Stem Cell Treatment for Chronic Lung Diseases

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Key Words

Asthma · Chronic lung diseases · Chronic obstructive pulmonary disease · Cystic fibrosis · Idiopathic pulmonary fibrosis · Mesenchymal stem cells

Abstract

Chronic lung diseases such as idiopathic pulmonary fibrosis and cystic fibrosis or chronic obstructive pulmonary disease and asthma are leading causes of morbidity and mortality worldwide with a considerable human, societal and financial burden. In view of the current disappointing status of available pharmaceutical agents, there is an urgent need for alternative more effective therapeutic approaches that will not only help to relieve patient symptoms but will also affect the natural course of the respective disease. Regenerative medicine represents a promising option with several fruitful therapeutic applications in patients suffering from chronic lung diseases. Nevertheless, despite relative enthusiasm arising from experimental data, application of stem cell therapy in the clinical setting has been severely hampered by several safety concerns arising from the major lack of knowledge on the fate of exogenously administered stem cells within chronically injured lung as well as the mechanisms regulating the activation of resident progenitor cells. On the other hand, salient data arising from few 'brave' pilot investigations of the safety of stem cell treatment in chronic lung

diseases seem promising. The main scope of this review article is to summarize the current state of knowledge regarding the application status of stem cell treatment in chronic lung diseases, address important safety and efficacy issues and present future challenges and perspectives. In this review, we argue in favor of large multicenter clinical trials setting realistic goals to assess treatment efficacy. We propose the use of biomarkers that reflect clinically inconspicuous alterations of the disease molecular phenotype before rigid conclusions can be safely drawn.

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Introduction

Stem cells are considered to be cells that are capable of unlimited self-renewal and differentiation into several cellular subtypes depending on their origin and the resident microenvironment. In humans, stem cells can be

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subdivided into two main categories: embryonic stem cells (ESCs) and adult stem cells [1]. The latter are found in a number of tissues, including the bone marrow (BM), blood, adipose tissue, liver, kidney, heart and the lungs; they contribute to tissue repair and provide a continual source of cells throughout an individual's life span [1–3]. In the lung, current evidence suggests that these cells may participate in tissue homeostasis and regeneration after injury [2–7] and may originate from within the lung itself, nesting in protected niches in the distal airways [8], called resident progenitor cells (alveolar, endothelial and interstitial), or from distant sites such as the blood, BM and adipose tissue, namely endothelial progenitor cells (EPCs) [5] and mesenchymal stem cells (MSCs) [3, 9]. While it is possible that such cells will ultimately be useful as alternatives to ESCs to prevent or treat lung disease, the therapeutic potential of adult stem cells in patients with lung diseases has to be better defined.

Chronic lung diseases such as idiopathic pulmonary fibrosis (IPF) and cystic fibrosis (CF) or chronic obstructive pulmonary disease (COPD) and asthma are leading causes of morbidity and mortality worldwide with a considerable human, societal and financial burden; chronic lung diseases rank second after cardiovascular diseases and amount to a gross estimate of more than 11 million deaths in the United States in 2020 [6, 10].

During the past 5 years, since the first clinical trial of the feasibility, safety and ability of EPCs to treat idiopathic pulmonary hypertension [11, 12] was conducted, studies of stem cells and cell therapies in lung biology and diseases have continued to expand rapidly [13]. The latter scientific explosion reflects the amenable need of chest physicians to respond to the will of patients suffering from end-stage chronic lung diseases that will culminate into a fatal outcome irrespective of currently available treatments. Starting from seminal observations demonstrating the potency of stem cells, notably adult mesenchymal stromal cells, to differentiate into alveolar epithelial cell (AEC) and endothelial cell lineages and rapidly extending to experimental and human lung explant models providing evidence of their efficacy, we witnessed significant advances regarding the mechanisms of action, biological properties and regenerative capacity of adult stem cells [13].

Nevertheless, despite relative enthusiasm arising from promising therapeutic applications of stem cells in experimental models of chronic lung diseases, there has been a paucity of preclinical and clinical studies regarding frequent chronic destructive lung diseases, like IPF, CF and COPD. It is generally recognized that this significant lack

could be attributed to unresolved ethical and safety concerns mainly arising from the yet unknown disease immunopathogenesis and the need for a better understanding of the mechanisms underlying pleiotropic properties of adult stem cells. With a gradually increasing worldwide incidence and no proven therapies other than lung transplantations, the role of stem cells in the treatment of this group of diseases is of significant interest.

The main scope of this review article is to summarize the current state of knowledge, based on both human and experimental studies, regarding the application status of stem cell treatment in chronic lung diseases, including IPF, COPD, CF and asthma, to address some important safety and efficacy issues that pose significant limitations to their implementation in current clinical practice and to present future challenges and perspectives. More importantly, there is an urgent need for large multicenter clinical trials with a painstaking study design by setting realistic goals to assess efficacy using biomarkers that reflect clinically inconspicuous alterations of the disease molecular phenotype before rigid conclusions can be safely drawn.

