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Repairing damaged lungs using regenerative therapy

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Abstract

There is an urgent need for better treatment of lung diseases that are a major cause of morbidity and mortality worldwide. This urgency is illustrated by the current COVID-19 health crisis. Moderate-to-extensive lung injury characterizes several lung diseases, and not only therapies that reduce such lung injury are needed but also those that regenerate lung tissue and repair existing lung injury. At present, such therapies are not available, but as a result of a rapid increase in our understanding of lung development and repair, lung regenerative therapies are on the horizon. Here, we discuss existing targets for treatment, as well as novel strategies for development of pharmacological and cell therapy–based regenerative treatment for a variety of lung diseases and clinical studies. We discuss how both patient-relevant *in vitro* disease models using innovative culture techniques and other advanced new technologies aid in the development of pulmonary regenerative medicine.

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Introduction

Loss of functional lung tissue is a characteristic of a range of acute and chronic lung diseases. In patients with severe end-stage chronic lung disease who are refractory to treatment, the only solution is lung transplantation. The type of injury that results in loss of lung function varies between these disease entities and may include destruction or remodeling of the distal lung parenchyma, where gas exchange occurs in the alveoli. Alveolar damage occurs in acute respiratory disease (ARDS), whereas repetitive alveolar damage can result in parenchymal destruction as observed in emphysema in patients with chronic obstructive pulmonary disease (COPD). In contrast, extensive remodeling due to fibrotic processes is observed in idiopathic pulmonary fibrosis (IPF) and other interstitial lung diseases. The current COVID-19 crisis highlights the range of processes leading to loss of functional lung tissue because some patients with COVID-19 (especially those with severe disease) may not only suffer from acute alveolar damage but also suffer from subsequent fibrotic processes [1]. Airway remodeling is also an important cause of lung function loss and is observed in COPD, asthma, and cystic fibrosis or may result from stenosis due to, for example, tumor formation. Finally, vascular remodeling in patients with pulmonary arterial hypertension also results in not only problems in the lungs but also in the heart and may be a cause of the need for lung transplantation.

Lung transplantation can be a life-saving procedure in patients with end-stage lung disease, but shortage of donor lungs and rejection of the transplanted lungs are important limitations. Pulmonary regenerative medicine holds promise for patients with severe loss of lung function requiring transplantation and also for those with progressive loss of lung function. In many of these lung diseases, including COPD, IPF, ARDS, cystic fibrosis, and COVID-19, the epithelium is affected. This epithelium covers the conducting airways, as well as the alveoli located in the distal lung parenchyma.

In the last decade, our understanding of lung development and lung repair has markedly increased, and much of this research has focused on the epithelium and its stem or progenitor cells and the niche (i.e. extracellular matrix and surrounding cells) these cells reside in. Injury models in animals and cell culture models have shown that the adult lung has marked repair capacity. Interestingly, this is supported by observations in patients after pneumonectomy [2]. Use of this increased knowledge of lung development and repair has resulted in the realization that pharmacological approaches, cell therapy, and tissue engineering are realistic options

for a better treatment of a range of lung diseases. Therapeutic strategies to repair lung tissue include those aimed at regeneration (cell therapy, extracellular vesicles, and pharmacological interventions) and replacement (cell therapy and tissue engineering) [3]. In this review, we will focus on regenerative pharmacology and cell therapy as a regenerative approach. Readers interested in tissue engineering are referred to other excellent reviews on this topic [4].

Lung stem cells and their role in repair and regeneration

Injury models in animals, mainly mice, have markedly increased our insight into the identity and role of lung stem cell populations in repair. However, such models are increasingly replaced by cell culture models, using primary cells isolated from lung tissue or by using (human) induced pluripotent stem cells that can be differentiated into the various lung cell populations [5]. The need to use more human cell and tissue culture models also arises from the realization that there are limitations in the translation of findings from murine studies to the human situation, resulting from differences in anatomical organization, biology/physiology, and cell-ratio differences along the epithelium lining the lungs [6].

