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REVIEW

Creating superior lungs for transplantation with next-generation gene therapy during ex vivo lung perfusion

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Engineering donor organs to better tolerate the harmful non-immunological and immunological responses inherently related to solid organ transplantation would improve transplant outcomes. Our enhanced knowledge of ischemia-reperfusion injury, alloimmune responses and pathological fibroproliferation after organ transplantation, and the advanced toolkit available for gene therapies, have brought this goal closer to clinical reality. Ex vivo organ perfusion has evolved rapidly especially in the field of lung transplantation, where clinicians routinely use ex vivo lung perfusion (EVLP) to confirm the quality of marginal donor lungs before transplantation, enabling safe transplantation of organs originally considered unusable. EVLP would also be an attractive platform to deliver gene therapies, as treatments could be administered to an isolated organ before transplantation, thereby providing a window for sophisticated organ engineering while minimizing off-target effects to the recipient. Here, we review the status of lung transplant first-generation gene therapies that focus on inducing transgene expression in the target cells. We also highlight recent advances in next-generation gene therapies, that enable gene editing and epigenetic engineering, that could be used to permanently change the donor organ genome and to induce widespread transcriptional gene expression modulation in the donor lung. In a future vision, dedicated organ repair and engineering centers will use gene editing and epigenetic engineering, to not only increase the donor organ pool, but to create superior organs that will function better and longer in the recipient.

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Abbreviations: AAV, adeno-associated virus; Ad, adenoviral; CLAD, chronic lung allograft dysfunction; CRISPR, clustered regularly interspaced short palindromic repeats; Cas, CRISPR-associated protein; dCas, nuclease-inactivated Cas; EVLP, ex vivo lung perfusion; gRNA, guide RNA; IRI, ischemia-reperfusion injury; IL, interleukin; MHC, major histocompatibility complex; $MSC^{IL\text{-}10}$, mesenchymal stromal cells engineered for augmented IL-10 production; NOS, nitric oxide synthase; PGD, primary graft dysfunction; TGF-β1, transforming growth factor β1; TNF, tumor necrosis factor; VLP, virus-like particles

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Lung transplantation is often the only remaining option in treating patients with end-stage lung failure, and the number transplants reported to the International Society for Heart and Lung Transplantation is approaching 5,000 per year.[1] Although lung transplant results have improved over time, ischemia-reperfusion injury (IRI), primary graft dysfunction (PGD), acute and chronic rejection, and the side effects of immunosuppression remain important obstacles to long-term survival.[2–4] Engineering the donor organ before transplantation with gene therapy holds great promise, as the organ could be modified to be protected against transplant-related injury, immunological reactions and pathological fibroproliferation. This would also lower the burden of immunosuppressive drugs and their side effects such as infections, malignancies, and metabolic problems.

Rather than proceeding to lung transplantation immediately after organ procurement and static cold storage, the donor lung could be placed in an ex vivo lung perfusion (EVLP) system. During EVLP, the donor lung is ventilated and perfused in normothermia outside of the body, enabling precise diagnostic assessment of donor lung quality before transplantation.[5,6] EVLP has revolutionized lung transplantation as marginal lungs originally considered unusable can now be safely transplanted with excellent short- and long-term results.[7] EVLP would also be an ideal platform for donor lung gene therapies as they could be delivered into the isolated organ.[6,8]

Here, we review the status of lung transplant gene therapies and the potential benefits of using EVLP for donor lung gene therapy. We also outline how donor organ genetic engineering could be further leveraged, by using next-generation gene therapies that enable gene editing and epigenetic engineering (Table 1), to not only increase the donor organ pool, but also to create superior organs that will function better and longer in the recipient. Achieving this goal will require 1) detailed molecular-level understanding of the pathological processes involved in transplantation, 2) safe gene therapy approaches that efficiently target critical molecular pathways, and 3) improved EVLP systems that provide stable extended ex vivo support for the donor organ for sufficient time to accommodate the application of novel therapies. As each of these fields has progressed rapidly, we anticipate that future cycles of innovation in target-discovery, next-generation gene engineering, and ex vivo perfusion platforms will accelerate clinical translation of gene therapy in EVLP from bench to bedside (Figure 1).