Stem Cells

Mesenchymal Stem Cells

Among the stem cell population, MSCs are the most extensively studied and probably have the best results in medical research. Since their seminal identification, almost 40 years ago, as a nonhematopoietic stem cell of mesodermal origin with a fibroblast-like morphology and potency to differentiate into both mesenchymal and non-mesenchymal cell lineages, the body of evidence relating to their differentiation, immunophenotype, preclinical use as well as their mechanisms of action has increased dramatically [3, 7, 9, 13, 14]. MSCs, besides BM, can also be readily harvested from other tissues, including adipose tissue, skeletal muscle, dental pulp and cord blood [15–18]. Of special interest is adipose tissue since it represents an abundant and easily accessible source of MSCs denominated adipose-derived stem cells (ADSCs), also including stromal vascular fraction cells. MSCs possess outstanding pleiotropic properties, including differentiation and regenerative and migratory capacity. The latter characteristic allows them to home selectively to sites of tissue injury and exert their immunosuppressive activity with the secretion of angiogenic, anti-apoptotic and anti-inflammatory factors [15, 18–21], such as stromal-derived factor-1, monocyte chemoattractant protein-3, vascular endothelial growth factor (VEGF), hepatocyte growth

factor, stimulating angiogenesis, fostering a protected environment for host cell recovery and preserving or even rescuing injured tissue from damage [19–22].

The above impressive characteristics render MSCs major candidates for therapeutic applications in patients with chronic lung diseases and provide a strong rationale to explore their potentially beneficial use.

Endothelial Progenitor Cells

EPCs represent microvascular endothelial cells originating from circulating BM-derived vascular progenitor cells and expressing specific surface antigens. They are divided into two cell subsets, namely early and late outgrowth EPCs, respectively, based on their morphology, timing of appearance in colony assays and immunophenotypic and functional profile. In particular, early outgrowth EPCs express the leukocyte markers CD45, CD11 and CD14, the endothelial markers CD31 and VEGF-A and the hematopoietic marker CD133. They are now known to be derivatives of the hematopoietic lineage differentiating among the myeloid lineage in response to variable soluble mediators. The other type, called late outgrowth, is characterized by CD31, CD144, CD146, CD105 and VEGF-R2 expression and possesses the unique ability to participate in angiogenesis, i.e. the formation of new blood vessels [23–25]. Therefore, they have been directly implicated in the restoration of endothelial function and vascular structure. Nevertheless, studies have also revealed a dual role for EPCs because they may also be involved in vascular remodeling by differentiating into smooth muscle cells leading to intimal hyperplasia and vascular muscularization and vasoconstriction with increased vascular resistance [23–29].

The exact mechanisms orchestrating the differentiation of circulating EPCs towards either mature endothelial cells or smooth muscle cells are still a matter of debate, and several studies propose that the source of EPCs (BM, peripheral blood or umbilical cord) and the microenvironment of engraftment fostering neighboring cells, growth factors and cytokines are presumably the key regulators of the final decision on the fate of EPCs [23–29].

Given their opposing pleiotropic activities, circulating levels of EPCs have been the subject of extensive investigations in both health and disease. In specific, in healthy subjects, the number of circulating EPCs decreases with age and cigarette smoke exposure while the presence of cardiovascular risk factors results in similar effects. With respect to chronic lung diseases (most notably COPD), the evidence discussed below seems rather conflicting and controversial [5, 23, 30–33].

Alveolar Epithelial Progenitor Cells

The airway epithelium is a dynamic tissue that undergoes constant and rapid renewal in order to vigorously respond to repeated exogenous and endogenous threats and reestablish an epithelial sheet with normal structure and function, mainly based on niches of resident progenitor cells. These are multipotent stem cells programmed to move down a certain pathway of differentiation and are often called progenitors to avoid implying that they are totipotent, as it happens with ESCs. Although several lines of research suggest that basal and secretory/or Clara cells are multipotent and can reconstitute a full epithelium with the contribution of the unipotent type-II AECs, stem cell niches and their microenvironments within the human lung have not been extensively characterized [4, 8].

Most recently, it has been intriguingly demonstrated that the human lung contains identifiable stem cells that give rise to completely structured respiratory units comprising bronchioles, alveoli and pulmonary vessels when injected within damaged mouse lungs. Nevertheless, it is still debatable whether these respiratory units are fully functional and participate in gas exchange. In addition, there is no definitive knowledge whether rare airway epithelial progenitor/stem cells proliferate, migrate and differentiate in a highly orchestrated procedure similar to classical models of high turnover in tissues such as the liver or skin or alternative models of simple duplication of differentiated cells (e.g. in the pancreas) are better applicable in lung tissue. Furthermore, there is significant lack of knowledge on the endogenous signals that control type-II AEC proliferation and differentiation into type-I AEC [4].

On the basis of the above predicament, a major challenge arising from developmental biology is to induce lung tissue regeneration by administering endogenous signaling molecules that are essential for lung development and maintenance [34].

ESCs and Induced Pluripotent Stem Cells

ESCs are derived from the inner cell mass of the blastocyst and are considered totipotent in their ability to regenerate all three germ layers of an organism, whereas stem cells derived from the adult are considered multi- or unipotent, and able to give rise to one or several mature cell types. Induced pluripotent stem cells (iPSCs) are mature cells derived from several tissues, including the skin, liver, kidney and lung, that have been reprogrammed back to an embryonic-like state. They are an ethical alternative to cloning or destroying in vitro fertilization embryos for pluripotent stem cells [13].

During the past 5 years, although progress using ESCs for lung regeneration or repair has been accomplished, e.g. by the generation of cells with phenotypic characteristics of type-II AECs, derivation of fully functional airway epithelium from ESCs has proven even more elusive and controversial. To our disappointment, several ethical concerns have hampered the efforts of investigators, and thus the number of available studies on the effects of ESC administration to the lung in vivo is scarce, e.g. regarding survival of mouse ESC-derived type-II AEC and maintenance of pro-surfactant protein C expression for 1 day [35].

