, immunosuppression, and tolerance induction strategies, and identifies the key breakthroughs and integrated solutions needed for future clinical translation. By synthesizing existing knowledge and exploring future directions, we hope to provide a scientific reference to advance this highly promising field toward mature clinical application.

THE FIRST CLINICAL BREAKTHROUGH IN PIG-TO-HUMAN LUNG TRANSPLANTATION

The first reported transplantation of a pig lung into a brain-dead human recipient in 2025 marked a critical step for lung xenotransplantation, moving from prolonged animal experimentation into pivotal human validation (Figure 2). The research team knocked out three genes GGTA1, CMAH, and B4GALNT2, aiming to eliminate the major xenograft carbohydrate antigens α -Gal, Neu5Gc, and Sd(a) on porcine cell surfaces. This combination was intended to maximally deplete preformed natural antibodies in human serum, thereby blocking antibody-dependent cellular cytotoxicity (ADCC) and immediate activation of the complement classical pathway at its source. Results showed no typical widespread intravascular thrombosis or hemorrhagic necrosis in the early post-transplant period, histologically confirming that this strategy successfully prevented the hyperacute rejection previously deemed unavoidable. The team also proactively introduced transgenes for human complement regulatory proteins CD46 and CD55, as well as the anticoagulant protein thrombomodulin (TBM). CD46 and CD55 were intended to enhance the graft endothelial cells' ability to regulate the human complement system, particularly at the level of C3 convertase, to mitigate complement-mediated injury. The introduction of TBM aimed to partially correct the incompatibility between porcine vascular endothelium and the human coagulation system by activating the protein C pathway to exert anticoagulant and anti-inflammatory effects.²³

This gene-editing scheme, serving as the first framework validated in a

human recipient for pulmonary xenotransplantation feasibility, carries both continuity with past research and groundbreaking paradigmatic significance. The first-generation GalTKO pig models of the early 21st century established the core pathway of eliminating the major Gal xenoantigen to avoid hyperacute rejection.^{24,25} The widely used "triple-knockout, triple-insertion" scheme did not originate from a single design but was progressively assembled through iterative ex vivo validation. In 2013, Westall et al. systematically compared wild-type, GTKO/CD55, and GTKO/CD55/hHT pig lungs on an ex vivo perfusion platform, demonstrating that the human complement regulatory transgene hCD55 provided incremental protection but was insufficient to prevent graft failure.²⁶ In 2014, Burdorf et al. reported that expression of human CD46 in GalTKO lungs significantly reduced complement activation, platelet sequestration, and coagulation cascade activation, yet thrombin generation and platelet activation remained key determinants of early graft failure, shifting attention toward the coagulation cascade.²⁷ Between 2013 and 2015, triple-carbohydrate knockout (TKO) pigs targeting GGTA1, CMAH, and β 4GalNT2 were generated, and in 2015 Estrada et al. demonstrated that TKO porcine cells exhibited markedly reduced binding of human natural antibodies.¹⁵ In 2017, Laird et al. reported that additional expression of HLA-E on GalTKO.hCD46 lungs extended median survival to over 4 hours ($P = 0.012$) and attenuated the rise in pulmonary vascular resistance, providing the first direct evidence that human NK cells contribute to pulmonary xenograft injury.²⁸ Each of these bottlenecks, successively identified through ex vivo perfusion, motivated the corresponding insertion of human transgenes, progressively building the current multi-transgenic framework. Between 2018 and 2022, multiple research teams reported extended survival in pig-to-baboon lung xenotransplantation, with the longest reaching several weeks.²⁹⁻³¹ This marked the field's entry into a new stage investigating whether recipients could maintain stable survival for weeks with partial graft function. The expression of human CD47 was proven particularly crucial, as it effectively inhibits phagocytosis of porcine lung endothelial and alveolar cells by binding to the SIRP α receptor on primate macrophages,³² thereby lightening early damage from innate immunity. Based on these models, researchers like He and colleagues strategically streamlined the genetic design. The core of this

