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Lung Xenotransplantation: Recent Progress and Current Status

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Abstract

Xenotransplantation has undergone important progress in controlling initial hyperacute rejection in many pre-clinical models, with some cell, tissue, and organ xenografts advancing toward clinical trials. However, acute injury, driven primarily by innate immune and inflammatory responses, continues to limit results in lung xenograft models. The purpose of this article is to review the current status of lung xenotransplantation – including the seemingly unique challenges posed by this organ – and summarize proven and emerging means of overcoming acute lung xenograft injury.

Introduction

Lung transplantation improves survival and quality of life for appropriately selected patients with end-stage lung disease,^{1–4} but the availability of suitable allografts limits access to this therapy and results in substantial wait-list mortality.^{3,5,6} Although every field of organ transplantation is critically supply-limited, the lung donor pool is particularly constrained.^{7–9} Lungs have among the lowest recovery and transplantation rates per donor, despite the growing use of extended criteria donor lungs, emerging lung repair strategies and donation after cardiac death protocols.^{5,7,10–12} As a result, lung waitlist mortality is high, and many potential recipients are too ill to undergo transplantation once an allograft is allocated.^{2,6,13} Expanding use of extracorporeal membrane oxygenation as a bridge to transplantation for severe acute respiratory distress syndrome, influenza-associated lung failure and a variety of other acute and acute-on-chronic lung conditions could exacerbate this shortage by increasing the number of potential recipients.^{14,15} Unfortunately, even with optimized donation consent and organ recovery practices, the current potential donor pool is insufficient to provide therapy for all the patients who meet the current, stringent criteria for

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lifesaving transplantation.¹⁶ As a consequence, approximately 25% of patients listed for lung transplantation die while waiting or are delisted as “too sick to transplant”.^{2,9}

Xenotransplantation is a promising approach to address the critical shortage of allografts by providing a potentially unlimited and on-demand supply of organs.^{17–20} Lung xenotransplantation could increase the quality of clinical transplantation by avoiding the use of lungs compromised by the damage commonly induced by brain death, donor resuscitation and the ischemia/reperfusion injury associated with procurement.²¹ Ready access to healthy animal organs would enable elective implantation when the recipient is in clinically stable condition and permit tolerogenic immunologic preconditioning of the recipient to facilitate long-term graft survival.^{19,22,23}

As recently reviewed by Pierson *et al* and Ekser *et al*, significant progress has been made toward clinical use of non-lung xenografts using genetically modified porcine donors.^{17,18} For example, long-term survival – of months, and occasionally beyond a year – has been achieved in heterotopic heart xenograft models.^{24–26} In contrast, functional lung xenograft survival remains limited to hours despite control of previously characterized “hyperacute rejection” mechanisms.^{27–29} Fortunately, emerging interventions that target early, acute injury mechanisms specific to lung xenotransplantation are showing major promise in pre-clinical lung models. We believe that tackling the challenge of lung xenotransplantation based on control of these additional injury pathways is also likely to catalyze broader progress in advancing other fields of xenotransplantation toward clinical applications, to the extent that these non-immune mechanisms prove relevant to the delayed injury seen in heart, kidney and other xenografts.³⁰

The purpose of this article is to review the current status of lung xenotransplantation, including the status of pre-clinical models, barriers that appear to be uniquely important to the lung xenograft, and established and emerging interventions expected to enhance lung xenograft performance. Because of acute barriers to lung xenograft survival, innate immune and inflammatory responses will primarily be discussed. Most xenotransplantation research uses porcine donors for reasons adequately described elsewhere,^{17,20,31} so the review will focus on the progress and remaining barriers in pig-to-human lung transplantation. Finally, as the full range of available and potential porcine genetic modifications and their application to lung xenotransplantation has been recently summarized in detail,³⁰ this review will emphasize interventions with proven benefit in lung xenograft models.