Regarding iPSCs, rapid advances in isolation and generation technologies have raised hope that these cells could serve as reliable alternatives for tissue renewal and restoration [36–38]. As described below, these advances generated disease-specific human iPSCs from patients with both genetic and acquired chronic lung diseases, including CF, α_1 -antitrypsin deficiency and scleroderma [36–38].

Stem Cell Therapy in IPF

IPF is an irreversible, devastating, fibroproliferative disorder of the lung that culminates into a fatal outcome irrespective of treatment [39–41]. Despite extensive research and rapid expansion of scientific knowledge, IPF pathogenesis still remains with numerous question marks. Recent data strongly suggest that the mechanisms driving IPF reflect abnormal wound healing in response to multiple sites of ongoing alveolar epithelial injury of unknown origin leading to fibroblast activation and exaggerated accumulation of extracellular matrix in the lung parenchyma [42–45]. Therefore, our present understanding of the molecular and cellular pathways has resulted in the testing of therapeutic approaches that modulate specific inflammatory and fibrotic mediators, however with minimal results [40, 46, 47]. With a gradually increasing worldwide incidence and no proven therapies other than lung transplantations, IPF treatment represents a major challenge and bottleneck for chest physicians. Therefore, the role of stem cells in the treatment of this disease is greatly warranted [7].

A continuing accumulation of data in animal models suggests that cell-based therapies may be potential therapeutic approaches for lung regeneration and normal wound healing after injury (table 1). An attempt to address this crucial issue was made by Ortiz et al. [48] who reported diminished histological and inflammatory in-

jury in the bleomycin (BLM) model of pulmonary fibrosis following intravenous instillation of MSCs. Fueled by the same prospect, Rojas et al. [49] intravenously administered BM-derived MSCs to mice following BLM-induced lung injury and observed decreased expression of inflammatory cytokines; their findings were verified by two follow-up studies conducted by Germano et al. [50] and Zhao et al. [51].

In line with this evidence, a beneficial effect of intratracheal and systemic infusion of MSCs in the BLM model of lung injury, which was assessed by decreases in lung collagen accumulation, fibrosis score and matrix metalloproteinase levels, has also been reported by Moodley et al. [52]. Further extending the latter finding, the same group of investigators evaluated the role of human umbilical cord MSCs in the treatment of BLM-induced lung inflammation and fibrosis. MSCs were administered systemically 24 h after BLM instillation to immunodeficient mice and were notably visualized at areas of fibroblastic foci 14 days after BLM injection but they were absent thereafter (by 28 days). Although differentiation of MSCs into AECs failed in vitro, their administration resulted in a significant attenuation of inflammation and fibrosis, which was reflected by the reduction in collagen deposition as well as inflammatory and profibrotic mediators, including TNF α , TGF β and IL-10. Furthermore, BM-derived MSCs modified to express keratinocyte growth factor via an inducible lentivirus have been found to protect from BLM-induced lung inflammation and fibrosis, which was assessed by a significant reduction in lung collagen deposition and inflammatory cytokine production [53].

Similar protective potentially paracrine effects of systemically administered BM-derived MSCs in the BLM model of lung fibrosis have also been reported by Kumamoto et al. [54] as well as Lee et al. [55], who demonstrated improvement in the lung injury score and modulation of inflammatory cytokine production, respectively. Despite these interesting and very promising data, there are significant limitations mainly arising from the lack of a specific mechanism of action through which MSCs exert their beneficial role in the experimental model of lung fibrosis.

Results were further reproduced by Cargnoni et al. [56], who reported improved body weight and decreased histological fibrosis in BLM-injured mouse lungs following administration of allogeneic MSCs via three different routes.

Finally, there are only two published reports showing beneficial effects of ESC administration in experimental

Table 1. Experimental data of stem cell therapy in animal models of pulmonary fibrosis

Study year	Type of stem cells	Animal model	Route of administration	Outcome	Potential mechanisms of action
Ortiz et al. [48] 2003	Mouse BM-MSCs Plastic adherent	BLM	Intra-venous	Decreased histologic and inflammatory injury, hydroxyproline, matrix metalloproteinase-2 and -9	None specific Potentially soluble mediators
Rojas et al. [49] 2005	Mouse BM-MSCs	BLM	Intra-venous	Decreased expression of inflammatory cytokines	None specific Potentially soluble mediators
Germano et al. [50] 2009	Mouse BM-MSCs Plastic adherent	BLM	Intra-venous	Decreased systemic inflammatory cytokines (IL-1 β , IFN- γ , IL-6, IL-8, MIP-1 α)	None specific Potentially soluble mediators
Zhao et al. [51] 2008	Rat BM-MSCs Plastic adherent	BLM	Intra-venous	Decrease in histologic injury, hydroxyproline, laminin, hyaluronan, TGF β , PDGF, IGF	None specific Potentially soluble mediators
Moodley et al. [52] 2009	Human umbilical cord MSCs	BLM	Intra-venous	Reduction in histologic injury, collagen deposition, hydroxyproline, TIMP-2	None specific Potentially soluble mediators
Aguilar et al. [53] 2009	Mouse BM-MSCs	BLM	Intra-venous	Decrease in collagen deposition, α SMA, TNF α , CCL-2, CCL-9	Keratinocyte growth factor secretion
Kumamoto et al. [54] 2009	Mouse BM-MSCs Plastic adherent	BLM	Intra-venous	Decrease in histologic injury, hydroxyproline, inflammatory cells	None specific Potentially soluble mediators
Lee et al. [55] 2010	Rat BM-MSCs Plastic adherent	BLM	Intra-venous	Decreased inflammation (neutrophils, BALF inflammatory cytokines), collagen deposition	None specific Potentially soluble mediators
Cargnoni et al. [56] 2009	Placenta MSCs	BLM	Intra-venous	Improved body weight Decreased histologic fibrosis	None specific Potentially soluble mediators
Wang et al. [35] 2010	ESCs	BLM	Intra-tracheal	Abrogation of lung injury/fibrosis, improvement in tidal volume, gas exchange	Differentiation of human ESCs to type-II AECs
Spitalieri et al. [57] 2012	Human ESCs	Silica	Intra-tracheal	Diminished lung injury, decreased levels of TGF β , improved gas exchange	Differentiation of human ESCs to type-II AECs