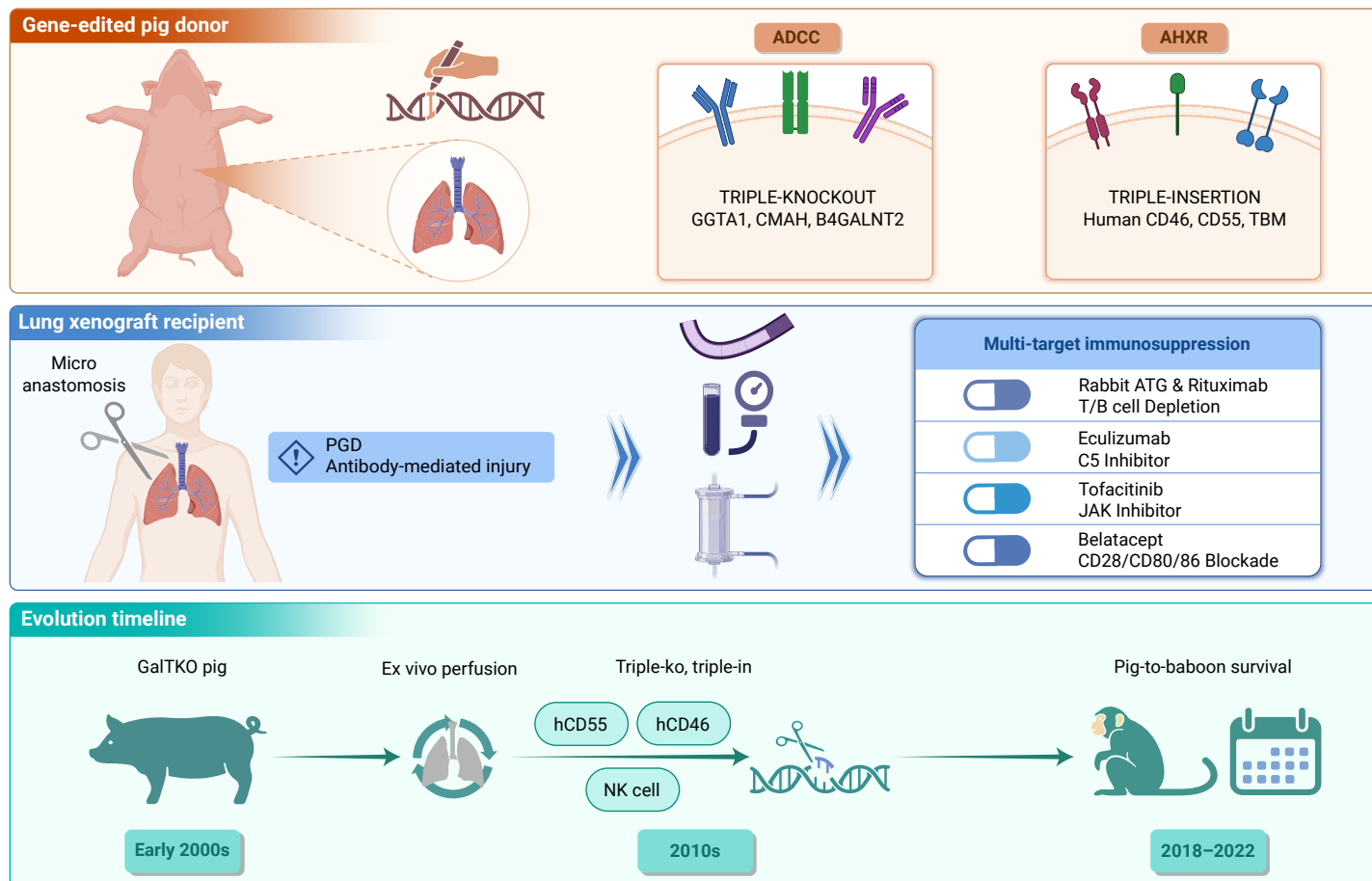


Figure 2. The first clinical breakthrough in pig-to-human lung transplantation.

strategy lies in using targeted genetic engineering to knock out key xenoantigen epitopes while coordinately regulating the complement cascade and coagulation balance, thereby addressing the critical obstacles of hyperacute rejection and complement-mediated damage. This reflects a conceptual shift from a universal model toward organ-customized xenotransplant design.

Potent multi-target immunosuppressive regimens have drawn attention in such preclinical experiments. The regimen used in the first pig-to-human lung xenotransplant combined rabbit anti-thymocyte globulin for T-cell depletion with the anti-CD20 monoclonal antibody rituximab for B-cell depletion. Simultaneously, C5 inhibitor (eculizumab) and JAK inhibitor (tofacitinib) were added to control complement activation and cytokine release, respectively. The regimens also attempted to use the CTLA-4-Ig fusion protein belatacept to block the CD28-CD80/CD86 costimulation pathway.³³ Novel therapeutic regimens different from traditional triple immunosuppression may be required for lung xenotransplantation, such as costimulation blockade schemes based on CD40³⁴ or CD154³⁵ antibody inhibition.

The implementation of this xenotransplant also benefited from standardized yet adaptable surgical techniques and meticulous perioperative management. The research team strictly adhered to the technical paradigm of classical lung transplantation, ensuring the reliability and reproducibility of the procedural workflow. This included standard hypothermic flush procurement of the donor pig's left lung, precise anatomical dissection, and end-to-end microanastomosis at the bronchus, pulmonary artery, and left atrial cuff following left pneumonectomy in the recipient.³⁶ Notably, despite anatomical differences in lobation and bronchial anatomy between pig and human lungs, the study cleverly circumvented adaptation issues by opting for a left single-lung transplant model. The team also established a multi-dimensional monitoring network, continuously tracking the recipient's hemodynamic status, mechanical ventilation parameters (including lung compliance and airway pressure), and regional blood gas sampled directly via catheters placed in the left pulmonary artery and pulmonary vein. Chest tube drainage was also

monitored. The high frequency of measurements and real-time dynamic adjustments aided in the timely identification and confirmation of severe primary graft dysfunction (PGD).

LIMITATIONS OF THE FIRST HUMAN STUDY AND THE SYSTEMIC CHALLENGES

The first pig-to-human lung transplant study provided the field with pivotal data that is both groundbreaking and defining. Its most direct contribution was the first confirmation within a human system that knockout of the GGT1, CMAH, and B4GALNT2 genes in donor pigs can effectively prevent hyperacute rejection targeting pig lungs. The absence of widespread thrombosis or hemorrhagic necrosis on early post-transplant biopsies successfully translated the core genetic strategy previously validated only in non-human primates to the human environment, completing a crucial proof-of-concept.

However, this milestone also illuminated the substantial gap between short-term feasibility and long-term clinical applicability. The nine-day observation window, while setting a record, could not provide information regarding mid-to-long-term rejection, chronic graft dysfunction, or long-term immunosuppression risks. Notably, the experiment was electively terminated on postoperative day 9 at the family's request, rather than due to irreversible graft failure. The partial graft recovery observed suggests that the current genetic modifications and immunosuppressive regimen may have been partially effective in controlling acute immune injury. The neuroendocrine dysregulation and systemic inflammatory background inherent in the brain-dead recipient necessitate caution when extrapolating these findings to living patients.³⁷

On a technical level, despite employing an advanced gene-editing scheme incorporating three human protective proteins (CD46, CD55, TBM), the transplanted lung rapidly encountered severe primary graft dysfunction (PGD) and active antibody-mediated injury. This outcome suggests that current genetic modifications, while sufficient to prevent hyperacute rejection, remain inadequate to fully address the complex, multifactorial pathophysiology of lung

xenograft injury. Future approaches may require the introduction of more lung-specific genetic modifications, such as enhanced expression of proteins targeting anti-inflammation (e.g., HO-1), anticoagulation (e.g., EPCR), or anti-phagocytosis (e.g., CD47). Furthermore, the clinical protocol did not include CD40–CD154 costimulation blockade. This strategy has been shown to significantly prolong xenograft survival and attenuate humoral responses in non-human primate models of cardiac xenotransplantation,³⁴ and its absence in this first lung xenotransplant may have contributed to the inability to adequately curb early antibody-mediated injury (Figure 3).

These limitations, revealed by the first human study, are not isolated technical gaps but rather symptoms of deeper, systemic challenges that must be addressed as the field moves toward clinical translation. These challenges span multiple dimensions, from donor organ production to surgical adaptation, from pathogen safety to the very design of translational pathways.