Lung Xenotransplantation Models

The gold-standard pre-clinical test of a lung xenograft is *in-vivo* orthotopic transplantation.³² This is the only model that enables study of long-term organ performance, and relevant immunosuppression and/or xenotolerance regimens.^{30,32} Typically, a left lung xenotransplant is performed in a baboon recipient.³² Ultrasonic flow probes on the aorta and donor pulmonary artery enable monitoring of cardiac output and blood flow through the xenograft, and occlusion of the contralateral native pulmonary artery enables evaluation of life-supporting function of the lung xenograft.^{32,33} An important limitation of this model is that, like pigs, nonhuman primates express N-glycolylneuraminic acid (NeuGc), an

important target of human xenoreactive antibodies.^{30,34} As such, anti-NeuGc antibody-mediated injury is not evident in *in-vivo* transplant studies using nonhuman primate recipients, indicating an important scientific role for *ex-vivo* experiments with human blood.

Ex-vivo pig lung xenoperfusion with human donor blood is an alternative to studying the effects of interventions on early xenograft injury and outcomes. In this model, explanted pig lungs are ventilated and perfused either together or in independent circuits;^{32,35–37} the latter configuration allows for treatment of one lung with the contralateral side serving as a paired control.^{32,37} Because this model is physiologically relevant, mechanistically informative and simulates xenotransplantation to a human recipient with less resource use than *in-vivo* studies, it plays an important role in lung xenotransplantation research and is our group's primary large-animal model.³²

Current status of lung xenotransplantation

The past decade has seen significant progress in xenotransplantation.^{17,20} Porcine islet and other cell-based therapies, such as hepatocyte liver support devices, have progressed to human trials.^{38–40} In whole organs, Yamada *et al* reported up to 83 days of life-supporting kidney xenograft function in baboons using α -1,3 galactosyltransferase knockout (GalTKO) pigs with a tolerance induction regimen.⁴¹ In heterotopic pig-to-nonhuman primate heart models, multiple teams have achieved several months of xenograft survival.^{25,26,42} Notably, Mohiuddin *et al* have achieved 1-year heterotopic heart survival with GalTKO pig donors transgenic for the human complement regulatory protein CD46 and human thrombomodulin (TBM), using experimental immunosuppression based on CD40/CD40L costimulation blockade combined with “induction” B-cell depletion.²⁴ Results in the life-supporting orthotopic pig-to-baboon heart transplantation model using conventional immunosuppressive regimens have progressed beyond hyperacute rejection and reached survival beyond five weeks.^{43–46}

Despite other xenotransplantation progress, the longest reported lung xenograft survival in an *in-vivo* orthotopic pig-to-nonhuman primate model has been 4.5 days (Table 1).^{47–49} Life-supporting function is more limited, and has consistently been less than 24 hours in reported series.^{27,30,33,49,50} Recently, our group has reached 24 hours of life-supporting lung function using a multi-transgenic pig with targeted donor and recipient treatment (Pierson *et al*, unpublished), indicating that hyperacute lung xenograft injury can be significantly delayed and likely overcome with optimized genetic and pharmacologic interventions. This result falls far short of a previously suggested preclinical target for proceeding to human trials: consistent 3-months of life-supporting function in a pig-to-nonhuman primate model has been proposed by the International Society for Heart and Lung Transplantation as an appropriate threshold for considering clinical application.¹⁹

We and others have observed that lung xenotransplantation has generally proven more difficult than other pig-to-primate organ xenografts. Our current working hypothesis posits that either: 1) lung xenografts are susceptible to organ-specific, mechanistically unique injury pathways; or that 2) lung vulnerability reflects, and is attributable to, physiologic or anatomic characteristics of lung tissue that unveil, or amplify, processes that are

mechanistically important to short and long-term survival of all porcine xenografts. While both may contribute, we consider the second postulate more likely. In either case, progress in addressing the lung's particular vulnerability will unveil either lung-specific or more general barriers broadly important to pig-to-human xenotransplantation, and enable progress toward clinical application.