α SMA = α -Smooth muscle actin; BALF = bronchoalveolar lavage fluid; IFN = interferon; MIP = macrophage inflammatory protein; PDGF = platelet-derived growth factor; TGF = transforming growth factor; TNF = tumor necrosis factor; TIMP = tissue inhibitor of metalloproteinases.

models of BLM- and silica-induced lung injury. Authors performed an impressive series of experiments and starkly demonstrated that intratracheal instillation of type-II AECs derived from human ESCs resulted in an intriguingly high percentage of prolonged engraftment in injured lungs while an attenuation of BLM- or silica-induced inflammation and fibrosis (assessed by both structural and functional measurements) was also notable [35, 57].

The above data indicate that it is conceivable to speculate that the beneficial effect of MSCs reported in the experimental model of lung fibrosis is mainly attributable to their immunomodulatory paracrine activity rather than their capacity to differentiate or alternatively

their potency to generate AECs. In line with this premise, a considerable number of studies have failed to demonstrate true phenotypic differentiation of adult MSCs of different origins into AECs. There was only one study in the literature so far (Sueblivong et al. [58]) which demonstrated that a small proportion of less than 5% of umbilical-cord-blood-derived MSCs cultured in special airway growth media preferentially expressed epithelial cell markers. Nevertheless, the above observation is weakened by the finding that only a minority of these cells engrafted into airway epithelium, indicating that most of the cells only temporarily lodged in the capillary beds of the pulmonary vasculature and were then either cleared or migrated to other sites. With regard to ESCs, it re-

Table 2. Experimental data of stem cell therapy in animal models of COPD

Study year	Type of stem cells	Animal model	Route of administration	Outcome	Potential mechanisms of action
Shigemura et al. [63] 2006	Rat ADSCs Plastic adherent	Rat elastase	Direct topical application	Improved histologic repair	Hepatocyte growth factor secretion
Shigemura et al. [64] 2006	Rat ADSCs Plastic adherent	Rat elastase	Intra-venous	Decreased apoptosis, improved histologic repair, improved gas exchange and exercise tolerance	Hepatocyte growth factor secretion
Zhen et al. [65] 2008	Rat BM-MSCs	Rat papain	Intra-venous	Decreased histologic injury and AEC apoptosis	None specific Potentially soluble mediators
Zhen et al. [66] 2010	Rat BM-MSCs	Rat papain	Intra-tracheal	Decreased histologic injury Restoration of VEGF lung expression	None specific Potentially soluble mediators
Katsha al. [67] 2011	Mouse BM-MSCs Plastic adherent	Mouse elastase	Intra-tracheal	Reduction in collagen deposition and inflammatory and profibrotic cytokines (TNF α , TGF β , IL-10)	None specific Potentially soluble mediators
Schweitzer et al. [68] 2011	ADSCs (mouse and human)	Cigarette smoke	Intra-venous	Decrease in collagen accumulation, fibrosis score, metalloproteinase levels, weight loss, BM suppression	None specific Potentially soluble mediators

mains to be elucidated whether their beneficial effect reflected structural engraftment and increased capacity to differentiate or was simply attributable to paracrine properties.

Stem Cell Therapy in COPD

COPD represents a disease paradigm where gas exchange abnormalities resulting in respiratory failure reflect pathogenetic dramatic changes in lung architecture, including loss of lung elasticity, vascular remodeling and luminal obstruction with inflammatory mucoid secretions, underlying the physiologic hallmarks of the disease [25, 59, 60]. Evidence accumulates that these key pathologic alterations are associated with an exaggerated inflammatory process in response to repeated inciting stimuli, including cigarette smoke exposure, and viral or microbial infections coupled with genetically predisposed accelerated mesenchymal cell senescence and potentially impaired mobilization of resident progenitor cells. This lethal combination of genetic susceptibility and epigenetic stimuli ultimately leads to endothelial dysfunction, destruction or stiffening of pulmonary capillaries with progressive accumulation of connective tissue and AEC apoptosis resulting in pulmonary hypertension and emphysema. On the basis of the above-mentioned different

aspects of disease pathogenesis and pleiotropic properties of stem cells, including immunomodulatory, anti-inflammatory and anti-apoptotic factors, a steadily increasing number of studies has evaluated the efficacy of systemic or intratracheal stem cells, notably MSC administration in a variable spectrum of lung injury models in mice [25, 34, 59–62] (table 2).

MSCs and Immunomodulation

The first attempt to address this crucial issue was made by Shigemura et al. [63, 64] who demonstrated that ADSCs ameliorated pulmonary emphysema in an experimental model by secreting large amounts of hepatocyte growth factor. Similar protective paracrine effects of BM-derived MSCs have also been demonstrated by several investigators in experimental models of pulmonary emphysema induced by papain [65, 66] or intratracheally administered elastase [67]. Based on the above observations, Schweitzer et al. [68] suggested that the protective effect of intravenously given human or mouse adult ADSCs in a model of inflammation and injury induced by cigarette smoke exposure was mediated via a paracrine pathway involving growth factors and angiogenic modulators. In addition, beneficial effects of ADSCs were also observed on a systemic basis, i.e. by the prevention of weight loss and restoration of BM dysfunction.