First, at the donor source level, a scaled-up production system for gene-edited pigs is a foundational condition supporting clinical translation.³⁸ Current production of gene-edited pigs still faces challenges such as low efficiency, high cost, and unstable lineages. Establishing standardized gene-editing platforms, optimizing somatic cell nuclear transfer technology, and perfecting breeding systems for specific pathogen-free herds are key steps for scaled-up production. Additionally, stringent quality control standards need to be established, involving comprehensive genomic, transcriptomic, and proteomic verification of gene-edited pigs to ensure their genetic stability and biosafety. Beyond production scale, donor–recipient size matching poses a lung-specific challenge, as the graft must fit within the fixed thoracic cavity,³⁹ unlike the heart or kidney. Substantial mismatch can lead to small-for-size or large-for-size syndrome. In human lung transplantation, significant undersizing is a known risk factor for primary graft dysfunction, residual pleural space, and reduced survival. Oversizing can cause hemodynamic compromise due to elevated intrathoracic pressure.^{40,41} These concerns are amplified in xenotransplantation, where the donor organ originates from a different species. Selecting smaller-stature pig breeds, such as Auckland Island pigs whose organ dimensions are well-suited for human transplantation, represents one practical strategy.⁴² In addition, growth-inhibiting genetic modifications, particularly growth hormone receptor knockout (GHR-KO), have gained substantial attention. GHR-KO pigs exhibit markedly reduced body weight and organ weight. A study by Hinrichs et al. demonstrated that at 6 months of age, GHR-KO pigs had a 61% reduction in body weight and a 63% reduction in heart weight compared with controls.⁴³ This enables the use of donor pigs at an age beyond the steepest phase of their growth curve and may prevent post-transplant xeno-organ overgrowth.⁴⁴ Therefore, integrating small-stature donor breeds with GHR-KO editing represents a tailored strategy to address the lung-specific size-matching challenge and warrants further investigation in preclinical lung xenotransplantation models.

Second, from a surgical and anatomical perspective, significant differences exist between pig and human lungs, including bronchial branching patterns, vascular distribution, and lymphatic drainage pathways. These differences can affect the technical difficulty of surgical anastomosis and may also have long-term impacts on postoperative physiological function recovery. Therefore, systematic comparative anatomical studies are needed, alongside the development of specialized surgical instruments and anastomosis techniques tailored for lung xenotransplantation.

Third, the issue of biosafety looms large. Risk control for cross-species pathogen transmission is an unavoidable safety issue in xenotransplantation. The presence of porcine endogenous retroviruses (PERVs) has raised widespread concern.^{45,46} Although current research suggests a low risk of cross-species infection in properly screened donor herds, a comprehensive screening and monitoring system remains necessary. Furthermore, other potential zoonotic pathogens carried by pigs require systematic evaluation.⁴⁷ Establishing specific pathogen-free breeding systems for donor pigs, developing highly sensitive pathogen detection technologies, and formulating peri-operative antimicrobial prophylaxis protocols are crucial components of building a robust biosafety framework. Long-term biosafety monitoring protocols should cover the entire post-transplant period, including regular pathogen testing of recipients, establishing a donor–recipient infection event reporting system, and conducting long-term epidemiological investigations. Simultaneously, dedicated xenotransplantation biobanks need to be established to systematically collect and preserve donor tissues, recipient biologi-

cal samples, and clinical data, providing resource support for long-term safety assessment and mechanistic research.

Finally, the translational research pathway itself requires careful design. Improvements in preclinical models should focus on enhancing their predictive value.⁴⁸ Current xenogeneic lung transplant research primarily uses non-human primate models, which are costly and subject to strict ethical constraints. Developing advanced ex vivo models that more accurately simulate human physiology and immune environments becomes crucial. For example, xenogeneic cross-circulation models using brain-dead recipients or ex vivo human donor lungs allow testing repair strategies and assessing immune compatibility directly at the human organ level, significantly improving the predictive value of preclinical research.^{49,50} Stepwise clinical research protocols should be planned following a principle of cautious and orderly progression. Initial clinical exploration could select critically ill patients with end-stage lung disease and no other treatment options as recipients, focusing on assessing surgical feasibility and short-term safety. As experience accumulates and technology matures, the scope of indications can be gradually expanded, and controlled studies initiated.

In summary, the first human study serves not as an endpoint but as a starting point. Its limitations define the scientific questions that must be answered, and the systemic challenges outlined above define the operational framework within which these answers must be pursued. The following sections will delve into the specific immunological and physiological barriers—innate immunity, adaptive immunity, and coagulation dysfunction—that lie at the heart of these challenges, and examine the intervention strategies being developed to overcome them.

IMMUNOLOGICAL BARRIERS AND DEVELOPMENT OF INTERVENTION STRATEGIES

Unlike the kidney or heart, the lung is densely populated by alveolar macrophages that launch a vigorous innate attack within minutes of reperfusion. Its vast endothelial surface amplifies the effects of molecular incompatibilities, and because the lung receives the entire cardiac output in a low-pressure, high-flow system, it is uniquely prone to platelet sequestration and microvascular thrombosis even when hyperacute rejection is avoided. Porcine coagulation and complement regulators function poorly in humans, further driving endothelial activation and microvascular occlusion. Therefore, lung xenotransplantation faces unique immune barriers (Figure 4; Table 1).

Recognition and control of innate immune responses

The core objective of intervention strategies targeting innate immunity is to establish effective molecular camouflage and active defense during the critical window following reperfusion. Both gene editing and targeted pharmacological agents contribute synergistically to this goal.

Current immunomodulatory strategies have evolved along a relatively clear stepwise pathway. The foundational and indispensable strategy is the complete knockout of the three key genes responsible for synthesizing the α -Gal, Neu5Gc, and Sd(a) antigens in the pig genome using technologies like CRISPR-Cas9. However, knockout alone has proven insufficient, as residual antibody binding and complement activation remain a threat. Consequently, the core of the second-generation strategy is the transgenic expression of human complement regulatory proteins such as CD46 (membrane cofactor protein) and CD55 (decay-accelerating factor). This aims to precisely mimic the natural protective mechanism of human endothelial cells, efficiently halting the complement cascade at the level of C3 convertase. In vitro functional assays have demonstrated that gene-edited porcine endothelial cells expressing CD46/CD55 exhibit significantly reduced C3b deposition and cytolysis upon exposure to human serum.^{51,52}

