Lung Progenitor and Stem Cell Transplantation as a Potential Regenerative Therapy for Lung Diseases

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Abbreviations

ATI, alveolar type I

ATII, alveolar type II

BCs, airway basal cells

BM, basement membrane

COPD, chronic obstructive pulmonary disease

ECM, extracellular matrix

EVLP, ex vivo lung perfusion

ILD, interstitial lung disease

IL-1β, Interleukin 1β

TNF-α, Tumor Necrosis Factor α

IPF, idiopathic pulmonary fibrosis

NKX2.1, NK2 Homeobox 1

NSG, NOD *scid* gamma

iPSCs, induced pluripotent stem cells

FGF, Fibroblast Growth Factor

CRISPR, Clustered Regularly Interspaced Short Palindromic Repeats

scRNA, single-cell RNA

DASCs, distal airway stem cells

Abstract

Chronic lung diseases are debilitating illnesses ranking among the top causes of death globally. Currently, clinically available therapeutic options capable of curing chronic lung diseases are limited to lung transplantation, which is hindered by donor organ shortage. This highlights the urgent need for alternative strategies to repair damaged lung tissues. Stem cell transplantation has emerged as a promising avenue for regenerative treatment of the lung, which involves delivery of healthy lung epithelial progenitor cells that subsequently engraft in the injured tissue and further differentiate to reconstitute the functional respiratory epithelium. These transplanted progenitor cells possess the remarkable ability to self-renew, thereby offering the potential for sustained long-term treatment effects. Notably, the transplantation of basal cells, the airway stem cells, holds the promise for rehabilitating airway injuries resulting from environmental factors or genetic conditions such as cystic fibrosis. Similarly, for diseases affecting the alveoli, alveolar type II cells have garnered interest as a viable alveolar stem cell source for restoring the lung parenchyma from genetic or environmentally induced dysfunctions. Expanding upon these advancements, the use of induced pluripotent stem cells to derive lung progenitor cells for transplantation offers advantages such as scalability and patient specificity. In this review, we comprehensively explore the progress made in lung stem cell transplantation, providing insights into the current state of the field and its future prospects.

Introduction

Respiratory diseases continue to rank among the leading contributors of morbidity and mortality on a global scale[1]. In the United States alone, over 34 million individuals are burdened with chronic lung conditions such as asthma, chronic obstructive pulmonary diseases (COPD), and interstitial lung disease (ILD)[2]. Notably, ILD, a class of pulmonary conditions characterized by progressive lung scarring, is on the rise, with idiopathic pulmonary fibrosis (IPF) at the forefront accounting for 37% of ILD diagnosis[3]. However, the spectrum of respiratory afflictions does not end here, and pulmonary malfunctions can also result from genetic mutations, as is the case in cystic fibrosis and primary ciliary dyskinesia[4,5]. Currently, no cures exist for any of the aforementioned diseases, emphasizing the critical need for innovative solutions capable of offering curative intervention. These chronic respiratory diseases carry significant fatal implications due to their potential to obstruct airflow, hinder mucociliary clearance, and destroy the intricate lung parenchyma architecture, ultimately compromising the lung's vital function to facilitate gas exchange[6,7], leading to hypoxia and respiratory acidosis[8], and threatening the overall homeostasis of the body[9,10].

The current treatment landscape for chronic lung diseases is generally limited to slowing disease progression as these conditions often cannot be cured, reversed, or halted[11,12]. Lung transplantation has traditionally served as the primary recourse for potential resolution of end-stage lung diseases[11,12]. However, numerous challenges, including shortage of eligible grafts[13], primary graft dysfunction following transplantation[14], and side effects of lifetime immunosuppression[15], restrict its feasibility for many patients in need[16,17]. Furthermore, while transplantation can potentially extend the life expectancy of the impacted patients, the stark reality is that only 50% of recipients survive beyond five years following transplantation. These challenges associated with donor organ transplantation have sparked strong motivations in the regenerative medicine community to develop

new strategies and technologies to address this urgent clinical demand for new curative solutions[18,19].

Considering a hallmark of chronic lung diseases is the compromised ability of the endogenous stem cells to regenerate healthy lung tissues, stem cell transplantation, a cell-based therapy involving the replacement of defective stem cells with their functional counterparts, has garnered interest as a promising therapeutic option. This approach aims to provide the diseased lungs with the necessary functional epithelial cells to restore physiologic tissue integrity and critical functions when they are compromised due to chronic epithelial injuries. Consequently, not only would the patient's respiratory symptoms be significantly ameliorated, but the very root causes of the disease would be addressed. In this review, we will elucidate the mechanisms of repair facilitated by lung epithelial stem cell transplantation, provide an overview of current research efforts aimed at assessing its promising potential, and discuss prospects and challenges for clinical translation.