EPCs and Endothelium Repair

As mentioned above, the exact pathogenetic mechanisms underlying restoration of endothelial dysfunction and pulmonary vascular remodeling are currently under investigation with studies supporting opposite roles for EPCs (both beneficial and detrimental) depending on the origin of these cells as well as their microenvironment comprising neighboring cells and soluble cytokines and growth factors. The latter seem to be responsible for the differentiation route of EPCs either towards mature endothelial cells reconstituting damaged endothelium or smooth muscle cells favoring vascular muscularization and deleterious remodeling.

In this context, a steadily increasing number of studies revealed distinct profiles regarding EPC numbers and function between COPD patients with stable disease and acute exacerbations. In specific, several lines of extensive investigation proposed that stable COPD is associated with a significant reduction in the number of circulating EPCs while a strong correlation with functional parameters of disease severity was also noted [5, 31, 33]. Moreover, a positive correlation between reduced EPC levels and a low body mass index, a marker of systemic impairment, has also been reported in COPD patients [32]. Nonetheless, circulating EPCs have been assessed using different methodology techniques among different studies with conflicting results [30]. On the other hand, it seems rationale to suggest that acute disease exacerbations are associated with a significant increase in CD34+ cells followed by upregulation of serum VEGF concentrations, reflecting either a compensatory response or simply a bystander effect [69].

The mechanisms orchestrating the reduction in EPCs in stable COPD patients have not been delineated yet. However, various premises have been suggested, e.g. impaired mobilization and the proliferative capacity of BM-derived EPCs driven by hypoxemia [31, 70] and/or increased apoptosis of EPCs due mainly to oxidative stress and inflammatory cytokines that directly affect BM production of progenitor cells [31, 71]. An alternative notion supports that diminished circulating levels of EPCs may simply reflect enhanced recruitment in injured pulmonary vessels [27, 71].

Alveolar Epithelial Progenitor Cells and Epithelium Restoration

An alternative approach to the complications arising from exogenously administered stem cells, including low engraftment levels and rejection, is to exploit the stemness of resident lung stem cells. It is becoming increas-

ingly apparent that a considerable number of stem cell populations with broad regenerative and differentiative capacity, such as basal, secretory or Clara cells and type-II AECs, reside in small protected niches across the tracheo-bronchial tree. While in otherwise healthy individuals the above cells are recruited to sites of injury to accelerate tissue repair and restoration, it is believed that in genetically predisposed individuals, e.g. patients with chronic lung disease experiencing repetitive injurious stimuli, impaired mobilization coupled with diminished regenerative capacity and/or limited reservoir of resident stem cells is responsible for abnormal wound healing.

It seems rationale that the re-awakening of developmental pathways being in a dormant state in adult or injured lung may provide an effective alternative to alveologenesis [34]. One such cascade is the retinoid pathway with the major candidate all-trans retinoic acid (atRA) derived from vitamin A (retinol). atRA functions via nuclear retinoic acid receptors and modulates the synthesis of elastin, an essential structural component of the lung matrix in neonatal fibroblasts [34]. Exogenous instillation of atRA in rats induced alveolization [72] whereas mice mutant for retinoic acid receptor genes exhibited disrupted alveolar formation [73].

On the basis of the above experimental data, clinical trials assessing the safety and efficacy of oral administration of atRA in patients with emphysema have been conducted and although an acceptable safety profile was demonstrated, efficacy results were rather disappointing, as evaluated by radiological and functional parameters [74, 75]. However, efficacy endpoints were assessed 6 months after drug administration, a time point which is too short to anticipate structural changes with clinically apparent functional effects. Other biological agents that have been reported to have a regulatory role in alveolar formation in experimental models, including estrogens, hepatocyte growth factor, granulocyte colony-stimulating factor, fibroblast growth factor-7 and statins, are currently under investigation while results from pilot clinical trials are greatly anticipated [34].

Stem Cell Therapy in CF

CF, the most common life-shortening genetic disorder in Caucasians, affecting approximately 70,000 individuals worldwide, is an autosomal recessive genetic disorder that affects most critically the lungs, but also the pancreas, liver and intestines. It is characterized by abnormal transport of chloride and sodium across the epithelium, lead-

ing to thick, viscous secretions. It is caused by a mutation in the gene for the protein CF transmembrane conductance regulator (CFTR) [76, 77]. This protein is required for the transport of chloride and sodium ions across the epithelial membrane. The most common mutation, $\Delta F508$, is a deletion in 3 nucleotides that results in a loss of the amino acid phenylalanine at the 508th position on the protein [76, 77]. The gradually increasing incidence and the poor survival estimated at 37.5 years with no firmly established treatments apart from lung transplantation have urged chest physicians to rapidly develop personalized therapeutic approaches to alter the basic defect in the disease using gene-class-specific therapy, the so called CFTR modulators [78]. The latter represent therapies directed towards specific disease-causing mutations and the molecular pathways that underlie their cause. Nevertheless, results are preliminary and treatment approaches are still in the developmental stages.