For macrophage and natural killer (NK) cell-mediated innate immune attacks, the core research strategy focuses on providing the xenogeneic cells with key signals recognizable as "self" by the human immune system. Transgenic expression of human CD47 on porcine cells can effectively bind to the SIRP α receptor on macrophages, delivering an inhibitory "do not eat me" signal and significantly reducing macrophage-mediated phagocytosis. Expression of human HLA-E provides NK cells with an effective inhibitory ligand, attenuating their cytotoxic attack. These strategies complement the aforementioned antigen knockout and complement regulation. In ex vivo pig-to-baboon lung perfusion experiments, gene-edited pig lungs co-expressing

Key Limitations & Systemic Challenges in Pig-to-Human Lung Xenotransplantation

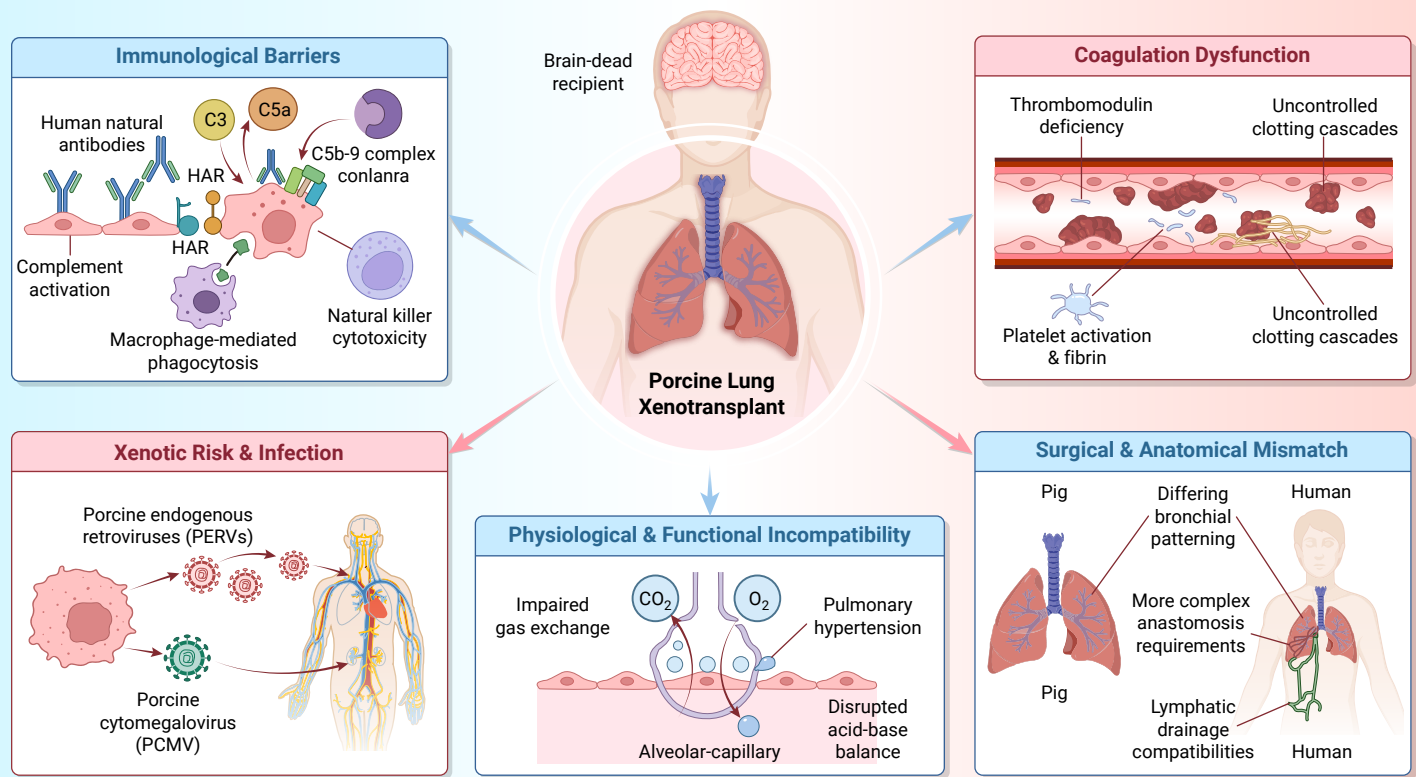


Figure 3. Key limitations and systemic challenges in pig-to-human xenotransplantation.

CD46 and HLA-E demonstrated less neutrophil sequestration and endothelial injury during perfusion.²⁸ This result provides experimental evidence for the synergistic effect of combinatorial genetic modification strategies in alleviating early inflammatory damage.⁵³

Regulation of adaptive immune responses

Once the graft survives the acute phase, the intervention focus shifts to managing the persistent immune response mediated by T and B cells.⁵⁴ Traditional calcineurin inhibitor-based regimens are inadequate in the xenogeneic setting, prompting a shift towards costimulation blockade and targeted cell depletion. Monoclonal antibodies targeting the CD28-CD80/CD86 or CD40-CD154 pathways have demonstrated efficacy in inhibiting xenograft rejection in preclinical studies.

The inhibition of CD40/CD154 costimulatory signaling represents a particularly promising approach, as evidenced by its success in preventing renal transplant rejection in non-human primate models. A systematic review and meta-analysis has confirmed the effectiveness of this strategy in transplantation settings.⁵⁵ The combination of anti-CD40 and anti-CD40L antibodies has demonstrated efficacy in preclinical cardiac xenotransplantation RCTs.⁵⁶ First-generation anti-CD154 antibodies, such as hu5c8, were associated with thromboembolic events in early clinical trials.⁵⁷ This was attributed to Fcγ receptor IIa-mediated platelet activation triggered by antibody-sCD154 immune complexes.⁵⁸ These concerns are especially salient in the coagulation-sensitive lung environment. To circumvent this limitation, novel agents have been developed through protein engineering. TNX-1500, an Fc-modified IgG4 anti-CD154 monoclonal antibody, retains the Fab region of hu5c8 while reducing FcγRIIIa binding, and has demonstrated prolonged allograft survival without thromboembolic events in nonhuman primate models of kidney and heart transplantation,^{59,60} with a phase 1 trial confirming no thrombotic complications in healthy volunteers.⁶¹ Frexalimab (SAR441344), a second-generation anti-CD40L antibody engineered with an inert Fc tail, is also under clinical investigation and has recently entered phase 3 trials for kidney transplant rejection in China. These advances in costimulation blockade carry particular relevance for lung xenotransplantation, where the dual

risk of antibody-mediated rejection and coagulation dysregulation demands both potent immunosuppression and an optimal safety profile. Furthermore, novel anti-CD40L antibodies like frexalimab have shown clinical efficacy in autoimmune conditions, suggesting their potential application in xenotransplantation.⁶² Combined use of anti-CD20 antibody for B cell depletion can further delay the development of de novo antibodies.