Recent reviews provide a detailed overview of the rapidly evolving field of research into lung stem/progenitor cell populations and associated models, to which we refer the interested reader to obtain further detailed information that is beyond the scope of this review [5,7−10]. Table 1 provides a summary of the main stem/progenitor populations in the mouse and human lung because these species have been most widely studied. Briefly, in the airway epithelium, the basal cells are the most widely recognized cell type that acts as a progenitor cell. These basal cells have the capacity to self-renew and to differentiate into the various types of luminal cells that constitute the airway epithelium: ciliated cells, secretory cells (mucus-producing goblet cells and club cells) and more rare cell types such as the cystic fibrosis transmembrane conductance regulator (CFTR)-expressing ionocytes, the chemosensory tuft or brush cells, and neuroendocrine cells [5,7,10−12]. At least two subpopulations of basal cells can be recognized, in which one serves as a self-renewing stem cell, whereas the other population mediates formation of the luminal cells during epithelial differentiation [13]. Single-cell analysis has, however, shown that these basal cells consist of various (and likely more than two) subpopulations that may have different characteristics and may differ in abundance in disease, as shown for instance in COPD [14]. A detailed understanding of the function of these subpopulations in health and disease is still missing. Especially after injury, and mainly demonstrated in mouse injury models, also luminal cells

may contribute to repair as shown for club cells [15]. Mouse studies showed that these cells may even give rise to basal cells after extensive injury [16], but it is unclear whether this also contributes to repair in homeostatic conditions and after milder forms of injury. Furthermore, to which extent these basal cells are different from 'conventional' basal cells remains to be determined. Furthermore, in the distal airways including at the junction between the bronchioli and alveoli, additional stem cell populations have been identified in the mouse that contribute to airway and alveolar (re) generation (reviewed in [5]).

There has also been a marked progress in our understanding of alveolar development and repair, not only through animal models but also especially through organoid studies including those with human cells [9]. Within the alveolus, the cuboidal alveolar type 2 cell (AT2) serves multiple functions: it produces surfactant to lower surface tension and prevents alveolar collapse, secretes host defense and inflammatory mediators, and serves as a progenitor for the flattened alveolar type 1 cells (AT1) that allow gas exchange. Importantly, AT2 cells also function as stem cells and have the capacity to self-renew [17,18]. WNT/β-catenin activation was reported to be central in self-renewal, whereas inhibition of WNT/β-catenin signaling is important for AT2-to-AT1 differentiation [18,19], although others report that activation is needed for this transition [20]. This illustrates not only that we are only beginning to understand alveolar development and repair but also the challenges and opportunities associated with pharmacological approaches based on this knowledge because the same pathway may regulate self-renewal and differentiation in different ways. Nevertheless, restoring disturbed WNT/β-catenin signaling in mouse emphysema models using blocking of inhibitory LTβR signaling [21] or direct activation of WNT/β-catenin signaling [22] was found to restore alveolar tissue, whereas inhibition of WNT/β-catenin signaling may exert antifibrotic effects in IPF [23,24].

Impact of lung tissue remodeling on repair

Lung epithelial cell function in lung repair and regeneration relies on essential cues from the surrounding niche. In lung diseases, profibrotic and proinflammatory microenvironmental cues, including those resulting from altered mesenchymal cell function, may contribute to dysregulated repair and lung tissue remodeling. Fibroblast activation by transforming growth factor (TGF)-β, a growth factor with increased expression in, for example, lung tissue from patients with COPD or IPF, was found to result in a loss of the ability of these cells to support lung epithelial cell organoid formation [25]. Furthermore, changes in extracellular matrix composition, as well as mechanical cues resulting from increased stiffness in fibrotic areas or alveolar stretch in

Table 1

Overview of the various stem/progenitor populations in the mouse and human lung.