Epithelial Stem Cells in Chronic Lung Diseases

To gain insight into the reparative mechanisms of lung stem cell transplantation, it is imperative to understand how the native repair takes place during routine lung injuries and discern its dysregulation in chronic pathogenesis. The lungs are comprised of two primary compartments: the proximal airways and distal alveoli[20]. The airways serve as conduits for airflow, where effective mucociliary clearance plays a crucial role in maintaining a clear and pathogen-free luminal environment to facilitate the free passage of air[20]. On the other hand, the alveoli, often referred to as the respiratory units of the lungs, are responsible for facilitating efficient gas exchange through their vast and extremely thin blood-air interface[20]. Together, these intricate structures ensure the proper functioning of the respiratory system. Both the airways and alveoli are covered by a vital lining known as the epithelium which serves as a crucial barrier protecting the lungs from airborne environmental

insults[21]. The epithelium of the airways and alveoli each house resident stem cells, known as airway basal cells (BCs) and alveolar type II (ATII) cells, respectively, which are essential for effective epithelial regeneration and injury repair[22]. These lung stem cells have the unique ability to self-renew and give rise to a variety of distinct, mature epithelial cells that perform specialized functions throughout the lungs[22].

When the epithelial lining routinely encounters external insults such as inhaled pollutants, toxins, or pathogens, a natural repair mechanism is activated[23,24]. This mechanism entails an acute inflammatory response characterized by the release cytokines such as Interleukin 1β (IL-1β), IL-6, IL-11, and Tumor Necrosis Factor α (TNF-α)[25]; immune cell recruitment of macrophages, neutrophils and T lymphocytes; and the migration and spreading of adjacent epithelial stem cells to the affected areas[23-26]. These activated stem cells, BCs and ATII cells, will then undergo differentiation into specialized airway (ciliated, secretory, mucosal[27]) cells and alveolar (ATI) cells[28], respectively, replenishing depleted populations with minimal disruption to physiological processes[23,24].

In the case of persistent lung injuries as observed in chronic pulmonary diseases such as ILD and COPD, the repair process takes on a pathological character[29-31]. Here, the repetitive injuries not only deplete the resident population of mature epithelial cells and stem cells, but also render the remaining stem cells dysfunctional in the widespread diseased tissue environment. This dysfunctionality is primarily attributed to factors such as chronic unresolved inflammation, persistent oxidative stress, and abnormal paracrine signaling[22,24,32]. Consequently, the pathogenic repair process tends to elicit a pro-fibrotic response rather than physiologic tissue regeneration, further restricting the reparative capacity of the lung epithelium and giving rise to the pathogenesis seen in many chronic pulmonary diseases[23,26,30,32-34]. Transplantation of exogenous stem cells holds promise for rectifying the chronic injury mechanism by providing a new population of fully functional epithelial stem cells to the diseased lungs. These cells have the capacity to re-initiate physiologic

repair pathways and promote appropriate tissue regeneration while simultaneously suppressing inflammation.

Lung Stem Cell Sources for Transplantation

While the promise of using lung stem cell transplantation to address chronic lung diseases is encouraging, its practical application is a multifaceted process that requires careful considerations. One critical aspect of this procedure is the identification of a suitable cell source (Fig. 1).

While autologous transplantation of a patient's own stem cells is a theoretical option (Fig. 1A), there are significant limitations with this approach. In many instances, the patient's endogenous stem cells may be rendered dysfunctional due to the underlying pathology[35]. Moreover, in the case of ATII cell transplantation, the acquisition of primary ATII cells requires invasive procedures as these cells reside deep in distal regions of the lungs. Additionally, the yield of primary stem cells is limited both in terms of the total number of cells that can be isolated and their subsequent expansion potential in vitro, making this a challenging choice for cell source[36-39].

Another potential cell source is allogenic stem cells isolated from lungs of deceased donors (Fig. 1A). While this generally means improved availability of larger tissue quantities for cell isolation and possibility of obtaining disease-free cells, the issue of limited in vitro expansion potential of the isolated cells remains an obstacle. Furthermore, the aspect of immunocompatibility and the likely need of lifelong immunosuppressive therapy adds another layer of complexity[40].

Given these inherent challenges with acquiring and expanding primary lung stem cells, there has been a growing interest in utilizing induced pluripotent stem cells (iPSCs) as an alternative cell source, opening new avenues for the development of cell-based therapies for epithelial repair in chronic lung diseases (Fig. 1B). By harnessing their pluripotent nature and ability to give rise to BCs

and ATII cells through directed differentiation, iPSCs offer the potential of overcoming the limitations associated with primary stem cells and provide an indefinite supply of patient-specific cells[41].

The initial steps for deriving lung epithelial cells from iPSCs follow a differentiation pathway that mimics embryonic lung development. This first involves the sequential induction of definitive endoderm and then anterior foregut endoderm, which is then ventralized to give rise to lung epithelial progenitors that are marked by the expression of the master transcriptional factor NKX2.1[42,43]. These lung progenitors have the potential to be further differentiated either towards a proximal BC lineage through a coordinated interplay of the WNT signaling pathway, Fibroblast Growth Factor (FGF)10, and FGF2[44,45]; or towards a distal ATII cell fate, with CHIR99021 (WNT agonist), Keratinocyte Growth Factor, cyclic adenosine monophosphate, and 3-Isobutyl-1-methylxanthine serving as effective inducers[46-48].