First-generation gene therapies in lung transplantation

Although lung transplant results have improved over time, the median survival is limited to 6.7 years.[9] Clinical studies have identified three distinct, but interrelated problems: PGD, acute rejection and chronic lung allograft dysfunction (CLAD) that contribute substantially to the early and late morbidity and mortality of lung transplant patients.[2–4,10] With the help of multiple "omics" approaches, detailed pathological pathways involved in lung transplantation are being unraveled,[11–18] and will likely reveal novel molecular targets (Figure 2). Specific pathways have been targeted in lung transplant experiments using first-generation gene therapies.[19]

Gene delivery systems

The vectors that deliver the genetic cargo into the target cells are important for successful gene therapy: the therapeutic effector should be produced in the right cells, at the

Table 1 Next-Generation Gene Therapy in Organ Transplantation

Gene editors	
Designer nucleases (ZNF, TALEN, CRISPR-Cas)	Site-specific DNA DSB, and gene disruption by deletions or insertions (NHEJ) or gene correction by homologous recombination (HDR)
Base editors	Accurate single-nucleotide conversions without DSB
Prime editors	Any single-nucleotide conversion or small insertions or deletions without DSB. Longer edits may be possible with emerging advancements delivering pairs of prime editing gRNAs
Epigenic editors	Transcriptional upregulation or downregulation determined by gRNA
Gene editing vectors	
AAV	Efficient expression and low immunogenicity but low cargo capacity
Liposomal nanoparticles	Low inflammation, gene editors can be transferred as mRNA or DNA requiring translation and transcription, or gene editors packaged as proteins or ribonucleoproteins
VLP	Includes viral structural proteins but instead of viral genetic material, carries mRNA, proteins, or ribonucleoproteins, combines the main benefits of viral and non-viral vectors
Therapeutic possibilities in donor organ genetic engineering	
Gene editing	Permanent deletion or modification of donor organ genes to achieve improved immunocompatibility or graft protection
Epigenic engineering	Transcriptional regulation of endogenous genes for donor organ protection and immunomodulation

AAV, adeno-associated virus; DSB, double-strand break; gRNA, guide RNA; HDR, homology-directed repair; NHEJ, nonhomologous end-joining; TALEN, transcription activator-like effector nuclease; VLP, virus-like particles; ZNF, zinc finger nuclease.

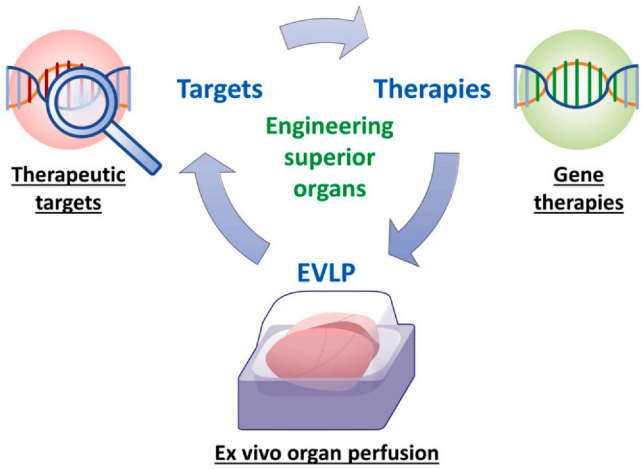

Figure 1 Innovations in target-discovery, gene therapies and ex vivo platforms in donor lung engineering. Detailed molecular-level understanding of the therapeutic targets, safe and efficient gene therapy tools and improved ex vivo lung perfusion (EVLP) systems will accelerate clinical translation of therapeutic donor lung engineering.