Cutting-edge exploratory strategies include local graft immunomodulation and hematopoietic chimerism induction. Transgenic overexpression of human PD-L1 on porcine endothelial cells suppresses human T-cell responses in vitro and in ex vivo kidney perfusion, but has not been directly tested in a lung xenotransplant model.^{53,64} The use of genetically immunodeficient pigs developed primarily for oncology research may serve as an additional ancillary immunomodulation strategy.⁶⁵ Recent breakthroughs include the development of homozygous editing techniques for multiple genes, which accelerate the generation of xenotransplantation pigs by simultaneously targeting several key genetic loci.⁶⁶ A more revolutionary intervention involves direct engineering of the donor thymus or combined hematopoietic stem cell transplantation.^{67,68} This strategy involves the combined transplantation of donor pig thymus tissue and lungs to establish donor-specific immune tolerance in the recipient. However, while thymic co-transplantation has been validated in renal models, its application in lung xenotransplantation remains in the proof-of-concept stage. The hybrid chimeric induction strategy aims to partially reconstruct the recipient's immune system through hematopoietic stem cell transplantation.⁶⁹ Lungs as an organ exposed to immune stimuli may uniquely influence on the homing and differentiation of hematopoietic stem cells. A study demonstrated that hCD47 transgenic donor marrow extended macrochimerism beyond 60 days and prolonged lung xenograft survival to 13–14 days,³⁰ representing a evidence to date for the chimerism strategy in a lung-specific context.

Correction of coagulation system dysfunction

Coagulopathy is a key factor leading to early failure in lung xenotransplantation. Intervention strategies aim to reconstruct normal anticoagulant and antithrombotic balance at the molecular level. Single-gene replacement has

Table 1. Immunological barriers and intervention strategies.

Barrier	Mechanism	Current Solution	Evidence Level	Key Limitation	Priority Next Step
Hyperacute rejection	Preformed natural antibodies (anti- α -Gal, anti-Neu5Gc, anti-SDa) bind porcine endothelium and activate complement	TKO of GGTA1, CMAH, β 4GalNT2 via CRISPR-Cas9	B	Residual non-Gal antibody binding persists; CMAH-KO introduces neo-antigen in Old World NHP models	Screen additional xenoantigen candidates; validate in human in vitro models
Complement-mediated injury	Residual antibody triggers C3/C5 activation, C3b deposition, and cytolysis	Transgenic hCD46 (MCP) and hCD55 (DAF)	B	Cannot fully block the alternative complement pathway	Explore combination with CD59 (MAC inhibition)
Macrophage-mediated phagocytosis	Porcine CD47 fails to engage human SIRP α , lacking the "don't eat me" signal	Transgenic hCD47 on porcine cells	C	Inconsistent hCD47 expression across cell types; SIRP α polymorphism	Optimize endothelial and alveolar epithelial hCD47 expression
NK cell cytotoxicity	Porcine SLA-I cannot engage human inhibitory KIR receptors	Transgenic HLA-E	C	Only inhibits NKG2A ⁺ NK cell subsets	Explore co-expression of HLA-G
Coagulation dysregulation	Porcine TBM, EPCR, TFPI are molecularly incompatible with human system; ATP/ADP promote platelet activation	Multi-transgenic combination: hTBM + hEPCR + hTFPI + hCD39	C	Single-gene replacement has limited effect; post-translational modification differences	Validate in brain-dead human lung model
T cell-mediated rejection	Xenogeneic antigen recognition via direct/indirect pathways; calcineurin inhibitor regimens insufficient	Costimulation blockade (anti-A CD40/CD154, anti-CD28/CD80/86); T cell depletion (rATG); B cell depletion (anti-CD20)		Chronic rejection remains difficult to control; NHP models differ from human immune responses	Validate TNX-1500 and frexalimab in NHP lung transplantation
Antibody-mediated rejection	Donor-specific antibodies bind endothelium, activate complement (C4d deposition) and ADCC	Anti-CD20 (rituximab); CD40/CD154 blockade; C5 inhibition (eculizumab)	D	Incomplete DSA elimination; memory B cells and long-lived plasma cells persist	Explore anti-CD38 (daratumumab) or plasma cell-targeted strategies
Primary graft dysfunction	Vicious cycle of innate immune activation, endothelial incompatibility, IRI, and coagulation dysregulation	Integrated strategy combining gene editing, immunosuppression, and coagulation regulation	C	Current strategies cannot fully prevent PGD	Optimize cold preservation solutions; explore gene/drug re-engineering during EVLP
Post-transplant infection	Intensified immunosuppression increases risk of opportunistic and porcine-derived pathogen transmission	SPF pig breeding; rigorous microbial screening; metagenomic/metatranscriptomic surveillance	D	Short monitoring window (9 days); long-term risk of latent pathogen reactivation unknown	Establish lifelong microbiological surveillance protocols

TKO, triple-carbohydrate knockout; IRI, ischemia–reperfusion injury; EVLP, ex vivo lung perfusion; PVR, pulmonary vascular resistance; MAC, membrane attack complex; DSA, donor-specific antibody; ADCC, antibody-dependent cellular cytotoxicity; SPF, specific pathogen-free; RCT, randomized controlled trial; NHP, nonhuman primate.

limited effect. The current most effective strategy is the systematic expression of a set of human coagulation regulatory proteins to cover different nodes of the coagulation cascade. TBM and EPCR work together to restore the anticoagulant function of the protein C system. Human tissue factor pathway inhibitor (TFPI) suppresses the initiation of the extrinsic coagulation pathway. CD39 mitigates platelet activation and inflammation at the source by degrading pro-inflammatory and pro-coagulant ATP/ADP. In pig-to-baboon lung transplant models achieving survival beyond 30 days, the donor pigs carried multiple modifications including hTBM, hEPCR, hCD39, or genes with similar functions.^{31,70}