Mechanisms of lung xenograft injury

General lung injury

Among organs, the lung is particularly susceptible to generalized injury and dysfunction, such as with ischemia/reperfusion during transplantation.^{51,52} As a “sentinel” organ, it is often the first to exhibit clinically important dysfunction in sepsis, complicated trauma and multisystem organ failure.^{53–56} The structure and function of pulmonary vascular and alveolar epithelial cells are constantly modulated by physiologic mechanical signal transduction.⁵⁷ In particular, the lung is highly sensitive to barotrauma associated with traumatic injury and mechanical ventilation, which can result in alveolar injury, endothelial dysfunction and local and systemic inflammation, resulting in significant pulmonary dysfunction.^{57–60}

Compared to the systemic vasculature, blood flow, shear stress and capillary characteristics within the pulmonary system are distinct in ways that specifically promote enhanced endothelial – neutrophil interactions, enabling extensive neutrophil adhesion and sequestration within the lungs.^{61–64} Uniquely among vascular beds in mammalian organs, the pulmonary vasculature flow decreases in response to insults such as hypoxia or inflammatory mediators,^{65,66} and the high endothelial surface area of the pulmonary vasculature renders the lung more susceptible to endothelial dysfunction and inflammatory injury.^{30,57} In particular, thromboxane is an important mediator of pulmonary vasoconstriction, inflammation and dysfunction during ischemia/reperfusion injury and lung transplantation.^{51,67–70} Because of these unique factors, lungs are relatively more susceptible to injury during procurement, preservation and implantation with both allo- and xenotransplantation.

Hyperacute injury and complement activation

Hyperacute rejection is the initial barrier to lung xenotransplantation, as it is for other vascularized xenografts. Pre-formed human IgM and IgG antibodies bind porcine antigens; this results in wild type (WT) lung xenograft failure within minutes (Table 2).^{28, 71–75} Galactose 1,3 α -galactose (Gal) is the primary antigenic target of hyperacute rejection in pig-to-human and pig-to-nonhuman primate transplantation,^{71,76,77} although other non-Gal porcine antigens, such as NeuGc also contribute.^{23,28,34,50,78–80} Antibody binding activates the classical complement pathway, resulting in endothelial injury, neutrophil recruitment, platelet activation and microvascular thrombosis.^{73,74} When WT pig lungs are perfused with human blood or transplanted into baboons, hyperacute lung rejection manifests most obviously in a rapid rise in pulmonary vascular resistance (PVR) and intense thrombosis.^{35,70} Lung failure typically ensues from a combination of loss of pulmonary

perfusion (elevated PVR), failure of vascular barrier function, alveolar injury and primary airway injury.^{28,33,35,37,71}

Xenogeneic thrombosis

Complement activation and thrombosis are interrelated in hyperacute xenograft rejection.^{81,82} However, even when complement activation is controlled, “physiologically inappropriate” thrombosis can independently and directly contribute to lung injury.^{35,71} In this setting, survival and function of xenoperfused lungs inversely correlates with the intensity of platelet consumption and coagulation activation.^{29,35} Similarly, *in-vitro*, resting porcine endothelial cells directly activate human thrombin in the absence of antibody binding, indicating failure of endogenous thromboregulation.⁸³

Importantly, cross-species molecular incompatibilities cause dysfunction of endogenous thromboregulatory mechanisms, which contributes to xenogeneic thrombosis in a variety of models.^{71,81,82,84} For example, the interaction between porcine endothelial TBM and human thrombin does not effectively catalyze protein C activation, resulting in a deficiency of this important endogenous anticoagulant.^{83,85,86} Similarly, binding between human von Willebrand factor (vWF) and human platelet glycoprotein (GP) Ib is normally regulated and shear stress dependent. However, porcine vWF – which is present at high levels in the lung relative to other porcine organs – binds human GPIb and activates platelets in the absence of shear force, facilitating non-physiologic platelet – endothelial adhesion.^{30,87,88}

Innate Immunity

Hyperacute lung xenograft rejection is associated with an intense innate immune response.^{30,74,89} For example, *ex-vivo* xenoperfused WT porcine lungs sequester 90% of circulating neutrophils within 5 minutes.²⁸ Even in the absence of anti-pig antibodies, human neutrophils and natural killer cells directly recognize and activate porcine endothelial cells.^{89,91–93} Although delayed in GalTKO ± hCD46 lungs, neutrophil infiltration still consistently occurs and correlates with lung survival.^{27–29}