In this context, stem cell technology has also penetrated into this fascinating therapeutic field leading to a number of ramifications, including the study and potential application of ESCs in patients with CF, as human $\Delta F508$ ESCs have been produced but not extensively studied [79]. So far the only studies reporting a method for producing patient-specific airway epithelial cells for disease modeling and in vitro drug testing were recently published by Wong et al. [80, 81]. In particular, investigators intriguingly demonstrated an in vitro differentiation protocol generating functional CFTR-expressing airway epithelium from human ESCs. A proof of concept analysis was also performed in a CF patient showing enhanced plasma membrane localization of mature CFTR protein. Another recent study reported the generation of disease-specific lung progenitor cells from human CF iPSCs that led to the formation of a fully functional respiratory epithelium, providing us with pivotal data for genetic lung diseases [82].

Stem Cell Therapy in Asthma

Asthma is a chronic inflammatory disorder of the airways in which many cells play an essential role. This chronic inflammation is associated with airway hyperresponsiveness that leads to recurrent episodes of variable airway obstruction that is often reversible either spontaneously or with treatment [83, 84]. Current treatment of asthma with inhaled corticosteroids and long-acting inhaled β_2 -agonists is highly effective, well tolerated, safe and relatively inexpensive, however many pa-

tients remain poorly controlled. While innumerable lines and funds of research are spent to improve these drug classes, major unmet needs, including better treatment of severe asthma (which shares several characteristics with COPD) as well as curative therapies for mild-to-moderate asthma that do not result in symptom recurrence when treatment is tapered or even discontinued, still represent considerable therapeutic shortcomings for chest physicians [83, 85].

In this context, a steadily increasing number of studies demonstrate the efficacy of either locally or systemically administered MSCs in a rapidly expanding spectrum of lung injury models in asthma (table 3). In specific, several groups of investigators either utilizing ovalbumin or even better airway allergens, including ragweed pollen, to produce a clinical and histological phenotype compatible with human asthma observed a beneficial effect of intravenously or intratracheally instilled MSCs, which was assessed by a significant decrease in airway hyperresponsiveness, eosinophilic Th2 inflammation, histologically documented injury and mucus metaplasia as well as abrogation of IgE serum concentrations [13, 86–90].

Despite the above promising data rendering MSCs wonderful candidates for fruitful therapeutic interventions in patients with severe persistent asthma nonresponding to conventional treatment, efforts to apply this evidence from bench to bedside have been restrained by considerable safety concerns, including possible malignant transformation of MSCs, on a longitudinal basis coupled with highly effective available current therapeutic regimens.

Future Challenges and Limitations

For these cell-based therapies to become truly evolutionary and be in the same trajectory as bronchodilators in the therapeutic field of chronic lung diseases, there is a number of challenges and limitations that should be addressed properly.

Lack of Stem Cell Clinical Trials in Patients with Chronic Lung Diseases

Although abundant lines of both human and experimental data have provided us with encouraging safety and efficacy data, unfortunately pulmonary and critical care medicine have traditionally lagged behind other fields, including hematology, cardiology and gastroenterology, in translational studies of potential new therapies, e.g. the use of reparative cells [13].

Table 3. Experimental data of stem cell therapy in animal models of asthma

Study year	Type of stem cells	Animal model	Route of administration	Outcome	Potential mechanisms of action
Bonfield et al. [86] 2010	Human BM-MSCs Plastic adherent	OVA-induced AR	IV	Improved histologic injury Decreased BALF inflammatory cytokines (IL-5; IL-13, IFN- γ), serum IgE, BALF iNOS	None specific Potentially soluble mediators
Cho and Roh [87] 2010	Mouse BM-MSCs	OVA-induced AR	IV	Decreased nasal inflammation, OVA-specific IgE, IgG1, IL-4, IL-5 Increased IFN- γ in cultured splenocytes	None specific Potentially soluble mediators
Nemeth et al. [88] 2010	Mouse BM-MSCs	Ragweed-induced AAI	1st IV 2nd IT	Decrease in histologic injury, BALF inflammatory cytokines, serum IgE	TGF- β 1 secretion
Park et al. [89] 2010	Mouse ADSCs	OVA-induced AAI	IV	Diminished airway hyperresponsiveness Decreased eosinophilic Th2 lung inflammation Th2 switching to Th1 immune response	None specific Potentially soluble mediators
Goodwin et al. [90] 2011	Mouse BM-MSCs	OVA-induced AAI	IV	Diminished airway hyperresponsiveness Decreased eosinophilic Th2 lung inflammation Th2 switching to Th1 immune response	None specific Potentially soluble mediators

AAI = Allergic airway inflammation; AR = allergic rhinitis; BALF = bronchoalveolar lavage fluid; IFN = interferon; iNOS = inducible nitric oxide synthase; IV = intravenous; OVA = ovalbumin..

More specifically, two published phase-I clinical trials of EPCs in primary pulmonary hypertension demonstrate both safety and efficacy [11, 91] while a third study exploiting the potential usefulness of autologous progenitor cells to act as vehicles of drug delivery, namely human endothelial nitric oxide synthase in patients with severe pulmonary artery hypertension, is currently under investigation. First anecdotal safety results from the latter study seem promising, and investigators are still recruiting eligible patients. On the other hand, evidence from a fourth study that has completed patient recruitment is still unknown.

Furthermore, the use of stem cell therapy is now being established in patients suffering from complications following acute myocardial infarction. In particular, six randomized placebo-controlled clinical trials estimating safety and efficacy of BM-derived stem cell therapy either locally or systemically administered to patients that have experienced acute myocardial infarction are now currently available in the medical literature. Although efficacy results arising from these studies seem rather confusing and conflicting with three trials reporting negative results and the remaining three demonstrating the effectiveness of stem cell treatment, which was assessed by the improvement in parameters such as left-ventricular function, quality of life and ventricular re-

modeling, nevertheless all of them were characterized by encouraging safety data [92–97].