Future optimization may focus on several directions. Precise spatiotemporal control of transgene expression using endothelial-specific promoters has been explored in vitro with promising endothelial specificity. For instance, the pig ICAM-2 promoter and inducible Tet-On systems have each been tested in porcine endothelial cells to restrict transgene expression or to regulate NF κ B activity.^{71,72} The functional impact of species-specific post-translational modifications has been demonstrated in the pig-to-baboon lung xenotransplant context.⁷³ The concept of re-engineering donor lungs during ex vivo perfusion has been established in lung allotransplantation through adenoviral IL-10 gene therapy,⁷⁴ MSC-based IL-10 delivery⁷⁵ and alpha-1 antitrypsin treatment.⁷⁶ This approach has not yet been widely applied to xenogeneic lungs. Pharmacological interventions have been tested in 10-gene-edited pig lungs during ex vivo human blood perfusion. These include selectin/integrin blockade combined with macrophage depletion.⁷⁰ Parallel in vivo and ex vivo testing of multi-gene-edited lungs is now being performed and represents an advanced evidence directly applicable to lung xenotransplantation.

Endpoints for evaluating intervention efficacy

The barriers and intervention strategies discussed above can be translated into a set of measurable translational endpoints. For hyperacute rejection, the endpoint is the absence of diffuse interstitial hemorrhage, vascular thrombosis, and complement C5b-9 deposition on graft biopsy within 48 h of reperfusion.²² For primary graft dysfunction, the endpoint is sustained PGD Grade 0–1 over the first 72 h post-transplant, as defined by ISHLT consensus criteria.⁷⁷ The first human study did not meet this benchmark, indicating that ischemia–reperfusion injury and innate immune activation remain inadequately controlled. Beyond the immediate perioperative period, graft function and immune protection must be maintained over time. Sustained gas exchange, defined by a PaO₂/FiO₂ ratio above 300 mm Hg without extracorporeal support, represents the functional threshold for independent graft performance and has been achieved during xenogeneic cross-circulation support of human donor lungs.⁷⁸ Freedom from antibody-mediated injury requires the absence of progressive C4d deposition on serial biopsies and undetectable levels of clinically significant anti-donor pig antibodies. Active AMR was documented on POD 3 and 6 in the first human study, confirming this endpoint remains unmet.

Two longer-term endpoints define the ultimate goals of clinical translation. Long-term survival that recipient and graft survival exceeding one year with stable pulmonary function and no histologic evidence of chronic graft dysfunction remains distant. Biosafety requires the absence of replication or dissemination of porcine-derived pathogens at any time post-transplant, confirmed by serial metagenomic screening, as emphasized by current consensus on infection surveillance in clinical xenotransplantation.⁷⁹ These endpoints collectively represent the milestones that must be reached before living-recipient trials can be responsibly initiated.

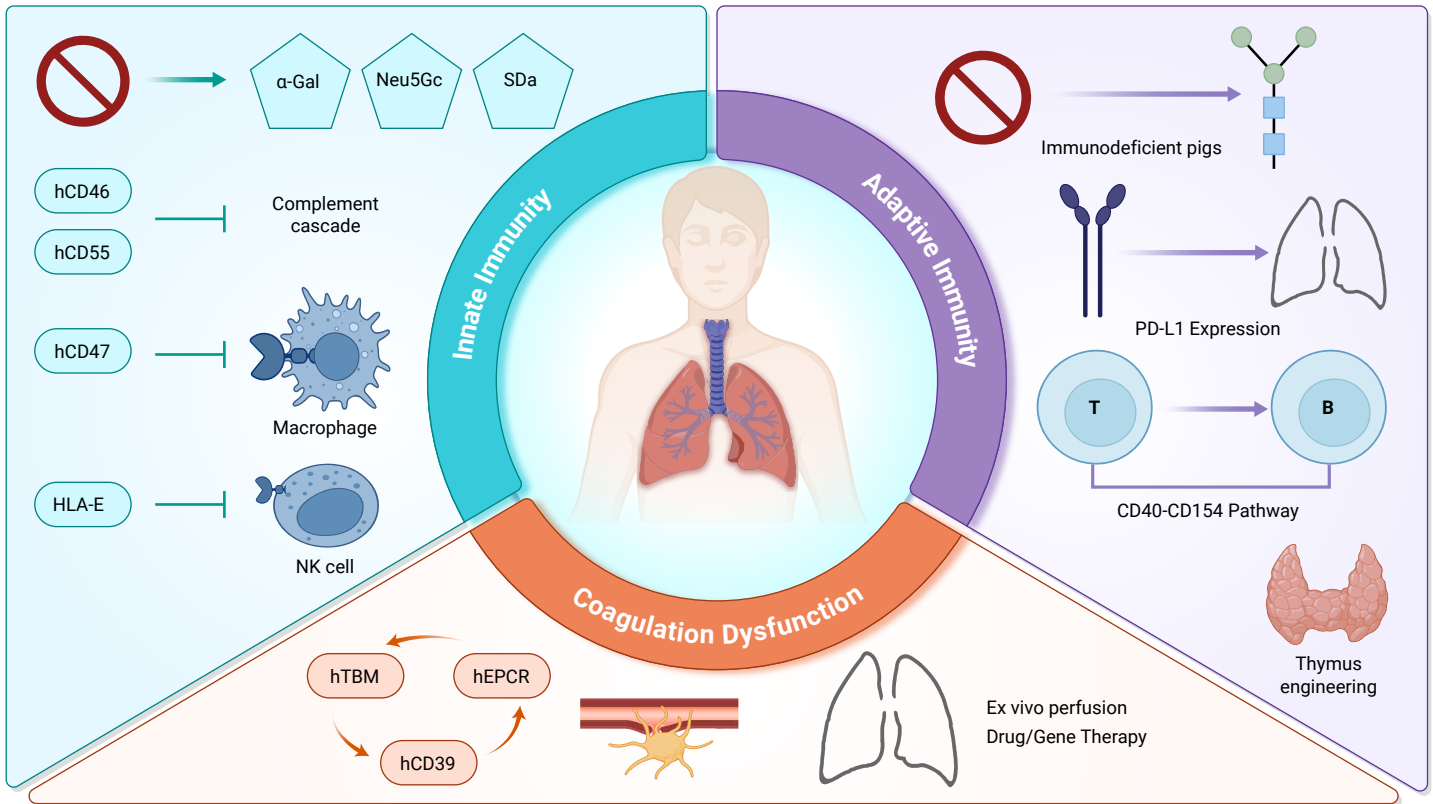


Figure 4. Immunological barriers and strategy in lung xenotransplantation.