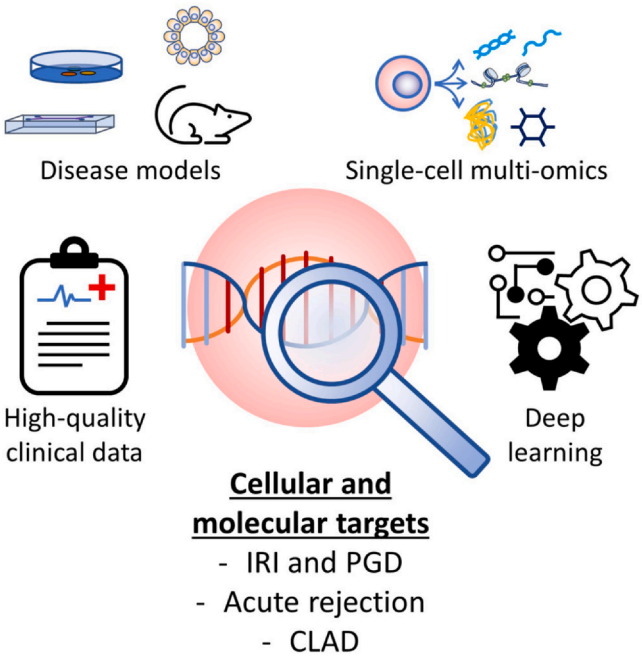

Figure 2 Cellular and molecular targets in transplantation. Advanced target discovery methods are needed to reveal and confirm novel molecular and cellular targets for ischemia-reperfusion injury (IRI), primary graft dysfunction (PGD), acute rejection and chronic lung allograft dysfunction (CLAD). This requires integration of high-quality clinical data, disease models such as small and large animal transplant models, cell culture, organoids, organs-on-a-chip, high-resolution multi-omics methods and bioinformatics.

right time, in the correct quantities, and without unwanted off-target effects or safety issues. Additionally, different gene therapy tools may be required to target distinct transplant phases (Figure 3).

Viral delivery systems

Viral vectors efficiently deliver genes to target cells and tissues. Three platforms are currently commonly used: adenoviruses, adeno-associated viruses (AAVs) and lentiviruses.[20] The modification of viral vectors usually includes removal of replication elements from the viral genome and insertion of the transgene and its regulatory elements. The structural and molecular properties of each vector are different, and have important implications for cell targets, transduction efficiency, transgene expression kinetics, host genome integration, immunogenicity, and the size of the genetic payload.[20–22]

Adenoviruses are double-stranded DNA viruses that efficiently transduce non-dividing and dividing cells, have good transgene carrying capacity (~36 kb), and result in a high and transient transgene expression. However, adenovirus vectors are immunogenic due to the capsid proteins and the remaining viral DNA, and pre-existent immunity is common.[20,22]

AAVs are single-stranded DNA human parvoviruses that are not known to cause any human disease. AAV vectors transduce dividing and non-dividing cells and generally result in sustained long-term transgene expression.[20] AAV immunogenicity is low, but the vectors have a limited packaging capacity (~5 kb). In addition, although the AAV genome mainly remains epigenomic in the target cell nucleus, genomic integration and potential for oncogenesis remains possible.[23]

Lentiviruses are retroviruses with single-stranded RNA that infect both non-dividing and dividing cells. Lentiviral vectors have a packaging capacity up to ~9 kb and enable long-term transgene expression. They have relatively low immunogenicity, but they do integrate into the host genome which predisposes to oncogenesis.[20,22]

Non-viral delivery systems

Therapeutic RNA and DNA can be also delivered into cells using non-viral vectors.[24–26] They are considered to circumvent some of the main concerns and limitations of the viral vectors such as pre-existing immunity, viral-induced immunogenicity, genome integration, payload size limitations and vector production difficulties. However, the main challenges for the non-viral platforms are low gene transfer efficiency and gene expression durability. Lipid nanoparticles can deliver siRNA, mRNA and DNA into cells, and have been instrumental in mRNA vaccines.[24]

Gene therapy for lung IRI

Restoration of circulation to the organ during the transplant operation leads to IRI[27,28] and results in PGD in approximately 30% of lung transplant recipients.[29] Mortality after severe PGD remains high[30] and treatment options are