While iPSC-derived lung epithelial cells hold substantial promise as a cell source for transplantation due to their accessibility and patient specificity, it is crucial to note the existing limitations associated with their use. A significant challenge lies in the general immaturity of iPSC-derived cells with transcriptional characteristics that are similar but remain distinct from their endogenous counterparts[49-51]. This discrepancy requires careful evaluation to what extent the iPSC-derived cells can differentiate or facilitate lung injury repair as compared to the endogenous cells[49-51]. Additionally, there remain concerns regarding the risk of tumorigenicity associated with the use of iPSC-derived cells due to the possible presence of any undifferentiated iPSCs within the cell population intended for transplantation[49-51].

In the context of treating genetic pulmonary diseases (such as cystic fibrosis), when considering autologous lung cells (primary or iPSC-derived) for transplantation therapy, genetic modification is necessary to rectify the underlying pathogenic mutations (Fig. 1A,B). This can be achieved using precision genome editing tools such as zinc finger nucleases or Clustered Regularly

Interspaced Short Palindromic Repeats (CRISPR)[52]. Research in this emerging field has demonstrated that in applications for cystic fibrosis, even a modest genetic correction of 30% of BCs can effectively restore the salt-water balance on the diseased airway epithelial surface[53]. While this finding highlights an encouraging prospect for clinical translation that full cellular replacement may not be required, its applicability to other types of genetic diseases impacting the respiratory systems needs to be further investigated on a case-by-case basis. A critical consideration with genetic modification pertains to the risk of genetic instability[50,54]. Manipulation of the genome can have unforeseen consequences on the global genetic profile, potentially inducing unintended variability in gene expression and DNA stability harboring implications that are still not entirely understood today[49-51]. Additional investigations are necessary to assess and mitigate potential off-target effects and the risk of tumorigenesis to ensure the safety and efficacy of gene correction approaches being used[55].

Current Approaches to Lung Stem Cell Transplantation

For lung stem cell transplantation to achieve meaningful therapeutic effects, several essential requirements must be fulfilled. First, the transplanted cells should engraft effectively into the target region of interest, either in the airways or alveoli, and maintain long-term viability. Second, these transplanted cells should possess the ability to further differentiate into mature cell types such ciliated and secretory cells in the airways to reconstitute the functional epithelium in a physiologically relevant manner. Lastly, these cells are preferred to have the self-renewal capability that is necessary to achieve long-lasting therapeutic effects. The upcoming section will shed light on recent studies that have made significant progresses towards addressing the challenges on the path to realizing these ambitious objectives, with a particular focus on the choice of animal models, preconditioning

techniques to enable foreign cell engraftment, and strategies to assess lineage contribution and outcome of transplanted cells.

Animal Models

Prior to advancing to clinical translation of lung stem cell transplantation to human studies, it is imperative to validate the effectiveness of the transplantation strategies in pre-clinical animal models. The selection of suitable animal models capable of replicating clinically relevant conditions for human translation remains a dynamic and ongoing process.

At present, rodents are most frequently used for evaluating lung stem cell transplantation efficacy across a wide spectrum of cell sources, preconditioning settings, and pathophysiological backgrounds. Table 1 provides an overview of the different immunocompatibility combinations of cell sources and recipients.

In syngeneic transplantation, the animals for lung stem cell sourcing and the recipient animals have nearly identical genetic makeup. By using syngeneic species, the risk of an adverse immune response is minimized, eliminating complications associated with rejection. This allows for investigation of the intrinsic engraftment and therapeutic efficacy of the transplanted cells without the interference of immune-related factors. Xenogeneic transplantation involves the use of human-derived lung stem cells transplanted into recipient animals. For this purpose, the NOD *scid* gamma (NSG) mice are commonly used as recipients, which have severe immunodeficiency due to the lack of T cells, B cells, and natural killer cells as well as defective innate immunity from deficient cytokine signaling[61,62]. Xenogeneic transplantation allows for studying the functional capabilities of human lung stem cells in a living system, facilitating the evaluation of their potentials for engraftment, differentiation, self-renewal, and delivering therapeutic benefits.

Preconditioning Techniques

The basement membrane (BM) is composed of thin layers of extracellular matrix (ECM) residing right underneath the luminal epithelium, and serves as a vital interface between epithelial cells and their surrounding tissue environment. The BM provides critical structural and biomechanical cues to support the proper homeostasis and functions of luminal epithelium in both the airways and alveoli[63,64]. Under physiological settings, the BM is fully covered by physical barriers of the lungs including the epithelial lining and mucosal layer, which routinely guards against inhaled particulates[65,66]. Accordingly, a prominent obstacle for lung stem cell transplantation involves enabling the transplanted cells to access the BM for effective engraftment and incorporation into host lung tissues (Fig 2). Overcoming this challenge requires deliberate alteration of the respiratory epithelial and mucosal barrier to partially expose the underlying BM to allow it to be effectively accessed by exogenous stem cells, a process referred to as “preconditioning”.

In recent studies, the selection of specific preconditioning agents has been largely motivated by two key objectives: first is to apply chemical agents to acutely compromise the protective barrier and expose the BM, thereby enabling engraftment of transplanted cells; and second is to simulate a pathological environment that inherently compromises the natural epithelial barrier within the lungs. This second strategy has facilitated studies of lung stem cell engraftment within the context of respiratory conditions such as pulmonary fibrosis, infection, or COPD, delivering critical information regarding the translational potential for clinical applications. Table 2 provides an overview on preconditioning techniques used in recent investigations, detailing their mechanisms of action and applications for lung stem cell transplantation.