Cell type	Marker expression	Differentiation/cell fate	Location/species	References
Basal cell	TP63, KRT5, NGFR	Self; airway luminal cells (including secretory goblet and club cells, and ciliated cells)	Airway Human/mouse	[11,12,14,49]
Club/secretory cell	Scgb1a1	Self; ciliated cells	Airway Human/mouse	[15,16]
Myoepithelial cell	TP63, Scgb1a1	Self; basal and luminal cells	Submucosal gland Mouse	[50]
Distal airway cell populations (BASC, DASC, LNEP, MHC^{high} [H2-K1^{high}])	Various markers (depending on the subpopulation)	Self; basal, club, ciliated cells; AT1, AT2	Distal airways Mouse	Reviewed in [5]
AT2	SFTPC, HT2-280, DC-LAMP, ABCA-3	Self; AT1	Alveoli Human/mouse	[17]
AEP	SFTPC, HT2-280, Axin2/TM4SF1	Self; AT2; AT1	Alveoli Human/mouse	[18,51]

ABCA-3: ATP-binding cassette sub-family A member 3; AEP: alveolar epithelial progenitor cell; BASC: bronchioalveolar stem cell; DASC: distal airway stem cell; DC-LAMP: dendritic cell lysosomal-associated membrane glycoprotein; H2-K1: mouse MHC class I marker; KRT5: keratin 5; LNEP: lineage-negative epithelial progenitor; NGFR: nerve growth factor receptor; Scgb1a1: secretoglobin family 1A member 1; SFTPC: surfactant protein C; TM4SF1: transmembrane 4 L six family member 1; TP63: tumor protein p63.

areas of tissue destruction or mechanical ventilation, may alter repair and differentiation processes, as illustrated by studies on AT2-to-AT1 differentiation [26,27]. Furthermore, although acute proinflammatory cues may promote AT2-to-AT1 differentiation and thus contribute to alveolar repair, prolonged inflammation may impair AT1 maturation and thus regeneration of alveoli [28].

Therapeutic approaches to achieve lung repair and regeneration

A better understanding of mechanisms involved in the respiratory developmental and repair pathways, the stem/progenitor cells and growth factors and other signaling molecules involved, provides clues for intervention strategies. Such interventions have been successfully evaluated in a variety of animal studies [29], but only few of these have reached the stage of clinical evaluation. Table 2 provides an overview of clinical studies using pharmacological or cell therapy approaches that have aimed at regeneration or repair of lung tissue and are discussed in the following.

Regenerative pharmacology

Insight into the important role of mesenchymal cell—derived fibroblast growth factors in lung development and repair [8] has resulted in clinical studies. Based on a variety of model studies, including a human lipopolysaccharide challenge model of acute lung injury that showed favorable effects [30], the effect of intravenous FGF7 (formerly named keratinocyte growth factor) was evaluated in patients with ARDS [31]. However, there was no clinical benefit or a change in physiological parameters, and study results even suggested that patients

on active treatment did worse than those on placebo. Retinoids and retinoic acid receptor agonists are another class of drugs that have been evaluated in clinical studies aimed at lung repair. These studies were a logical follow-up from the observation that retinoic acid is able to restore alveolar tissue after elastase-induced emphysema in rats [32]. Although these initial observations were confirmed in various—but not all—studies in rodents, it proved difficult to translate these preclinical findings to effective treatment in patients with meaningful outcomes (see Table 2 for details). A third category of drugs that are now close to evaluation for their capacity to stimulate repair and regeneration of lung tissue in clinical trials are those that target senescence. The realization that senescence impairs repair and that lung function declines with aging has led to the discovery that some lung diseases display features of accelerated aging, as demonstrated, for example, in COPD [33] and IPF [34,35]. Accelerated aging is accompanied by increases in senescent cells that have a decreased repair capacity and secrete proinflammatory mediators. These observations have prompted researchers to investigate the effects of senotherapy (using senolytics and senostatics) in animal models of these diseases [33,34], but so far, this has not yet resulted in published clinical studies although some of the drugs used in animal models have been approved for human use for other clinical indications.

Cell therapy

In addition to pharmacotherapy, cell therapy holds promise for pulmonary regenerative medicine. This has been demonstrated in various animal models of lung injury that have been reviewed elsewhere [5,36]. So far,

Table 2

Clinical studies on regenerative therapies in human lung disease.