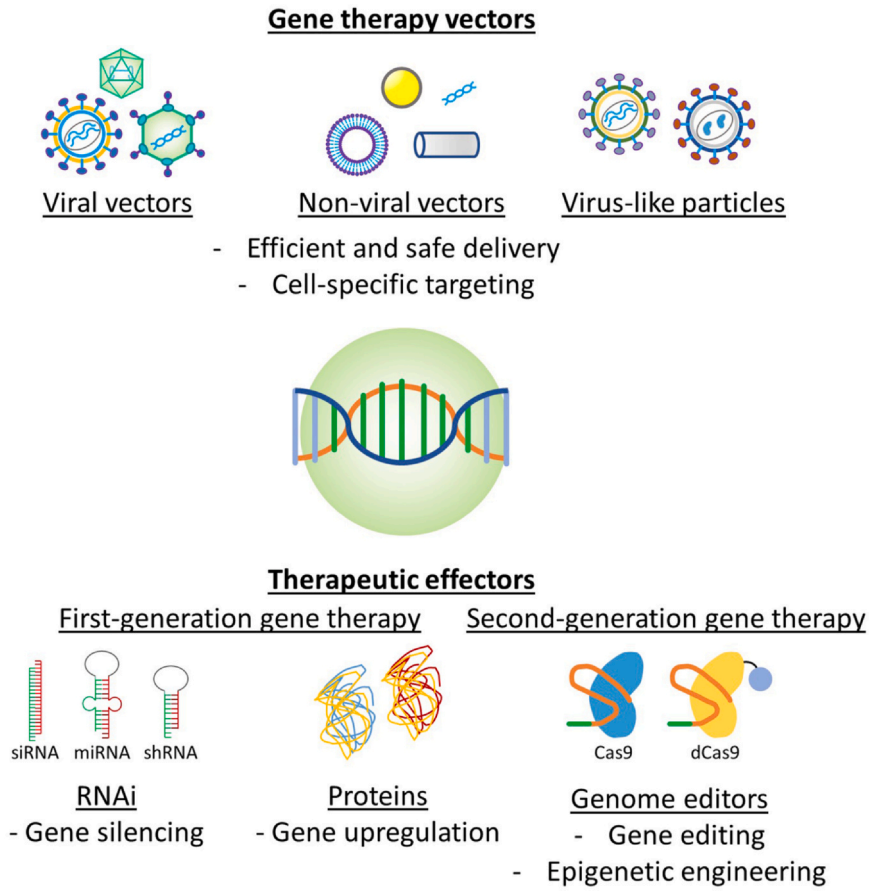

Figure 3 Gene therapy vectors and effectors. Commonly used gene therapy vectors include viral and non-viral delivery systems and virus-like particles. First-generation therapeutic effectors concentrate on gene silencing and upregulation. Next-generation gene therapy enables gene editing and epigenetic engineering.

limited.[31,32] The clinical importance is further emphasized by the negative long-term effects.[33]

Several of the pathological pathways important for lung IRI have been targeted in gene therapy experiments.[19,22] Inspired by the anti-inflammatory effects of interleukin-10 (IL-10),[34] adenoviral IL-10 (AdhIL-10) delivery to rat donor lungs showed protective effects.[35] Further studies revealed that AdhIL-10 decreased inflammation and cell death and improved lung function.[36–38] Additionally, low-dose gene transfer[39] and high-dose methylprednisolone treatment reduced vector-related inflammation,[40] and translational porcine lung transplant experiments have paved the way towards clinical applications.[41]

Upregulation of other genes such as heat shock protein 70,[42] endothelial nitric oxide synthetase (NOS),[43] transforming growth factor β1 (TGF-β1),[44,45] tumor necrosis factor (TNF) inhibitor,[46] soluble type I IL-1 receptor,[47] Bcl-2,[48] and IκB superrepressor[49] have been beneficial in lung IRI. Similarly, lung protection has been achieved with siRNA-mediated downregulation of caspase-3,[50] p38α[51] and Fas.[52]

Gene therapy for acute lung rejection

Acute rejection remains a significant problem after lung transplantation, and 27% of the recipients experience at least one acute rejection episode during the first year.[9] Furthermore, recurrent rejection episodes are problematic and increase the risk of later development of chronic rejection.[53,54]

Due to the interaction of innate and adaptive immunity, gene therapy strategies against acute rejection overlap with the approaches that are effective against lung IRI. For example, IL-10 and TGF-β have been extensively tested in both IRI and acute rejection.[55–62] In addition, TNFα receptor,[63] NOS,[64] NFκB pathway,[49,65] Bcl2 and FasL[48,66] have been targeted by lung transplant gene therapy.