Chemical agents, such as polidocanol and naphthalene, are common preconditioning agents utilized to acutely disrupt the epithelial barrier in order to expose the BM. Polidocanol is recognized for its capacity to induce concentration-dependent cellular injury and death in the airways[67]. Along

the same vein, naphthalene induces epithelial injury in the airways when transformed into its toxic counterpart upon metabolic activation by the cytochrome P450 enzyme[68]. The partial airway epithelial ablation resulting from polidocanol and naphthalene treatment is instrumental for creating openings for transplanted cells to effectively access and engraft onto the airway BM.

Bleomycin is another preconditioning agent recognized for its ability to impair the lung epithelial barrier following acute exposure and to induce fibrosis phenotypes when administered at high doses. This is achieved through the induction of toxicity and cell death, which not only damages the epithelial barrier but is capable of driving an influx of inflammatory and immune cells to the injured region ultimately leading to fibrosis over chronic pathogenesis[56,69]. Nichane et al. 2017 demonstrates the utility of bleomycin to facilitate engraftment when employed at a low dose (1.5 U/Kg). Here, the transplanted cells were introduced three days post-injury, emphasizing the acute role of bleomycin treatment in enabling epithelial engraftment[69]. In contrast, Alvarez-Palomo et al. (2020) utilized a higher dose of bleomycin (2.5 U/kg) to induce chronic lung fibrosis, allowing investigation of not only the engraftment of transplanted cells but also their potential to address the induced pathology. In this case, cells were transplanted fifteen days post-injury, revealing the capacity of the transplanted cells to expedite the resolution of the fibrotic lung conditions[59].

In the context of simulating diseased conditions, researchers have employed elastase and lipopolysaccharide to model COPD. Elastin, a critical ECM protein highly enriched in the alveoli, plays a crucial role in maintaining alveolar structural integrity as it facilitates elastic recoil in respiratory cycles[81]. Elastase administered into the airspace enzymatically digests the alveolar elastin, leads to destruction of the intricate parenchymal architecture, and promotes an emphysema-like pathology. In parallel, lipopolysaccharide simulates the pulmonary response to bacterial infection with elevated paracellular permeability and inflammation[73]. Together, the use of these agents can effectively precondition the respiratory epithelium for foreign cell engraftment[58], replicate several key

pathological hallmarks of COPD, and provide valuable information towards clinical applications of stem cell transplantation to address chronic lung conditions.

Additionally, bacterial and viral infection to the lung can be mimicked with the introduction of Pseudomonas aeruginosa bacteria or influenza virus, respectively. The Pseudomonas aeruginosa infection replicates the pathology of pneumonia and causes damages to resident lung cells through cell lysis and necrotic death, thereby creating gaps in the epithelial lining of the alveoli [75,76]. Comparably, the influenza virus causes cell death through viral invasion and replication leading to the deterioration of the epithelial lining throughout the lungs and induction of inflammation[79].

Studies that implement preconditioning conditions for acute impairment of the epithelial barrier are primarily focusing on evaluating the ability of the transplanted lung stem cells for successful engraftment and restoration of the damaged epithelium (Fig. 2). When applied to pathologically relevant models, disease phenotype resolution will usually be studied in addition to assessment of transplanted cell engraftment and epithelial reconstitution. These studies help to bring insights regarding whether and to what extent restoring the resident epithelial stem cell pool can lead to resolution of the underlying disease pathologies.

Lineage Tracing and Outcome Assessment

Upon delivery of exogenous lung stem cells, it is necessary to track them together with their progenies to assess their long-term viability, determine their self-renewal and differentiation into specialized mature cell types, and examine their abilities to deliver desired therapeutic benefits. Lineage tracing generally involves the use of genetically integrated reporter genes, such as those expressing fluorescent or bioluminescent proteins, into the exogenous cells prior to transplantation as lineage tracers to distinguish transplanted cells from the endogenous counterparts[82]. Another

approach for lineage tracing tracks the Y chromosome in transplanted cells derived from male donors in female recipients using fluorescent in situ hybridization techniques[56].

To assess the phenotype maintenance and transition (i.e., differentiation) of transplanted cells, multiplexed immunofluorescence detection of lineage-specific markers in conjunction with lineage tracers is commonly employed through flow cytometry and histological analysis. In particular, for the airways, the BC identity is marked by the expression of Cytokeratin 5 and P63, and the emergence of Club cells (Club Cell Secretory Protein$^+$), goblet cells (Mucin 5AC$^+$), and multiciliated (Acetylated-α-Tubulin$^+$, FOXJ1$^+$) indicates further differentiation of the engrafted BCs. Similarly, ATII cells as the alveolar stem cells are marked by expression of Surfactant Protein C and NK2 Homeobox 1 (NKX2.1), and their further specification into ATI cells is signified by the expression of Aquaporin 5 and Advanced Glycosylation End-Product Specific Receptor. In recent years, single-cell RNA (scRNA) sequencing has emerged as a powerful analytical approach for more comprehensive evaluation of the lineage contribution of transplanted cells[83,84]. ScRNA sequencing enables transcriptomic analysis at single-cell resolution within a complex cell mixture, such as cells from the entire lungs following exogenous stem cell transplantation, and thereby delineates lineage relationships and trajectories of cellular differentiation[85,86]. By analyzing the whole-transcriptome in an unbiased manner, scRNA sequencing facilitates detection of transitional cell states and reveals key information regarding the overall physiological status of each transplanted cell regarding survival, proliferation, migration, and ECM interaction[85,86].