Unlike xenogeneic thromboregulatory dysfunction, porcine endothelial adhesion molecules efficiently interact with human leukocytes to facilitate firm attachment.^{94,95} These interactions are associated with endothelial and leukocyte activation, endothelial injury and generation of inflammatory mediators.^{28,89,96} In addition to the role of human leukocytes, abundant resident porcine pulmonary intravascular macrophages (PIMs) are a prolific source of thromboxane, and contribute to activation of inflammatory and thrombotic pathways.^{30,70,97,98}

Inflammatory lung injury

Inflammatory mediators generated during reperfusion represent a final common pathway linking antibody binding, thrombosis, innate immunity and tissue injury with lung xenograft failure.⁷¹ In particular, an intense elaboration of thromboxane and histamine is directly associated with endothelial dysfunction, elevated PVR and lung injury.^{29,68,70,71,75} The lung has high thromboxane synthetic capacity as well as sensitivity to thromboxane’s effects,^{67–69} a response that appears to be amplified during lung xenotransplantation.^{29,30,75}

Finally, xenoperfusion of pig lungs results in generation of both porcine and human cytokines, including pig interleukin (IL) 8 and human IL6 and IL8, resulting in complex local and systemic inflammation.²⁸ By extrapolating from the well-defined roles of these cytokines in other models of tissue injury, we infer that inhibiting these pathways is likely to improve intermediate (days) and perhaps long-term (weeks to months) lung xenograft survival.

Proven and Emerging Protective Strategies

Prevention of antibody mediated hyperacute rejection

The development of GalTKO pigs was a major advance in lung xenotransplantation. While immunoadsorption or depletion of recipient anti-Gal and other antibodies prior to engraftment is partially beneficial,^{33,50,74,80,87,99} knocking out α -1,3 galactosyltransferase blocks expression of the Gal epitope,^{49,71,100,101} providing a more effective and durable means of limiting hyperacute rejection (Table 3). With *ex-vivo* xenoperfusion, median survival time is increased from 10 minutes for WT lungs to 120 minutes for the GalTKO genotype,²⁸ a finding that correlates with improved life-supporting function *in-vivo*.²⁷ In these models, absence of Gal was associated with reduced IgM and complement deposition.^{27,28} Indeed, the GalTKO genotype is associated with improved function and survival of other porcine xenografts,^{26,41} and serves as the genetic background for additional donor modifications.^{18,49}

GalTKO lungs and other organs are protected from hyperacute rejection mediated by anti-Gal antibodies,¹ but complement fixation by non-Gal antibodies contributes to endothelial injury, thrombosis and subsequent xenograft failure.^{28,50,78,79,102–104} The NeuGc residue appears to be an important secondary xenoantigen, as approximately 85% of humans have antibodies against NeuGc.^{30,34} Cells from pigs that are double-knockouts for Gal and NeuGc expression elicit a weaker human antibody response and subsequently reduced cytotoxicity.¹⁰⁵ As has been suggested for other xenografts, the removal of this antigen is likely to improve protection against residual antibody-mediated injury in lung xenotransplantation.^{18,30,105}

Control of complement activation

Addition of a human complement regulatory protein, such as hCD46 or hCD55 (membrane cofactor protein and complement decay accelerating factor, respectively), to the GalTKO platform by minigene transfection suppresses residual complement activation.^{29,30,35,106} In particular, the GalTKO.hCD46 genotype reduces complement activation and associated thrombosis and neutrophil sequestration in xenoperfused lungs.²⁹ The largest experience with complement control in lung xenografts has been with GalTKO.hCD46 donors, but transgenic expression of other complement regulatory proteins appears beneficial. For example, expression of hCD55 and/or hCD59 (membrane attack complex inhibitor) decreases complement deposition and markers of thrombosis, and improves lung function and survival.^{30,35,107–109} Given the benefit of combinations of modifications acting in concert to control different aspects of complement activation, it is likely that further progress will rely on multiple complement regulatory proteins. Indeed, our group's longest

duration of life-supporting lung xenograft survival was achieved with lungs that expressed both hCD46 and hCD55 within a cassette of other transgenes (Table 1; Pierson *et al*, unpublished).