Indication/therapeutic area	Registration nr. and design	Treatment/dosing/timing	Outcome	References
LPS-induced human model of lung injury (n = 36)	ISRCTN 98813895 Randomized, double-blind, placebo-controlled trial	KGF (palifermin) or placebo 60 µg/kg/day (i.v.) 3 days	Increased Sp-D, increased alveolar IL-1Ra, MMP-9, GM-CSF (pro-repair/pro-resolving effect)	[30]
ARDS (n = 29)	ISRCTN95690673 Randomized, double-blind, placebo-controlled trial Phase 2	KGF (palifermin) or placebo 60 µg/kg (i.v.) 6 days	No/worsening effect; fewer ventilator-free first 28 days; higher mortality at day 28	[31]
AATD-emphysema (n = 262)	Randomized, double-blind, placebo-controlled trial Phase 2	RARγ-agonist (palovarotene-retinoid) 5 mg/day (oral) 12 months	No effect	[52]
COPD-emphysema (n = 148)	Randomized, double-blind, placebo-controlled trial Phase 2	All-trans retinoic acid (ATRA), 13-cis retinoic acid (13-cRA:) or placebo ATRA: 1 mg/kg/d or 2 mg/kg/d (oral) 13-cRA: 1 mg/kg/d (oral) 6 months followed by a 3-month crossover period	No effect	[53]
COPD (n = 6)	Randomized, double-blind, placebo-controlled trial Pilot trial	FGF-2 2.5 ng by inhalation 3 times a day during two weeks	Safe and well-tolerated No significant improvements in respiratory symptoms and lung function	[54]
Mild/severe COVID-19 (n = 18)	NCT04288102 Non-randomized, controlled trial Phase 1	UC-MSC 3×10^7 cells/infusion (i.v) Day: 0, 3, and 6	Safe and well-tolerated	[55]
Advanced COPD-emphysema (n = 4)	NCT01110252 Single-arm trial Phase 1	Mononuclear cells (BMMC) 1x i.v. BMMS (autologous)	Safe and well-tolerated	[56]
Moderate to severe COPD-emphysema (n = 62)	NCT00683722 Randomized, double-blind, placebo-controlled trial Phase 2	BM-MSC $4x\ 100 \times 10^6$ cells/infusion (i.v.; allogeneic) Monthly	Safe and well-tolerated, trend toward lower CRP	[57]
Severe COPD-emphysema (n = 8)	NCT01306513 Single-arm trial Phase 1	BM-MSC $2x\ 1–2 \times 10^6$ BM-MSCs/kg (i.v.; autologous) 1 week apart	Safe and well-tolerated; increase in alveolar septal CD31	[58]
Severe COPD-emphysema (n = 10)	NCT01872624 Randomized, patient-blinded, placebo-controlled trial Phase 1	BM-MSC $1x\ 10^8$ cells (i.v.; allogeneic) Combined with EBV insertion	Safe and well-tolerated, increased QoL, decreased CRP, decreased BODE and MMRC.	[59]
Mild-to-very severe stable COPD (n = 9)	ANZCTR12614000731695 Single-armed trial Phase I	BM-MSC $2x\ 10^6$ cells/kg (i.v.; allogeneic) 1 week apart [111]indium-labeled MSC	Safe and well-tolerated; Reduced MSC uptake in emphysema lungs, decreased systemic inflammation	[60]
COPD (n = 5)	Open-label, Single-armed trial Phase (1/2)	UC-MSC $4x\ 1–2x10^6$ BM-MSCs/kg (i.v.)	Safe and well-tolerated; no significant effects	[61]

Table 2 (*continued*)