These first-generation gene therapy experiments demonstrate that donor lung immunomodulation is possible by overexpressing anti-inflammatory mediators[55–62,64] or by inhibiting key regulatory molecules using ligand-sequestering soluble receptors,[63] oligodeoxynucleotide decoys or modified suppressors.[49,65] These studies have mainly used rodent lung transplant models with lipid or adenoviral vectors delivered through the airways or the vasculature but additional translational acute rejection studies are needed to establish efficacy and safety. It will be important to use large animal studies with prolonged follow-up to investigate for example whether immunomodulatory gene therapies predispose to adverse effects such as oncogenesis or infection, and what would be the best way to manage conventional immunosuppression in conjunction with immunomodulatory gene therapies.

Gene therapy for CLAD

CLAD leads to progressive and irreversible deterioration of the transplant function and remains the leading cause of death after lung transplantation.[67,68] Unfortunately, the current therapeutic options for CLAD are limited, and retransplantation is possible only for highly selected patients.[69] It is believed that chronic immunological and inflammatory injury to the lung is central in causing pathological distal airway fibroproliferation and CLAD.[68,70–72]

In experimental rat tracheal transplant models that simulate the airway obliteration related to CLAD, beneficial effects have been achieved using adenoviral delivery of anti-inflammatory IL-10[73] or soluble TGF-β type III receptor that functions as a ligand trap.[74] In addition, adenoviral overexpression of hypoxia-inducible factor-1α has attenuated pathological fibrotic remodeling,[75] and vascular endothelial growth factors[76,77] have been targeted to control the immune responses related to CLAD.

Stable transgene expression for up to 15 months in the lung has been achieved with lentiviral vectors in mice,[78] but the duration of the transgene expression is limited especially in full major histocompatibility complex (MHC)-mismatched transplant models.[79] Highest transgene expression levels are achieved with multiple lentiviral doses given before and after transplantation.[80] Importantly, lentiviral IL-10 therapy has decreased IL-17, airway obliteration and lung fibrosis.[78–81]

All these studies have used adenoviral or lentiviral vectors to induce ligands that have anti-inflammatory or protective properties,[73,75,78,79] or to upregulate soluble receptors leading to inhibition of the respective ligands.[74,77] As most have used rodent tracheal transplant CLAD models,[73–77] more translational studies need to be conducted to determine whether prevention of CLAD or even reversing established disease is possible in models that better resemble clinical CLAD. Of these goals, reversing the complex established fibroproliferative disease may be harder to achieve, and it is also uncertain, whether the presence of chronic injury in the lung itself affects the efficacy of gene therapy. On the other hand, the therapeutic options for patients with severe CLAD are very limited, and exploring novel treatments would be warranted.

EVLP and donor lung gene therapy

EVLP would be an ideal gene therapy platform,[19] compared to gene vector delivery in vivo during donor lung procurement or administration to the recipient, as gene vectors could be easily delivered either through the airways or vasculature, into an isolated organ without risks of off-target effects, potentially resulting in increased efficacy and decreased vector-related inflammation. In addition, if gene therapy is performed during EVLP, pre-implantation, it may be possible to target the early phase of transplantation as target cell transduction and transgene expression and translation take time.

Gene therapy during EVLP

AdhIL-10 therapy has been tested in large animal EVLP and lung transplant models and in human lungs rejected from clinical transplantation. In pig EVLP, adenoviral vector mediated transgene expression was detected at 8 hours.[82] When EVLP was followed by single lung transplantation, increased circulating IL-10 levels were observed within hours after transplantation, peaked at 5 days, and remained elevated for the 7-day study period. No systemic adverse effects were identified in the recipient animals,[83] and decreased vector-related inflammation has been noted in experiments using AdhIL-10 administration during EVLP.[84] Importantly, large animal studies show that AdhIL-10 therapy during EVLP reduced inflammation and attenuated allo-specific $CD4^+$ T cell responses.[83] Moreover, AdhIL-10 delivery to human lungs rejected from clinical transplantation improved lung function, inflammation and alveolar–blood barrier integrity.[82] Gene therapy has also been combined with cell therapy. Administration of mesenchymal stromal cells, pre-engineered with AdhIL-10 ($MSC^{IL\text{-}10}$), during EVLP resulted in rapid IL-10 upregulation in human lungs rejected from clinical transplantation.[85,86] Although this type of cell and gene therapy combination, using pre-engineered and cryopreserved cells, was feasible during clinical-grade EVLP, potential immunomodulatory effects of $MSC^{IL\text{-}10}$ therapy need to be explored in large animal transplant models. Also, as the poor metabolic conditions related to severe lung damage were found to influence $MSC^{IL\text{-}10}$ function,[85] the target lung environment needs to be taken into consideration when using genetically modified cell therapy.