Assessment of the therapeutic benefits of lung stem cell transplantation depends on the specific anatomical regions of interest (airways and/or alveoli) and underlying pathophysiological conditions of the recipient lungs. Histological analysis of lung tissues at different time points following cell transplantation provides direct visualization of changes in the tissue architecture and structural integrity[51,57-59,71]. Connected to evaluating physiological functions, analysis of the proximal airways

generally focuses on mucociliary clearance efficiency, inflammation, and antimicrobial properties[51,60,69]. In the context of alveolar functionality, measurement of gas-exchange efficiency and lung compliance are commonly employed[56-59].

In summary, Table 3 provides a comprehensive overview of recent research in lung stem cell transplantation, highlighting the combinations of animal models, cell sources, preconditioning techniques, lineage tracing methods, and functional outcomes.

Future Directions and Translation Potential to Human Studies

As introduced above, transplantation of lung epithelial progenitors holds significant potential for addressing the underlying pathology of various chronic respiratory diseases that currently lack curative treatment options. However, the translation to human trials is currently constrained by several factors. Firstly, the scarcity of clinically relevant animal models that accurately mirror the scale and characteristics of human respiratory diseases limits our ability to gain in-depth insights into the therapeutic efficacy of the transplanted cells to address human pathological conditions[55,88,89]. Most current animal models of lung diseases represent oversimplified forms of human diseases, particularly for chronic respiratory conditions that can take decades to develop in human patients, making the extrapolation of findings from animal models to clinical settings challenging[89].

Secondly, for transplanted lung epithelial cells to contribute to lung repair and regeneration, orthotopic cell transplantation is generally required, which remains a substantial hurdle. As successful cell engraftment demands direct contact with the epithelial BM, this necessitates at least temporal removal of the luminal epithelial barrier that generally involves the use of epithelium-damaging chemicals[90]. However, for patients already grappling with chronic lung conditions, exacerbating damage is not ideal in human translations. Consequently, there is a pressing need to explore more efficacious methods for facilitating repair that do not further deteriorate the lungs or

compromise the quality of life for these patients. In contrast, in organ systems that do not require orthotopic procedures, such as endocrine organs like the pancreatic islets, therapeutic benefits can be achieved through cell transplantation in a heterotopic manner[91].

To bridge the gap to human trials, a key consideration is the cellular yield necessary to achieve a therapeutic effect in humans. Current research indicates an average use of 3 x 10^6 epithelial cells for the lungs of a mouse (Table 3). Extending this insight to human proportions with consideration of the larger volume of human lungs (6,000 mL compared to 1 mL in mice[88]) we can project that roughly 6,000 times the quantity of cells employed in mice might be essential to achieve comparable therapeutic outcomes in humans. This estimation serves as an initial benchmark as we progress toward human trials. Related to obtaining sufficient cells, it is essential to ensure the isolated or derived lung stem cells can maintain their desired phenotypes over the extensive in vitro expansion. Most current approaches for lung stem cell expansion commonly involve embedding culture within Matrigel, a murine tumor derived ECM material[36,51,92] , posing limitations regarding batch-to-batch consistency, cost, and scalability. Accordingly, future efforts are needed to further understand the signaling pathways and substrate supports necessary for in vitro maintenance of the proliferation and differentiation potential of lung stem cells.

Despite the complexities impeding the progression of lung stem cell transplantation into human application, this technology is versatile in its implementations and can offer therapeutic benefits in conjunction with ex vivo lung perfusion (EVLP). EVLP is a technique for isolated perfusion and maintenance of donor lungs outside the body under the normothermic condition, providing an opportunity to evaluate, optimize, and potentially repair the lung graft prior to transplantation[93]. Thus, EVLP represents a simpler and better controlled setting for the introduction of lung epithelial stem cell therapy compared to direct in vivo cell delivery and offers a promising avenue for enhancing donor lung graft quality by leveraging the regenerative and reparative potential of lung stem cells.

Similar concept has been explored for the delivery of mesenchymal stem cells during the ex vivo lung and kidney perfusion, where promising protective effect against ischemic injury was observed[94,95].

In conclusion, lung stem cell transplantation presents a substantial potential as a prospective curative solution for chronic lung diseases, addressing a significant cause of morbidity and mortality globally. While navigating the complexities of translation into clinical applications remains a challenge, the existing body of knowledge and research is encouraging. It is evident that further dedicated efforts and research endeavors are required to transform this potential into a viable reality, offering new hope for the effective treatment of chronic lung diseases on a broader scale.