Indication/therapeutic area	Registration nr. and design	Treatment/dosing/timing	Outcome	References
Moderate-to-severe COPD (n = 20)	ISRCTN70443938 Placebo-controlled trial Phase 1/2	UC-MSC 1x 10^6 cells/kg (i.v.)	Safe and well-tolerated; decreased MMRC and CAT score, lower number of exacerbations	[62]
Moderately severe IPF (n = 8)	NCT01385644 Single-arm Phase 1b	PL-MSC 2.5×10^6 cells/mL (i.v.)	Safe and well-tolerated	[63]
Moderate and progressive IPF (n = 16)	Single-arm Phase 1	Alveolar type II cells 4x $1–1.2 \times 10^9$ cells bronchoscopic instillation 15 days apart	Safe and well-tolerated	[64]
Non-CF bronchiectasis (n = 2)	NCT02722642 Single-arm Pilot	SOX9+ basal cells 10^6/kg body weight bronchoscopic instillation	Recruitment ongoing (n = 20 estimated)	[65]

BMMC: bone marrow mononuclear cell; BM-MSC: bone marrow–derived MSC; PL-MSC: placenta-derived MSC; RARγ: γ-type retinoic acid receptor; UC-MSC: umbilical cord–derived MSC.

cell therapy related to lung regeneration in patient studies has focused on mesenchymal stromal cells (MSCs) [36], although also other cell types have been evaluated including alveolar epithelial cells (Table 2). MSCs are cells of nonhematopoietic origin that have the capacity to differentiate into multiple mesenchymal cell lineages, that is, chondrocytes, osteoblasts, and adipocytes. Cell culture, animal studies, and clinical studies in graft-versus-host disease and inflammatory bowel disease have shown that MSCs may modify immune and inflammatory responses and possibly increase repair and regeneration. MSCs may help create a more favorable microenvironment for lung repair to occur through their immunomodulatory activity, or they may enhance repair by secreting mediators that enhance repair. MSCs have been used in clinical studies for a variety of indications, including COPD, ARDS, IPF, and COVID-19 (Table 2), but so far, clinical efficacy of MSC treatment for lung diseases remains to be demonstrated.

This lack of a clinical benefit of MSC treatment for lung diseases may be explained by the fact that they enter inflamed lung tissue. Lung inflammation has indeed been found to modify MSC behavior, as demonstrated in a recent study in which human bone marrow–derived MSCs were cultured with bronchoalveolar lavage (BAL) fluid from patients with ARDS or patients with lung diseases other than ARDS (non-ARDS). Incubation of MSCs with BAL from non-ARDS resulted in a different gene expression pattern in MSCs and in a stronger anti-inflammatory phenotype in monocytes exposed to bone marrow–derived MSC conditioned medium than using ARDS BAL [37]. Evidence was provided that especially IL-6 in conditioned medium from ARDS BAL–exposed MSCs contributed to this effect. The authors stressed that also other factors of the (diseased) lung environment may affect MSC behavior, including oxygen levels, tissue stiffness, and mechanical forces such as stretch.

In addition to using, for example, pharmacotherapy to reduce lung inflammation at the time of MSC infusion, there are several other options to improve treatment outcomes of MSC administration. First, the route of administration needs to be considered. Clinical studies have used intravenous administration, whereas local delivery into the lung has been widely used in animal models and only in few human studies (Table 2). Second, conditioning of MSCs by treatment with selected mediators or gene editing may enhance regenerative properties of MSCs [36] and can be considered in clinical studies.

Conclusions and future directions
Although a multitude of pharmacological and cell therapy approaches have been evaluated in animal models (reviewed in several recent reviews; [8,29,38]), the

number of regenerative approaches evaluated in clinical trials for acute or chronic lung disease has so far been limited. Clearly, there is difficulty in translating outcomes from animal models to the human situation, and there are ethical concerns regarding the use of animals in research. This has important consequences for drug discovery, which previously largely relied on animal models and observations in human lung tissue. Rapid developments in *in silico* approaches and culture methods, such as organoids, lung-on-chip, and precision-cut lung slices (PCLSs), have markedly contributed to the tool box for regenerative drug development