The latter studies offered pivotal clinical insights that helped clinicians to overcome potential safety concerns emerging from the local or systematic delivery route of MSCs. Furthermore, in one of the aforementioned studies, authors came up with an exploratory finding as they reported lung function improvement (FEV₁) in the majority of patients, indicating either a hemodynamic effect on ejection fraction or alternatively homing of a large proportion of infused cells to the lungs and induction of anti-inflammatory and regenerative processes. The latter observation attracted the interest of chest physicians and triggered the launch of two clinical trials assessing the safety and efficacy of intravenous infusion of BM-derived MSCs in patients with moderate and severe COPD.

The first study was recently published by Ribeiro-Paes et al. [98] who reported a marginal statistically significant improvement in functional parameters as well as in exercise capacity in patients with severe COPD after an intravenous administration of autologous BM-derived MSCs. However, this trial was severely hampered by the limited number of patients enrolled (only 3 patients eligible for analysis) posing major limitations to the data presented. Regarding the second phase-II clinical trial, sponsored by

Table 4. Human data of stem cell therapy in patients with chronic lung diseases

Study year	Disease	Type of stem cells	Route of administration	Patients n	Outcome	Potential mechanisms of action
Ribeiro-Paes et al. [98] 2011	COPD	BM-MSCs	Intra-venous	3	Marginal improvement in FEV ₁	None specific Potential soluble mediators
Osiris Pharmaceuticals, 2012	COPD	BM-MSCs	Intra-venous	62	No improvement in FEV ₁	None specific Potential soluble mediators
Tzouvelekis et al. [100] 2011	IPF	ADSCs-SVF	Endo-bronchial	15	Disease stabilization 1 year after first infusion No improvement in FVC, DL _{CO} , 6MWD, high-resolution CT Improvement in QoL parameters	None specific Potential soluble mediators
Wong et al. [81] 2012	CF	ESCs	Intra-venous	1	Enhanced plasma membrane localization of mature functional CFTR	Construction of mature functional CFTR

6MWD = 6-Minute walking distance; DL_{CO} = diffusing lung capacity for carbon monoxide; FEV₁ = forced expiratory volume in 1 s; FVC = forced vital capacity; QoL = quality of life; SVF = stromal vascular fraction.

Osiris Pharmaceuticals, recruitment has been completed and a total of 62 patients with a diagnosis of moderate (n = 23) or severe (n = 39) COPD, based on the recent GOLD functional criteria [99], were enrolled and being followed for a period of 2 years in a placebo-controlled study. Despite the great hype that was generated, first anecdotal results are rather disappointing, highlighting the need for a careful study design before rigid conclusions can be drawn. Official findings and publication are greatly anticipated.

On the other hand, safety concerns based on IPF pathogenesis, which is still elusive and controversial, coupled with issues reflecting the origin and the potential of MSCs to differentiate into fibroblasts have severely hampered clinicians' efforts to apply, so far, cell-based therapies in the treatment of this dismal disease. To address the above concerns and to establish a rigid basis for future efficacy trials, we have conducted a nonrandomized unicentric, dose-ranging safety study of endobronchial infusion of autologous ADSCs (stromal vascular fraction) in IPF patients with moderate disease (FVC >50%, DL_{CO} >35%) pattern. As secondary exploratory endpoints, parameters reflecting functional and radiological disease status have been assessed. A total of 15 patients have been enrolled so far, and although interim analysis [100] in the first 12 recruited patients showed improvement in indicators of exercise capacity and quality of life, final evaluation failed to show such a benefit. Nonetheless, the safety data reported raised cli-

nicians' hopes and helped them to overcome their fears and concerns accelerating the conduction of well-designed randomized controlled clinical trials in the near future (table 4).

Setting Realistic Endpoints to Assess the Effectiveness of Stem Cells in Clinical Trials

Based on the above data from pilot studies estimating the safety and efficacy of the intravenous administration of either allogeneic or autologous BM-derived MSCs, it is essential to underline the need for a careful study design by setting feasible primary endpoints in order to reliably assess efficacy. In other words, it seems illusory to anticipate that stem cells promote lung renewal and completely reverse a disease phenotype that advances through years assessed by ameliorations in functional and radiological parameters, exercise capacity or quality of life within a period of 1 year or after 1 single MSC administration. It is far more reasonable to evaluate the effectiveness of cell-based therapies using biomarkers that reflect changes in the molecular phenotype of the airway epithelium and endothelium and estimate whether these alterations produce beneficial effects that are clinically occult but may be of primary clinical importance ultimately.

Precautions of Stem Cell Treatment

Currently, despite sound information supporting the application of stem cells either as first-line or adjuvant

treatment for chronic lung diseases, there are several safety precautions precluding their global applicability in the everyday clinical practice that should be addressed properly.

Firstly and most importantly, a still opening question is related to the yet unknown fate and potential mechanisms of actions of these cells once engrafted into a highly inflammatory and potentially dysplastic microenvironment. A panel of experts strongly believes on the fibrogenic and/or tumorigenic capacity of these cells on a longitudinal basis given the close association of chronic lung injury with malignant transformation. Since the risk of malignancy or even ectopic tissue formation is present with any cell type that is propagated *ex vivo*, experts in the field support the use of minimally manipulated cells, such as unfractionated stromal vascular fraction cells, versus more than minimally manipulated MSCs, such as culture-expanded BM-derived MSCs [3, 7, 101]. At this point, it is crucial to highlight that the use of either allogeneic or autologous MSCs is safe and well tolerable since extensive human and experimental data have starkly demonstrated that MSCs derived either from BM or adipose tissue do not express HLA-DR and seem to be less immunogenic than other cell types since they exert powerful immunosuppressive properties [3, 7, 101]. Furthermore, the therapeutic strategy of re-activating endogenous signals to induce lung regeneration may harbor detrimental effects leading from cell senescence to cell immortality and carcinogenesis.