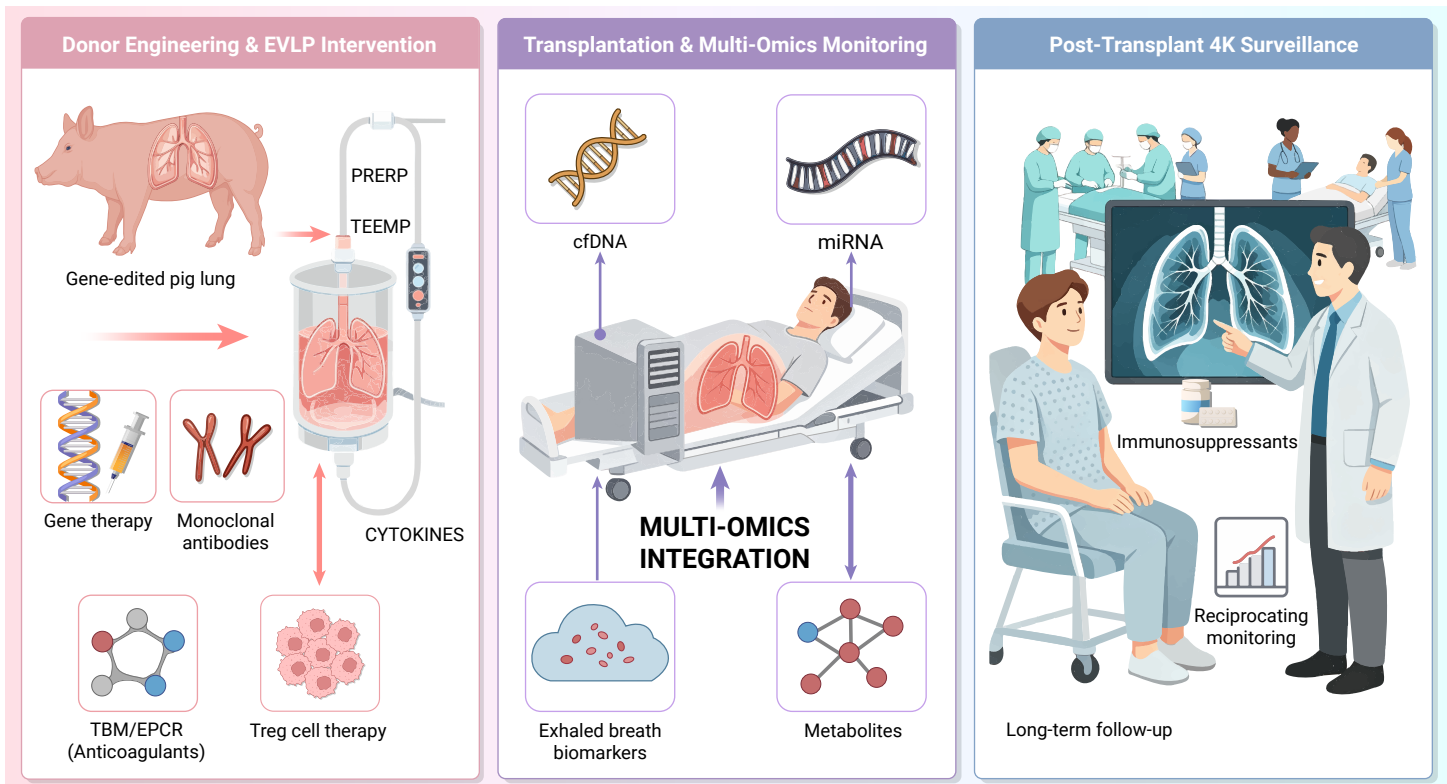


Figure 5. Future pathways of lung xenotransplantation.

FUTURE PATHWAYS OF LUNG XENOTRANSPLANTATION

Ex vivo lung perfusion: From preservation platform to intervention hub

The functional expansion of ex vivo lung perfusion (EVLP) represents one of the most transformative developments in lung transplantation research.⁸⁰

Traditionally employed for organ preservation and quality assessment, EVLP is now evolving into a versatile delivery platform for organ repair, gene therapy, and immune intervention. This evolution holds particular promise for xenotransplantation, where the donor organ could potentially be modified or

preconditioned before implantation. Notably, EVLP is distinct from normothermic machine perfusion (NMP), a term historically associated with perfusion strategies for kidneys, hearts, and livers, though the two terms are sometimes used interchangeably in the literature.

During EVLP, critical biological processes remain active, providing a unique window for intervention. Complement activation occurs in both porcine and human discarded kidneys during perfusion, highlighting the importance of complement regulation during ex vivo organ support.^{81,82} This knowledge directly informs the development of lung perfusion strategies that minimize complement-mediated damage. Moreover, EVLP serves not only as a preservation technique but also as a model of reperfusion to deliver novel therapies and assess inflammation and immune activation.^{83,84}

Particularly notable is the emergence of xenogeneic cross-circulation support systems. By connecting a human donor lung to the circulatory system of a live animal, these systems can provide several days of comprehensive physiological support and a repair window for severely damaged donor lungs.⁵⁰ More importantly, studies evaluating this platform have reported that this xenogeneic cross-circulation environment did not trigger hyperacute rejection against the human lung, potentially providing a safe time window for simultaneous immune tolerance induction or other interventions during the repair process. Detailed immunological characterization of xenogeneic cross-circulation systems helps elucidate the immune changes experienced by human organs in the context of xenogeneic blood contact, thereby identifying potential risks and protective pathways.⁸⁵

Monitoring and assessment: Non-invasive platforms and multi-omics integration

Parallel to advances in intervention platforms, the field requires equally sophisticated tools for monitoring graft status and understanding rejection mechanisms.

Establishing a non-invasive biomarker monitoring system is crucial for postoperative management. Early diagnosis of xenotransplant rejection currently still relies on invasive biopsies. Detection of biomarkers based on peripheral blood or exhaled breath condensate may offer a safer and more dynamic monitoring approach. Recent research focuses on donor-derived cell-free DNA, microRNA profiles, and metabolomic characteristics as early indicators of rejection. Standardization and clinical translation of these non-invasive monitoring technologies would likely enhance the precision of post-transplant management for lung xenotransplantation.