Figure 1

Current Opinion in Pharmacology
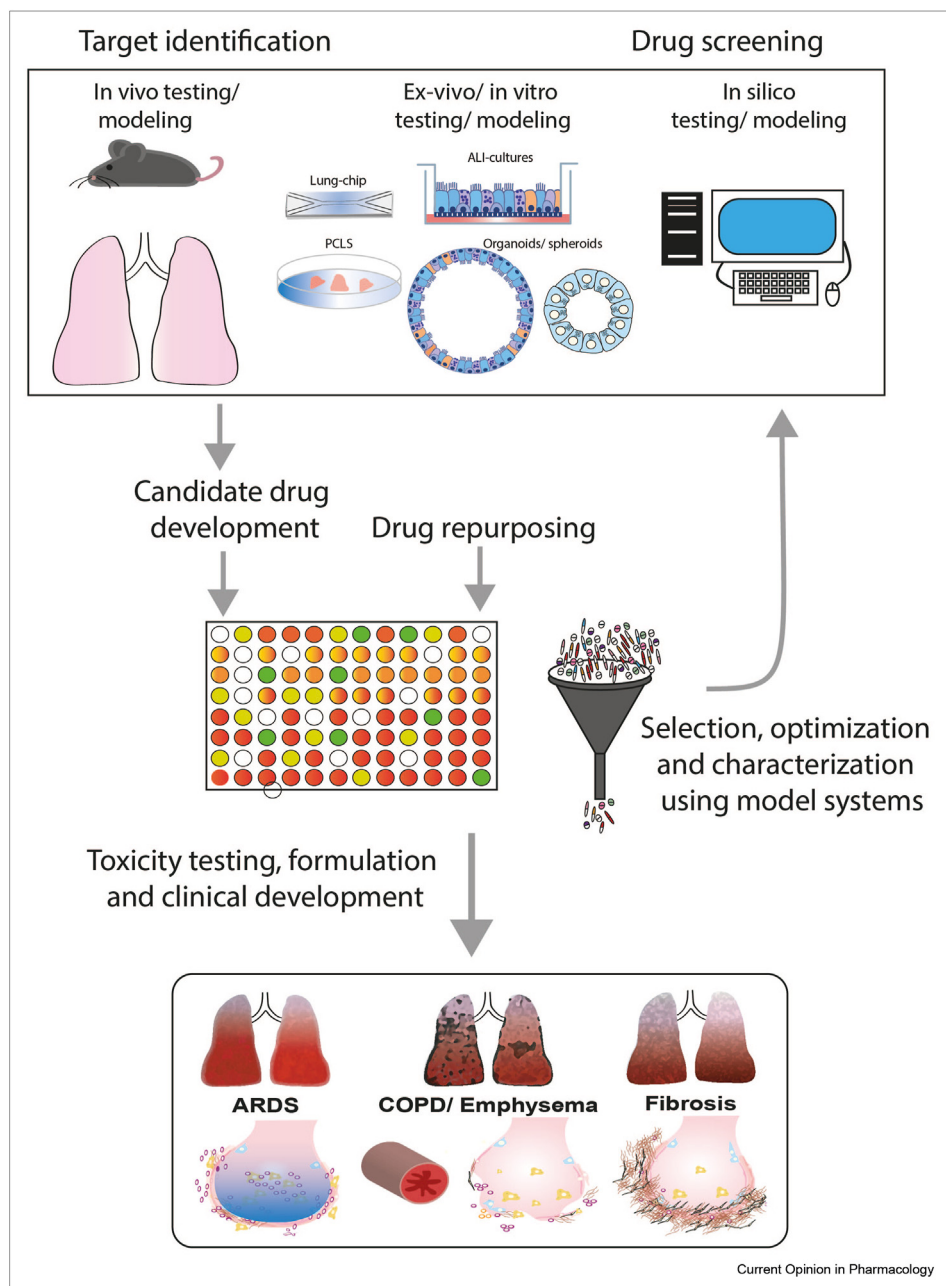
Approaches to drug discovery in pulmonary regenerative medicine. *In vitro, in vivo, ex vivo*, and *in silico* modeling of respiratory lung diseases may be used for target identification as well as serve as the model to test drug efficacy. Thus, these complementary approaches contribute to development of new drug candidates, and these can be used in (semi) high-throughput screening approaches (depending on the technique). Such screening approaches are also particularly worthwhile for drug repurposing. Candidate drugs selected using such screens can be further analyzed and validated using the abovementioned *in vivo*, *in vitro*, and *in silico* approaches and lead to regenerative pulmonary medicine for a variety of lung diseases.