Lentiviral vectors have been also delivered during pig EVLP for donor lung vascular endothelial immunoengineering. In this approach, shRNA-mediated targeting of beta-2 microglobulin and class II-transactivator transcripts silenced swine leukocyte antigen I and II, respectively,[87] potentially decreasing donor organ immunogenicity.[87,88]

Taking into consideration all the previous experimental data, anti-inflammatory and immunomodulatory IL-10 can be viewed as the proof-of-concept first-generation lung transplant gene therapy molecule with potential for clinical translation. Early, robust and transient upregulation of IL-10 in the donor lung could be achieved with adenoviral vectors, and vector-related inflammation would be best avoided by using EVLP as the gene therapy platform and by recipient methylprednisolone treatment. Similarly, long-term durable IL-10 expression in the donor lung could be achieved with lentiviral or AAV vectors. However, it is unclear whether gene therapy with IL-10 or with other similar anti-inflammatory genes has a therapeutic window, and how recipient immunosuppressive medication should be managed in conjunction with the gene therapy. Optimally, immunomodulation of the donor lung would enable reduction in the intensity of conventional immunosuppression. On the other hand, too excessive local immunosuppression could predispose the lung graft and the recipient to infections and oncogenesis.

Next-generation gene therapies in lung transplantation

First-generation gene therapies have focused on transgene delivery resulting in gene augmentation or silencing in the target cells. The emergence of *gene editing* technologies will enable next-generation gene therapies in lung transplantation (Table 1).[89–91]

Therapeutic possibilities with gene editing and epigenetic engineering

Transplantation of an allograft involves transfer of a significant amount of foreign genetic material from the donor to the recipient. Permanent modification of critical genes in the donor organ genome would significantly improve immunocompatibility and transplant results.[92] With new and improved gene editing tools, it may be possible to engineer human donor organs ex vivo prior to transplantation. This could include for example deletion of MHC genes in the donor cells,[87] and sophisticated gene editing tools could even enable precise modification of the MHC locus. Deleting or engineering donor blood type antigen genes may generate universal blood type organs for transplantation across the ABO blood type barrier.[93,94] Moreover, epigenome editing could regulate expression of multiple protective and immunomodulatory genes in the transplant.[92]

Gene editing tools

Gene editing became possible with designer nucleases that generate double-strand breaks at specific DNA locations. The zinc finger nucleases and transcription activator-like effector nucleases relied on protein-DNA interactions that required synthesis of a specific protein for each target site which is expensive and time-consuming. These problems were largely overcome by using nuclease systems that include Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and CRISPR-associated protein (Cas) which are naturally involved in the adaptive immunity of bacteria and archaea against bacteriophages.[90–92] When using the CRISPR-Cas9 system for gene editing, a small guide RNA (gRNA) directs the Cas nuclease to a precise DNA location, and genetic editing of a different target therefore requires only designing and synthesis of gRNA specific for that DNA sequence. Knock-out mutations can be achieved in non-dividing cells through nonhomologous end-joining that induces random insertions or deletions in the DNA cleavage site. In dividing cells, specific mutations and insertions can be achieved through homologous repair when a template for the repair is also provided.[90–92]

Recently, refined gene editors based on the CRISPR-Cas systems have been developed, and base editors and prime editors enable precise gene editing without the need for double-strand breaks in the DNA.[90,91] Base editors can be used to obtain accurate single-nucleotide conversions to correct disease-causing point mutations or to achieve single-nucleotide variants.[95] Development of prime editors has further expanded the toolbox available for gene editing, enabling installation of any single-nucleotide conversion, and permitting small insertions or deletions.[90,96,97] In addition to editing specific genes, the CRISPR-Cas system has been used for epigenome engineering. These applications use nuclease-inactivated Cas (dCas) proteins, and fusion of dCas with gene-regulatory proteins enables transcriptional upregulation or downregulation at the site determined by the gRNA.[89,98]