Multi-omics integrated analysis platforms are becoming essential tools for understanding rejection mechanisms.⁸⁶ By integrating transcriptomic, proteomic, and metabolomic data, researchers can more comprehensively reveal the molecular networks underlying xenotransplant rejection, identifying key pathways and intervention targets.⁸⁷ The application of such platforms may help optimize immunosuppressive regimens and develop novel therapeutic strategies from a systems perspective. Recent advances in multi-omic analysis have enabled detailed characterization of pathological states, as demonstrated in studies of right ventricular dysfunction,⁸⁸ providing a framework for similar comprehensive analyses in xenotransplantation research.

Advanced imaging and assessment tools are also being developed to better understand and evaluate lung anatomy and pathology. Computer vision systems have been developed for scoring swine lung images, showing comparable performance to human evaluators.⁸⁹ Such technologies could facilitate more objective assessment of donor lung quality and surgical outcomes. Additionally, novel simulation models for thoracic procedures, such as tube thoracostomy,⁹⁰ provide valuable training platforms for surgeons preparing for xenotransplantation procedures.

Anticipated breakthroughs and collaborative pathways

Synthesizing the current state of the field, we outline several anticipated pathways for technological progress.

(1) Through the optimization and scaled-up production of multi-gene edited pigs, the field will move toward constructing ideal donors with lower immunogenicity and better coagulation compatibility.⁹¹ This will involve not only more comprehensive gene editing but also tissue-specific expression systems that achieve localized expression of protective genes, particularly in

lung tissues and vascular endothelial cells, avoiding the potential risks associated with systemic expression.

(2) The development of xenotransplant-specific immunosuppressive protocols will continue to evolve, combining established strategies such as costimulation blockade with emerging approaches including cell therapy, to achieve more precise immune regulation.⁹² Insights from other disease areas such as thromboinflammation targeting strategies developed for antiphospholipid syndrome⁹³ and multi-epitope nanovaccine platforms designed for oncology applications^{94,95} may inform future therapeutic approaches.

(3) The establishment of comprehensive perioperative management systems and long-term monitoring protocols will be essential to ensure surgical safety and long-term efficacy. The integration of advanced technologies from other fields may support progress in lung xenotransplantation, though at differing levels of direct validation. Computer vision systems have been developed for scoring swine lung images.⁸⁹ Multi-omic analysis platforms, originally applied in cardiovascular and kidney research, provide a methodological framework that may be adapted to dissect the molecular networks underlying xenograft rejection.⁹⁶

Crucially, lung xenotransplantation is inherently a systems engineering endeavor, requiring the establishment of efficient cross-disciplinary and cross-institutional collaboration mechanisms. It is recommended to form a lung xenotransplantation Research Consortium, integrating basic scientists, clinicians, bioengineers, ethicists, and regulatory experts. This consortium should develop unified research standards and technical specifications, promoting resource sharing and data interoperability. Simultaneously, a fast track from basic discovery to clinical translation should be established, accelerating knowledge translation and technology integration through regular symposia, joint research projects, and personnel exchange.

Ethical And Regulatory Dimensions

As lung xenotransplantation gradually moves towards clinical application, ethical and regulatory frameworks need to be refined in parallel. On the ethical front, areas requiring further clarification include the ethical boundaries of research involving brain-dead recipients, formulate protection norms for special populations, and establish fair recipient selection criteria.⁹⁷ On the regulatory front, dedicated guidelines for lung xenotransplantation should be developed promptly, clarifying requirements for preclinical studies, standards for clinical trial design, long-term follow-up protocols, and clear criteria for pausing and withdrawal mechanisms with independent Data and Safety Monitoring Board (DSMB) oversight.⁹⁸

The success of lung xenotransplantation depends not only on technological progress but also on the rational understanding and cautious acceptance of this innovative medical approach by society as a whole. Through rigorous scientific research, transparent communication, and reasonable and effective regulation, lung xenotransplantation holds the promise of becoming an important means to address lung failure in the future, offering new hope to patients with end-stage lung disease. The main translational pathways, from donor engineering and ex vivo lung perfusion (EVLP) to transplantation, multi-omics monitoring, and long-term post-transplant surveillance, are summarized in Figure 5.

CONCLUSION AND PERSPECTIVES

Lung xenotransplantation is transitioning from basic research to clinical exploration. The first pig-to-human lung transplant in a brain-dead recipient demonstrated that the current multi-gene editing framework can block hyperacute rejection, yet early primary graft dysfunction, antibody-mediated injury, and coagulation dysregulation all emerged within nine days, confirming that existing genetic and immunosuppressive strategies remain insufficient. The core task ahead is to systematically identify the residual molecular incompatibilities beyond the three carbohydrate xenoantigens and current human transgenes that drive graft injury.

Progress requires refining three interconnected elements in parallel: a donor genetic background iteratively validated against human blood on a standardized ex vivo perfusion platform, an immunosuppressive backbone that balances costimulation blockade with the lung's inherent infection risk,⁹⁹ and non-invasive monitoring tools, notably donor-derived cell-free DNA and exhaled breath biomarkers, capable of detecting rejection before irreversible

injury occurs.^{100,101} Advancements in gene editing technology provide powerful tools for donor optimization, while innovations in immune management strategies open new paths for controlling rejection. These elements must be coordinated within a unified regulatory framework that addresses the infectious and ethical dimensions specific to pulmonary xenotransplantation.¹⁰²

Ultimately, the success of lung xenotransplantation rests on both technological progress and societal acceptance. Through rigorous scientific research, transparent communication, and reasonable and effective regulation, it holds the promise of offering new hope to patients with end-stage lung disease.

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AUTHOR CONTRIBUTIONS

Yubing Fang: Writing – original draft; Writing – review & editing; Visualization; Investigation. Jiani Gao: Writing – review & editing; Investigation; Validation. Minjue Shan: Writing – original draft; Investigation; Visualization. Di Lu: Conceptualization; Supervision. Shusen Zheng: Conceptualization; Supervision. Chang Chen: Conceptualization; Supervision; Project administration. All authors contributed to and approved the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

DATA AND CODE AVAILABILITY

No new data were created or analyzed in this study.