Reducing platelet and coagulation activation

Although improved complement control reduces thrombosis and organ injury in lung xenotransplantation models,^{27–29,110} coagulation and platelet activation persists, necessitating additional targeted genetic modifications. For example, adding human CD39, which degrades prothrombotic adenosine diphosphate, to the GalTKO.hCD55.hCD59 background appears to improve lung function.³⁵ Similarly, expression of human tissue factor pathway inhibitor (TFPI) and a direct thrombin inhibitor in cultured porcine endothelial cells reduces thrombosis instigated by porcine tissue factor.^{111,112} If incorporated into the donor genome, such strategies could reduce thrombosis locally and potentially reduce the need for systemic antiplatelet and anticoagulation therapies.

More recent investigation has focused on multi-transgenic pigs that express human TBM or endothelial protein C receptor (EPCR).³⁰ Indicating this approach may successfully restore dysfunctional thromboregulatory mechanisms, transgenic hTBM expression enhances activated protein C generation.^{113,114} hEPCR expression is particularly promising, as it improves lung performance during *ex-vivo* xenoperfusion.¹¹⁵ In multi-transgenic donors, hEPCR contributes to improved life-supporting function in an *in-vivo* pig-to-baboon model, including up to 24 hours (Table 1; Pierson *et al*, unpublished). Co-expression of hTBM and hEPCR may be synergistic, enhancing constitutive activated protein C synthesis and EPCR-mediated cytoprotective signaling.^{30,114}

A range of genetic modifications for porcine donors has been reported.¹⁸ These should have broad benefit in the field, and have been specifically discussed in the context of lung xenotransplantation.³⁰ Although most have promising results in cellular or non-lung models, few have been tested in lung xenografts (Table 3). Considering advances in multi-transgenic genetic engineering and the apparent benefit of using transgenes in combination to target similar (eg: hCD55.hCD59) or disparate (eg: GalTKO.hCD46.hEPCR) processes contributing to xenograft injury, future developments will incorporate expanded blocks of transgenes to reduce xenogeneic incompatibilities and enhance xenograft protection.³⁰

Donor therapies

Genetic engineering has yielded major pre-clinical advances in xenotransplantation as a field, but further donor interventions are required to optimize lung xenograft function and survival. For example, pretreating pigs with desmopressin prior to lung procurement depletes pulmonary vWF reserves and attenuates complement activation.^{116,117} It also decreases platelet adhesion and endothelial activation resulting from dysfunctional porcine vWF – human GPIb binding during subsequent perfusion of the xenograft.^{30,87,88} Similarly, pretreatment with liposomal clodronate to deplete pulmonary macrophages protects lungs from sterile and xenogeneic injury by blunting activation of inflammatory and thrombotic pathways.^{47,70,97,98}

Genetic and pharmacologic interventions to control complement and pulmonary macrophage activation both target the triggers underlying thromboxane elaboration.⁷⁰ However, downstream thromboxane elaboration can also be controlled by donor treatment. For example, we routinely administer a synthetic thromboxane synthase inhibitor (1-benzylimidazole) prior to pig lung procurement to limit thromboxane generation during *ex-* and *in-vivo* experiments.^{27,29} Some of these additional pathways could be targeted by genetic modifications, such as the deletion of porcine vWF or addition of human vWF.^{30,47} However, given the range of biologic interactions and limitations of genetic engineering, there will likely remain an important role for donor pretreatment in xenotransplantation protocols.