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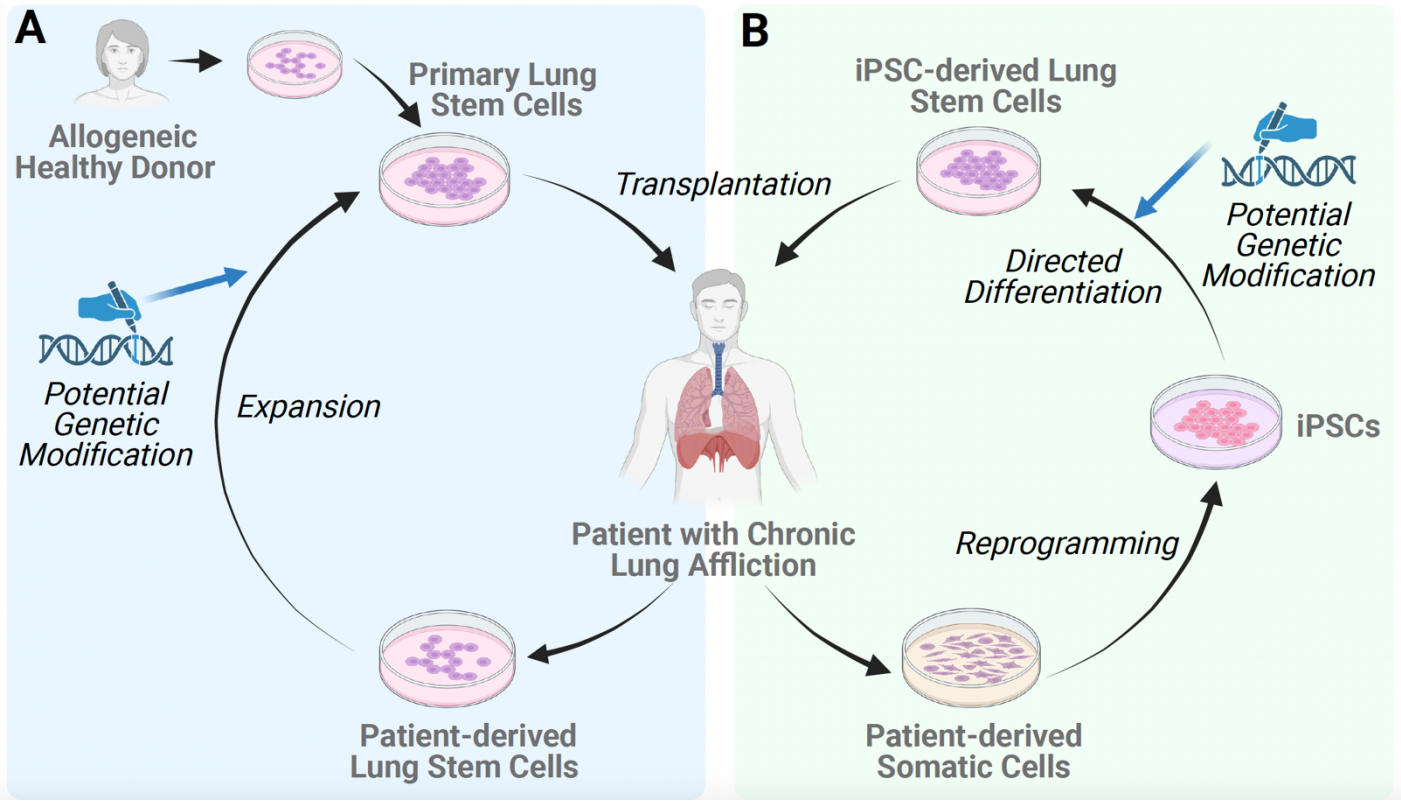

Figure 1. Potential pathways for lung stem cell transplantation for treating chronic lung diseases. **A)** Primary lung stem cells can be isolated from the patient and undergo genetic modification to correct disease-causing mutations, or isolated from a healthy donor. These primary cells can be expanded to a therapeutic yield and transplanted into the patient's lungs. **B)** Somatic cells can be isolated from patients with chronic lung diseases and undergo iPSC reprogramming. At this stage, these iPSCs can receive genetic modification to rectify the disease-causing mutations in genetic disease applications. In their pluripotent state, iPSCs can undergo directed differentiation towards lung stem cells of the airways (BCs) or alveoli (ATII cells), and then be transplanted back into the patient's lungs. Figure created with Biorender.com.