(Figure 1). PCLSs provide an *ex vivo* tool to mimic and study mechanisms operational in pulmonary diseases and provide a platform to screen (novel) regenerative medicine approaches. PCLSs enable studying the various cell types present in the lung in their inherent surrounding, while maintaining tissue structure and integrity, as reviewed in [39]. However, the contribution of, for example, circulating factors and exposure to air cannot be included in PCLS models, but this limitation is shared with conventional cell culture models. Furthermore, so far, PCLSs are mainly used in short-term studies because prolonged culturing of these *ex vivo* tissue slices proved to be difficult. However, recent studies have shown that improvements such as use of customized hydrogels may support extended culture duration [40]. The advantage of using the PCLS derived from patient tissue for studying lung regeneration is illustrated by a study showing that activation of WNT/β-catenin signaling in the PCLS from patients with COPD resulted in an increase in markers of alveolar repair [41]. In addition, in cell culture models, inclusion of patient-derived cells may increase their relevance. Depending on availability, such cells can be obtained from patients, but (human) induced pluripotent stem cells have also shown potential for the development of patient-specific and disease-relevant *in vitro* models, as illustrated for Herman-Pudlak syndrome type 2, associated with pulmonary fibrosis [42]. As shown in Figure 1, these complementary approaches contribute to target discovery and development of new drug candidates, and culture methods can be adapted for use in (semi) high-throughput screening approaches using, for example, WNT/β-catenin reporters or, for instance, functional assays for which organoids may be suitable. Such screening approaches are also particularly worthwhile for drug repurposing. Candidate drugs selected using such

Figure 2
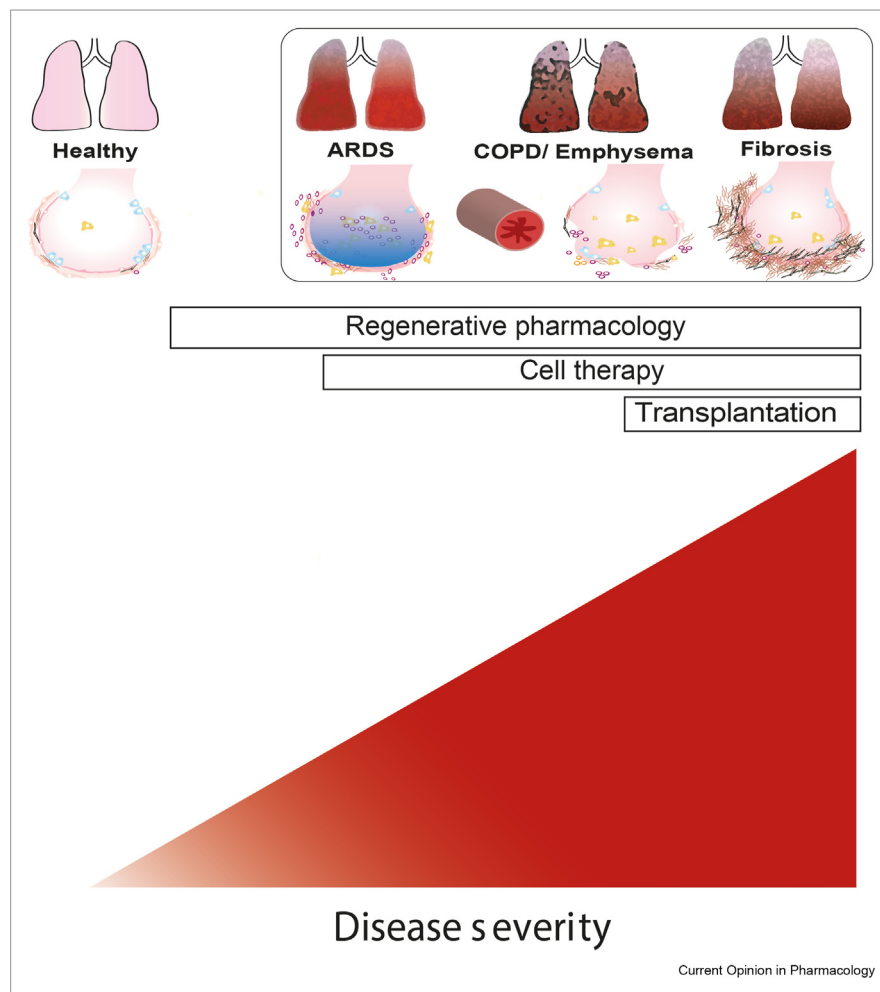
Regenerative approaches for lung diseases and the role of disease state. Lung diseases present with heterogenous characteristics, which emphasizes the need to consider different treatment approaches and combinations for pulmonary regenerative medicine. In addition, the choice for a therapy may be determined by the stage of the disease. ARDS, COPD, and fibrosis are presented as examples of diseases that may benefit from a regenerative therapy approach. See text for further details.