Ex-vivo lung perfusion (EVLP), a technique used clinically to evaluate and rehabilitate lung allografts prior to transplantation,^{10,118} may provide a novel platform for xenograft-directed interventions prior to transplantation. As EVLP attenuates markers of pulmonary ischemia/reperfusion injury,¹¹⁹ it may supplant standard cold ischemia for routine lung preservation while additionally providing an opportunity for lung preconditioning,²¹ and delivering pulmonary gene and pharmacologic therapy without systemic toxicities.^{12,120} For example, pretreating GalTKO.hCD46.hEPCR endothelia with human activated protein C reduces thrombosis during subsequent xenoperfusion,¹²¹ a finding that emphasizes both the limitation of transgenic manipulations alone and the potential role for xenograft pretreatment to augment self-protective mechanisms.

Recipient treatment

Despite progress with genetic engineering and donor-directed treatments, recipient treatment is required to fully limit lung xenograft injury. Pharmacologic complement suppression, such as by administration of complement 1-esterase inhibitor immediately before lung xenograft reperfusion, augments the effect of GalTKO and transgenic hCD55 expression.^{27,108} Because thrombotic xenograft injury persists despite genetic advances and systemic anticoagulation,^{27–29,87} there is an important role for additional platelet and coagulation inhibition. For example, recipient treatment with a GPIb antagonist decreases dysfunctional porcine vWF – human platelet binding and subsequent thrombosis.^{30,87,122} Similarly, GPIIb/IIIa blockade provides another mechanism to limit thrombosis and the potent pro-inflammatory and cytotoxic consequences of platelet activation and degranulation.^{117,123,124} Finally, generation of inflammatory mediators during evolving xenograft injury represents an additional target for recipient treatment after reperfusion, both as a general approach and, where indicated, specifically targeted to xenogeneic mechanisms.^{27,70} For example, preliminary data suggests additional thromboxane synthase inhibition and histamine receptor blockade significantly improve PVR and *ex-vivo* lung survival by decreasing residual thromboxane and histamine elaboration.¹²⁵

Long-term xenograft survival has only been achieved using intensive, multimodal immunosuppression.^{24,25,41,125,126} While some pig-to-baboon lung xenotransplantation experiments have utilized conventional cyclosporine-based immunosuppression,^{33,50,87} selection of an effective, clinically applicable regimen will not become critical until the barriers of acute prothrombotic and innate immune responses are overcome. Success from

xenotransplantation of other organs suggests that multimodal immunosuppression (including induction lymphocyte depletion, plus maintenance therapy with IL-2 antagonism, steroids, antimetabolites and perhaps T cell costimulation pathway blockade) will be necessary and sufficient for lung xenograft recipients.^{24,41,125,126} If, as expected, our ongoing efforts to address more proximate barriers are successful, we look forward to working toward our eventual goal of eliciting cross-species immunologic tolerance to replace chronic immunosuppression.²³

Conclusion

The inherent challenges unique to lung transplantation are compounded between species. As such, lung xenograft survival is still measured in hours whereas there have been major pre-clinical and clinical advances in other xenotransplantation fields. Advances in porcine donor genetics, lung xenograft preservation and conditioning, and donor and recipient treatments are poised to contribute to significant progress toward clinical reality.

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Abbreviations

BIA	1-benzylimidazole
C1inh	C1 esterase inhibitor
DDAVP	desmopressin acetate
EPCR	endothelial protein C receptor
EVLP	<i>ex-vivo</i> lung perfusion
Gal	galactose 1,3 α -galactose
GalTKO	α -1,3 galactosyltransferase knockout
GP	glycoprotein
h	human
HRB	histamine receptor blockade
IL	interleukin
mab	monoclonal antibody

NeuGc	N-glycolylneuraminic acid
PIM	pulmonary intravascular macrophage
PMN	polymorphonuclear neutrophil
PVR	pulmonary vascular resistance
TBM	thrombomodulin
TFPI	tissue factor pathway inhibitor
vWF	von Willebrand factor
WT	wild type

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Table 1

Summary of reported pig-to-baboon orthotopic lung xenotransplantation experiments.