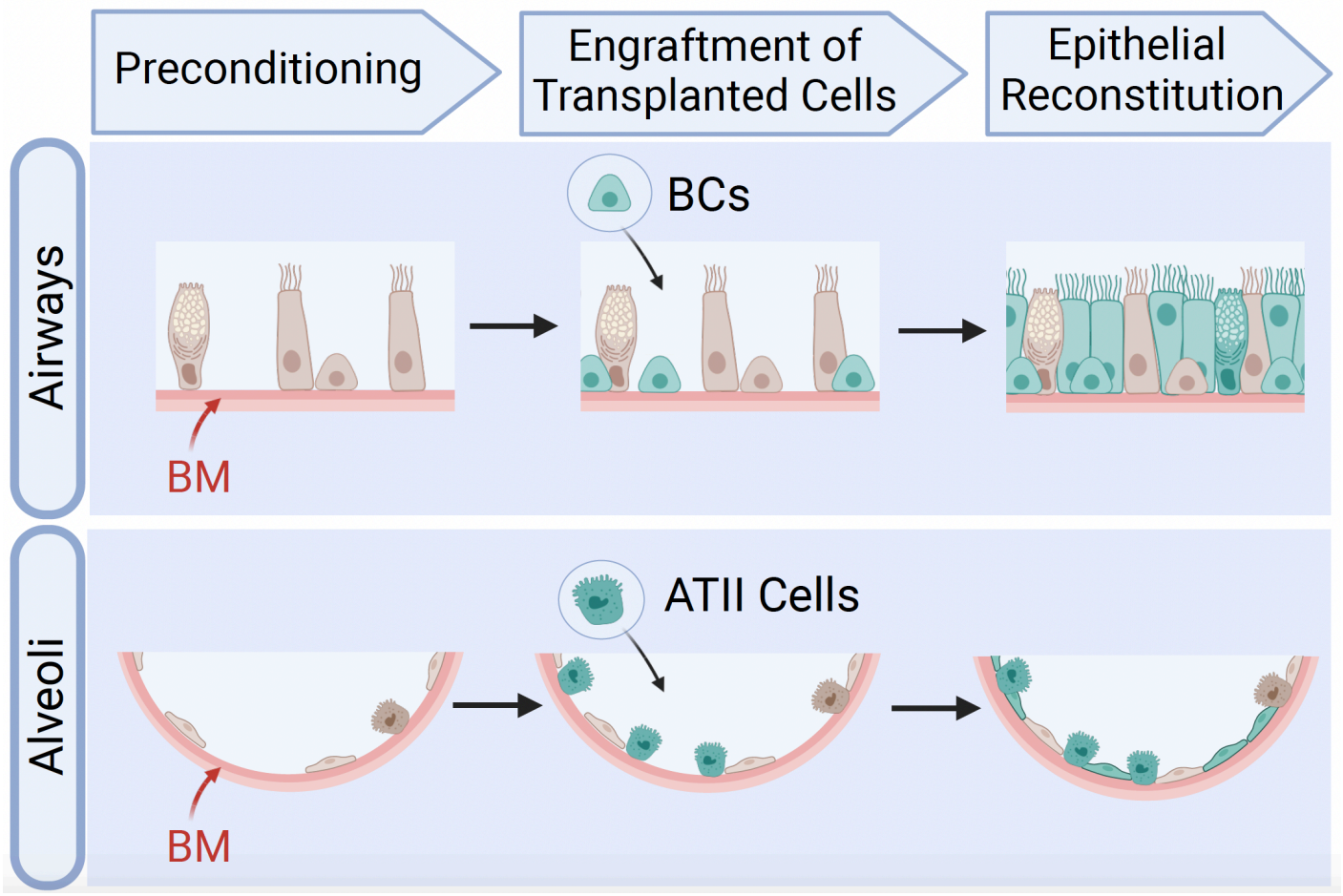

Figure 2. Preconditioning as a means to facilitate engraftment of transplanted lung stem cells and subsequent repair of diseased lungs. Preconditioning of the airways and alveoli will expose the BM underlying luminal epithelium, allowing engraftment of the transplanted lung stem cells (BCs for the airways, and ATII cells for the alveoli), which will then reconstitute the damaged or diseased epithelium through self-renewal and differentiation. Figure created with Biorender.com.

Immunocompatibility	Cell Source	Recipient	Example
Syngeneic	C57BL/6J mice	C57BL/6J mice	– Ma et al, 2023[51]
	Lewis Rats	Lewis Rats	– Serrano-Mollar et al, 2007[56]
	129X1/S1 Mouse	129X1/S1 mice	– Herriges et al, 2023[57]
Xenogeneic	Human	NSG mice	– Ma et al, 2023[51] – Herriges et al, 2023[57] – Wang et al, 2021[58] – Alvarez-Palomo et al, 2020[59] – Ghosh et al, 2017[60]

Table 1: Immunocompatibility models utilized to study lung stem cell transplantation.

Preconditioning Reagent	Mechanism of Action	Acute or Chronic	Primary Anatomical Site Affected	Pathological Model	Example
Polidocanol	Detergent/sclerosing agent that induces epithelial sloughing[67]	Acute	Airway	NA	– Ma et al, 2023[51]
Naphthalene	Causes Clara cell death and epithelial exfoliation[68]	Acute	Airway	NA	– Nichane et al, 2017[69] – Ghosh et al, 2017[60]
Bleomycin	Induces interstitial edema with influx of inflammatory and immune cells. Short-term exposure induces acute injuries to alveolar epithelial cells, long-term exposure leads to pulmonary fibrosis[70]	Acute	Alveoli	NA	– Nichane et al, 2017[69] – Serrano-Mollar, 2007[56]
		Chronic		Fibrosis	– Serrano-Mollar, 2007[56] – Shi et al, 2019[71] – Alvarez-Palomo, 2020[59] – Herriges, 2023[57]
Elastase	Enzyme that digests elastin and destroys alveoli architecture[72]	Chronic	Alveoli	Emphysema	– Wang et al, 2021[58]
Lipopolysaccharide	Increases paracellular permeability, cell leakage and induces acute lung inflammation mimicking pulmonary response following exposure to bacteria[73,74]	Chronic	Airway	Airway Inflammation	– Wang et al, 2021[58]

Pseudomonas aeruginosa infection	Bacterial infection causing lung injury and inflammation due to secretion of toxins[75,76]	Chronic	Alveoli	Pneumonia	– Zhou et al 2020[77]
Influenza Virus	Viral infection that causes cell death through viral invasion and replication leading to destruction of epithelial lining and inflammation[78,79]	Chronic	Airway and Alveoli	Influenza viral infection	– Weiner, 2019[80]

Table 2: Pre-conditioning techniques to promote engraftment of transplanted BCs or ATII cells. Acute injury pertains to transplantation administered within one to three days post-injury, and chronic injury refers to transplantation administration following more than three days post-injury.