Vectors for gene editing

Gene editing agents can be delivered into cells in the form of DNA or mRNA that encode the editors, or directly as proteins or ribonucleoproteins.[90] Viral vectors and non-viral vectors have been utilized to transfer gene editing agents, and AAV vectors and liposomal nanoparticles are commonly used.[26,90,92] In addition, virus-like particles (VLP), that include viral structural proteins but do not contain viral genetic material, but instead can carry mRNA, proteins, or ribonucleoproteins, are increasingly studied. VLP vectors combine main benefits of viral and non-viral vectors as they efficiently achieve intracellular delivery but, unlike viral vectors, the gene editing agents are transiently transferred as mRNA or proteins, which limits problems such as off-target effects and genomic integration.[90]

Next-generation gene therapy in lung transplantation

With the advances in gene editors and vectors and ex vivo organ perfusion systems, it is plausible that precise genomic engineering of donor organs will become possible. In a proof-of-principle epigenome engineering study, a CRISPR-Cas technique was used to enhance expression of endogenous IL-10 in rat donor lungs. Adenoviral delivery of CRISPR-Cas9-based activators resulted in IL-10 upregulation in isogeneic and allogeneic recipients after transplantation and multiplex gene modulation was demonstrated in vitro.[99] It remains to be seen, whether similar epigenome engineering or gene editing can be achieved in human lungs.

Improving EVLP systems for next-generation gene therapies

Standard clinical EVLP provides stable support of the donor lung for 6 to 12 hours, but consumption of glucose and accumulation of metabolic by-products in the EVLP perfusate have been noted in metabolomics studies[100] and extending ex vivo perfusion eventually results in graft failure. It is likely that achieving next-generation gene therapy, and completing the intended genomic changes, will require extension of the time that the donor lung is kept on EVLP. However, the exact needed EVLP duration probably depends on vector and gene editor kinetics and dynamics, and on the therapeutic and molecular goal. For example, gene editing would be faster when the gene editors are

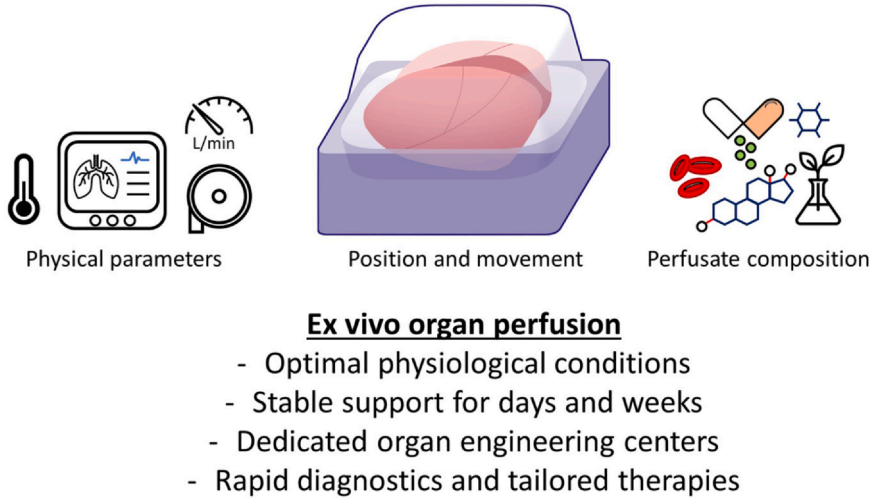

Figure 4 Improvements in ex vivo organ perfusion systems. Optimization of physical parameters, organ physiologic assessment, and perfusate composition may provide ideal physiological conditions to enable stable ex vivo support for days or even weeks.

transferred as proteins or ribonucleoproteins using liposomal nanoparticles or VPL vectors compared to for example viral vector mediated delivery of mRNA or DNA that encode the editors.[101] Similarly, it would take less time to achieve upregulation of immunomodulatory mediators through epigenomic engineering than to accomplish complete production, turnover and replacement of an existing cell surface receptor with a gene edited version. Although the gene editing phase with CRISPR-Cas systems may occur in minutes-hours rather than several hours,[102] it is likely that accomplishing vector delivery, editor assembly and nuclear translocation, gene editing and production of the engineered product[103] during EVLP will take at least 12 to 24 hours.