Authors	Year	Regimen	Survival
Daggett <i>et al</i> ³³	1998	Donors: Wild type Recipients: immunodepletion + CSA + AZA + steroids	11 hr *
Yeatman <i>et al</i> ¹¹⁰	1998	Donors: transgenic hCD55/hCD59 Recipients: complement depletion	3 hr
Gonzalez-Stawinski <i>et al</i> ⁵⁰	2002	Donors: transgenic hCD46 Recipients: anti-Gal antibody depletion	12 hr
Gaca <i>et al</i> ⁸⁷	2002	Donor: transgenic hCD46 donor Recipient: immunoadsorption + anti-C5 mab induction	9 hr
Gaca <i>et al</i> ¹²⁸	2002	Donors: transgenic hCD46 Recipients: anti-GP1b mab	3 hr
Cantu <i>et al</i> ⁹⁸	2006	Donors: transgenic hCD46 + clodronate liposomes Recipients: anti-Gal antibody depletion	24 hr
Cantu <i>et al</i> ⁴⁷	2007	Donors: vWF deficient + clodronate liposomes Recipients: anti-Gal antibody depletion	109 hr
Nguyen <i>et al</i> ²⁷	2007	Donors: GalTKO Recipients: C1Inh + BIA + HRB	4 hr *
Pierson <i>et al</i> , unpublished	2014	Donor: GalTKO.hCD46.hCD55.hEPCR.hTFPI.hCD47 + DDAVP + BIA Recipient: C1Inh + anti-GP1b mab + BIA + HRB	24 hr *

* Experiment with reported life-supporting function. CSA: cyclosporine, AZA: azathioprine, h: human, mab: monoclonal antibody, GP1b: glycoprotein 1b, vWF: von Willebrand factor, Gal: Galactose 1,3 α -galactose, C1Inh: C1 esterase inhibitor, BIA: 1-benzylimidazole (a thromboxane synthase inhibitor), HRB: histamine receptor blockade, DDAVP: desmopressin acetate.

CD55: decay activating factor, CD59: membrane attack complex inhibitor, CD46: membrane cofactor protein, GalTKO: α -1,3 galactosyltransferase knockout, EPCR: endothelial protein C receptor, TFPI: tissue factor pathway inhibitor, CD47: integrin associated protein.

Table 2

Xenogenic processes contributing to lung xenograft injury.

Mechanisms
Antibody mediated
Galactose 1,3 α -galactose (Gal)
N-glycolylneuraminic acid (NeuGc)
Other xenoreactive antigens
Innate immunity
Complement activation
Donor PIM activation
Recipient PMN sequestration & activation
Thromboregulatory dysfunction
Impaired activated protein C generation
Direct thrombin activation
Porcine vWF mediated platelet adhesion
Inflammation
Thromboxane generation
Histamine release
Cytokine synthesis

Gal: Galactose 1,3 α -galactose, NeuGc: N-glycolylneuraminic acid, PIM: pulmonary intravascular macrophage, PMN: polymorphonuclear neutrophil, vWF: von Willebrand factor

Table 3

Summary of porcine genetic modifications with proven benefit in lung xenotransplantation.

Pathway	Effect	Citation(s)
Hyperacute rejection		
GalTKO	Improves function & survival, reduces complement deposition	27,28,35
Complement control		
hCD46	w/ GalTKO, reduces complement, platelet & inflammation activation	29
hCD55	Improves function & survival, reduces complement deposition	75,107,108,129
hCD59	Improves function & survival, delays complement deposition	109
hCD55/hCD59	Improves function & survival, reduces complement & thrombosis	35,36,110
Thrombosis control		
hEPCR	w/ GalTKO.hCD46, improves lung function & survival	115
vWF deficient	w/ PIM depletion, improves lung survival	47
hCD39	w/ GalTKO.hCD55.hCD59, improves lung function	35

GalTKO: α -1,3 galactosyltransferase knockout, EPCR: endothelial protein C receptor, vWF: von Willebrand factor, PIM: pulmonary intravascular macrophage.

CD46: membrane cofactor protein, CD55: decay activating factor, CD59: membrane attack complex inhibitor, CD39: ectonucleoside triphosphate diphosphohydrolase 1.