Area of Repair	Transplanted Cells	Transplant Recipient / Disease Model	Lineage Contribution	Therapeutic Outcome of Transplanted cells	Example
Proximal Airway	Primary murine BCs (6×10^6 cells/animal)	C57BL/6J mice preconditioned with polidocanol	– Basal cells – Ciliated cells – Secretory cells – Neuroendocrine cells – Tuft cells – Hillock cells – Ionocyte cells	– Airway reconstitution with long-term self-renewal and multipotent differentiation capacity for at least two years in vivo	Ma et al, 2023[51]
	Murine iPSC-derived BCs (6×10^6 cells/animal)		– Basal cells – Ciliated cells – Secretory cells		
	Primary human BCs (6×10^6 cells/animal)	NSG mice preconditioned with polidocanol	– Basal cells – Ciliated cells – Secretory cells – Goblet cells		
	Human iPSC-derived BCs (6×10^6 cells/animal)		– Basal cells – Ciliated cells – Secretory cells		
	Primary human BCs (1×10^6 cells/animal)	NOD *scid* mice preconditioned with naphthalene	– Basal cells – Ciliated cells – Secretory cells	– Repopulated proximal airway epithelium within two weeks	Ghosh et al, 2017[60]
	Embryonic murine $SOX9^+$ progenitors ($3\text{-}5 \times 10^5$ cells/animal)	NSG mice preconditioned with naphthalene	– Ciliated cells – Club cells – Basal cells – Goblet cells – Neuroendocrine cells	– Engrafted into airway epithelium and persisted for at least eight weeks	Nichane et al 2017[69]

Distal Alveoli	Primary murine epithelial cells (6×10^5 cells/animal)	C57BL/6J mice acutely preconditioned with bleomycin	– ATI cells – ATII cells	– Persisted for at least 6 months post-transplantation – Demonstrated long-term survival and differentiation potential	Herriges et al 2023[57]
	Primary murine epithelial cells isolated from distal fetal lung bud tip ($5\text{-}7 \times 10^5$ cells/animal)	129X1/S1 mice acutely preconditioned with bleomycin			
	Human distal airway stem cells (2×10^6 cells/animal)	NOD *scid* mice preconditioned with lipopolysaccharide and elastase to model COPD	– ATI cells – ATII cells	– Engrafted into both the airway and alveolar epithelium – Demonstrated long-term self-renewal and differentiation capacity – Alleviated inflammation – Improved gas exchange	Wang et al 2021[58]
	Human iPSC derived ATII cells (3×10^6 cells/animal)	Sprague-Dawley rats chronically preconditioned with bleomycin to model pulmonary fibrosis	– ATII cells	– Reduction in fibrotic and parenchymal lesions – Decrease in edema	Alvarez-Palomo et al 2020[59]

	Primary murine ATII cells (9×10^5 cells/animal)	C57BL/6J mice preconditioned with influenza virus	– ATII cells – ATI cells	– Robust engraftment into alveolar epithelium – Demonstrated self-renewal and differentiation capacity – Improved gas exchange	Weiner et al 2019[80]
	Embryonic murine $SOX9^+$ progenitors ($3\text{-}5 \times 10^5$ cells/animal)	NSG mice acutely preconditioned with bleomycin	– ATI cells – ATII cells	– Engrafted into alveolar epithelium and persisted for at least eight weeks	Nichane et al 2017[69]
	Male murine ATII cells (2.5×10^6 cells/animal)	Lewis rats chronically preconditioned with bleomycin	– ATII cells	– Robust engraftment into injured lung – Improved gas exchange – Reduction in fibrotic lesion	Serrano-Mollar et al 2017[56]
	Wild-type primary murine ATII cells	129/Sv mice with homozygous mutation in Sftpc to model childhood interstitial lung disease, preconditioned with bleomycin	– ATII cells	– Demonstrated robust, long-term engraftment into alveolar epithelium – Reintroduced surfactant protein – Attenuated fibrosis for up to four months post-transplantation	– Iezza et al 2023[87]
	Primary murine distal airway stem cells (DASCs) genetically modified to express LL-37	Mice infected with Pseudomonas aeruginosa	– DASCs	– Demonstrated engraftment into alveolar epithelium with normal stem cell properties – Enhanced antimicrobial functions and host defense capability (from LL-37 expression)	– Zhou et al 2020[77]

Table 3: Overview of recent research in lung stem cell transplantation highlighting combinations of animal models, cell sources, preconditioning techniques, lineage tracing methods, and functional outcomes.