screens can be further analyzed and validated using the abovementioned *in vivo*, *in vitro*, and *in silico* approaches. More advanced culture models, such as lung-on-chip models in which mechanical forces of breathing (airflow and stretch) [43,44] or culture of, for example, alveolar cells on curved membranes to mimic the morphology of the alveolus [45], have the potential to better mimic the lung environment and may help reducing the need for animal models.

These new approaches may help to improve the selection of candidate drugs for human studies. Here, there is also a need for better outcome markers. Especially in COPD, relevant clinical outcomes often require treatment for years to observe, for example, a slowdown of the progressive decline in lung function. This illustrates the need for surrogate (bio)markers of repair or a focus on the exacerbation of disease as a window of accelerated lung function loss and intensified disease burden. Analysis techniques such as proteomics, metabolomics, single-cell/nucleus RNA sequencing, mass cytometry, and advanced imaging techniques also hold great promise for target discovery and biomarker identification.

The type of regenerative treatment may depend on the stage of the disease, with pharmacological interventions being feasible at earlier disease stages than cell therapy (Figure 2). Timing and duration of treatment may be crucial because some compounds have differential effects on differentiation and proliferation, as shown for retinoic acid [46]. The same holds true for anti-inflammatory treatment that may preferentially be used before starting a repair-inducing treatment. Combined treatments indeed have the potential to enhance the efficacy of therapies. Cell therapy with MSCs or lung progenitors, either derived from induced pluripotent stem cells or from progenitor populations isolated from tissue, may be more effective when combined with pharmacological approaches aimed for instance at improving the microenvironment or niche in which these cells are supposed to act. Furthermore, clearly, the development of additional lung injury should be slowed down or stopped when applying regenerative treatment. In emphysema, alveolar destruction needs to be halted, and repair and regeneration need to be stimulated. Smoking cessation is the most effective intervention to slow down lung function decline in COPD and restrict alveolar destruction, but inflammation does persist for at least some time after cessation [47]. Interestingly, some interventions may have the opportunity to inhibit ongoing destruction while also enhancing repair. This is, for instance, not only likely for anti-inflammatory treatments but also for those targeting mitochondrial dysfunction because this may both trigger inflammation and restrict repair [48]. In lung fibrosis, there is an urgent need to block ongoing fibrosis, and new classes of antifibrotics have shown promise in slowing down

progression in, for example, patients with IPF. Combining such an antifibrotic approach with treatment that helps restoring or repairing damaged lung tissue may be most suitable for IPF and other interstitial lung diseases. The need to consider different treatment approaches and combinations for pulmonary regenerative medicine is illustrated in Figure 2.

CRediT author contribution statement
Padmini Khedoe: Writing — original draft, Writing — review & editing. Xinhui Wu: Writing — review & editing, Reinoud Gosens: Conceptualization, Writing — review & editing. Pieter Hiemstra: Conceptualization, Writing — original draft, Writing — review & editing.

Conflict of interest statement
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* of special interest
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