Optimally, the donor lung could be supported ex vivo for days or even weeks, enabling complex organ engineering before transplantation. Some evidence exists that this goal could be reached by keeping the organ in as physiological conditions as possible, as demonstrated by the ex vivo preservation of human livers for 1 week by integrating multiple core physiological functions to the perfusion system,[104] and by some studies with whole blood cross-circulation between human donor lungs and pigs as hosts.[105] Similarly, the physical and physiological parameters and perfusate composition have been modified to extend the time that the donor lung can be supported ex vivo (Figure 4).

Regarding temperature,[106–110] the current EVLP protocols all use normothermia. While this temperature is probably best for lung assessment, some studies indicate that subnormothermic EVLP reduces inflammation.[108,109] In addition, successful 3-day preservation of pig lungs was achieved by using a protocol that used cold static preservation at 10°C, which is considered to improve mitochondrial health, and intermittent EVLP cycles at normothermia.[107,110]

The impact of perfusate has also been investigated in several studies by modifying the perfusate composition, or by cytokine filters or continuous dialysis. Metabolic enhancement during EVLP with total parenteral nutrition has decreased inflammation and improved lung function.[111,112] Cytokine adsorption has reduced perfusate proinflammatory cytokine levels[113–116] and improved lung function and edema during EVLP and after transplantation.[113–115] Also, incorporation of continuous dialysis into the EVLP circuit has been effective in maintaining normal metabolic levels[117–119] but it remains to be determined whether it also affects lung function or prolongs EVLP duration.[120]

In addition to these aspects, also different lung positions, ventilation strategies and perfusate flow rates have been investigated. Placing the donor lungs in prone position instead of the typical supine position results in more even distribution of lung edema and in improved lung function.[107,110] Regarding alternative ventilation strategies, airway pressure release ventilation[121] and flow-controlled ventilation[122] have improved lung oxygenation and compliance compared to volume-controlled ventilation. In addition, negative-pressure ventilation that resembles normal breathing, reduced lung inflammation, edema and injury,[123] and good post-transplant results were achieved.[124] In one study, further reduction of EVLP perfusion flow from 40% to 20% of cardiac output was investigated in pig lungs, and it improved lung function, reduced edema, and attenuated inflammation after transplantation.[125]

In conclusion, it is likely that longer EVLP durations can be achieved by optimizing different physical and physiological parameters and the perfusate composition. However, integration of several core upgrades to the current EVLP systems may be needed to enable stable ex vivo duration needed for next-generation gene therapy.

Current limitations

Although progress has been made in several key areas, important questions must be addressed before it is possible to proceed to clinical donor organ gene therapy trials. As the current studies have been predominantly done in

preclinical animal models, the safety and efficacy of potential gene therapy applications need to be thoroughly investigated using human cells and organs, for example by using organoids, or by treating human lungs during EVLP. However, as these methods do not recapitulate the complex in vivo environment and donor-recipient interactions, large animal transplant studies with extended follow-up[126] are required to address potential concerns of oncogenesis, inflammation and infection.[21,127] In addition, proof-of-concept human transplant studies, similar to what have been done in xenotransplantation,[128] may be needed. Also, before initiating larger clinical trials, regional gene therapy restricted to a specific area of the donor lung could be considered to maximize safety, as the affected region could be resected if unexpected adverse effects are encountered.

Summary

Recent advances in multiple "omics" methods, gene therapy and EVLP platforms have brought lung transplant gene therapy closer to clinical reality. Further innovations in next-generation gene therapy tools will enable exciting possibilities for sophisticated ex vivo genetic engineering of donor organs, and it may become possible to create superior lung transplants that will function better and longer in the recipient.

Conflict of interest

A.I.N. declares no conflict of interest. S.K. serves as Chief Medical Officer of Traferox Technologies; S.K. receives personal fees from Lung Bioengineering and Traferox Technologies. S.K. is an inventor of IP licensed to Traferox Technologies. M.L. serves as a consultant to Traferox Technologies. The authors fully adhere to policies at University Health Network that ensure academic integrity and management of potential conflicts of interest.

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