Secondly, faulty or aberrant engraftment of these systemically administered cells may pose other still under-

estimated potential risks, including lethal pulmonary emboli or differentiation into bone or other inappropriate cellular structures.

Conclusions

Based on the above data, evidence indicates that an increasing, although not uniform, body of data supports to move forward from animal studies to clinical trials. Salient information arising from seminal clinical observations gives credence to the view that cell-based therapies may be a fruitful therapeutic strategy for lung repair and remodeling after injury. In the past 5 years, we witnessed major advances that increased our current state of knowledge from theoretical discussions to practical considerations. It is anticipated that the few 'brave' pilot investigations of the safety of stem cell treatment in chronic lung diseases will excite new fields of research to improve our current understanding of the mechanisms orchestrating lung renewal and sparking the design of large multicenter clinical trials, as it happens in other fields of medicine. Lastly, but most importantly, we should always bear mind that separating the hope from the hype when informing end-stage lung disease patients represents the most crucial step for the moment.

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References

- 1 Fuchs E, Segre JA: Stem cells: a new lease on life. *Cell* 2000;100:143–155.
- 2 Gomperts BN, Strieter RM: Stem cells and chronic lung disease. *Annu Rev Med* 2007;58:285–298.
- 3 Rankin S: Mesenchymal stem cells. *Thorax* 2012;67:565–566.
- 4 Crystal RG, Randell SH, Engelhardt JF, Vovnow J, Sunday ME: Airway epithelial cells: current concepts and challenges. *Proc Am Thorac Soc* 2008;5:772–777.
- 5 Huertas A, Palange P: Circulating endothelial progenitor cells and chronic pulmonary diseases. *Eur Respir J* 2011;37:426–431.
- 6 Siniscalco D, Sullo N, Maione S, Rossi F, D'Agostino B: Stem cell therapy: the great promise in lung disease. *Ther Adv Respir Dis* 2008;2:173–177.
- 7 Tzouvelekis A, Antoniadis A, Bouros D: Stem cell therapy in pulmonary fibrosis. *Curr Opin Pulm Med* 2011;17:368–373.
- 8 Kajstura J, Rota M, Hall SR, Hosoda T, D'Amario D, Sanada F, Zheng H, Ogorek B, Rondon-Clavo C, Ferreira-Martins J, Matsuda A, Arranto C, Goichberg P, Giordano G, Haley KJ, Bardelli S, Rayatzadeh H, Liu X, Quaini F, Liao R, Leri A, Perrella MA, Loscalzo J, Anversa P: Evidence for human lung stem cells. *N Engl J Med* 2011;364:1795–1806.
- 9 Rankin SM: Chemokines and adult bone marrow stem cells. *Immunol Lett* 2012;145:47–54.
- 10 Barnes PJ: Chronic obstructive pulmonary disease: effects beyond the lungs. *PLoS Med* 2010;7:e1000220.
- 11 Wang XX, Zhang FR, Shang YP, Zhu JH, Xie XD, Tao QM, Zhu JH, Chen JZ: Transplantation of autologous endothelial progenitor cells may be beneficial in patients with idiopathic pulmonary arterial hypertension: a pilot randomized controlled trial. *J Am Coll Cardiol* 2007;49:1566–1571.
- 12 Zeng C, Wang X, Hu X, Chen J, Wang L: Autologous endothelial progenitor cells transplantation for the therapy of primary pulmonary hypertension. *Med Hypotheses* 2007;68:1292–1295.
- 13 Weiss DJ, Bertoncello I, Borok Z, Kim C, Panoskaltis-Mortari A, Reynolds S, Rojas M, Stripp B, Warburton D, Prockop DJ: Stem cells and cell therapies in lung biology and lung diseases. *Proc Am Thorac Soc* 2011;8:223–272.

- 14 Wolf D, Wolf AM: Mesenchymal stem cells as cellular immunosuppressants. *Lancet* 2008; 371:1553–1554.
- 15 Garcia-Gomez I, Elvira G, Zapata AG, Lama-na ML, Ramirez M, Castro JG, Arranz MG, Vicente A, Bueren J, Garcia-Olmo D: Mesenchymal stem cells: biological properties and clinical applications. *Expert Opin Biol Ther* 2010;10:1453–1468.
- 16 Gronthos S, Mankani M, Brahmi J, Robey PG, Shi S: Postnatal human dental pulp stem cells (DPSCs) in vitro and in vivo. *Proc Natl Acad Sci USA* 2000;97:13625–13630.
- 17 Kotton DN, Fine A: Lung stem cells. *Cell Tissue Res* 2008;331:145–156.
- 18 Gimble JM, Guilak F, Bunnell BA: Clinical and preclinical translation of cell-based therapies using adipose tissue-derived cells. *Stem Cell Res Ther* 2010;1:19.
- 19 Hong SJ, Traktuev DO, March KL: Therapeutic potential of adipose-derived stem cells in vascular growth and tissue repair. *Curr Opin Organ Transplant* 2010;15:86–91.
- 20 Kondo K, Shintani S, Shibata R, Murakami H, Murakami R, Imaizumi M, Kitagawa Y, Murohara T: Implantation of adipose-derived regenerative cells enhances ischemia-induced angiogenesis. *Arterioscler Thromb Vasc Biol* 2009;29:61–66.
- 21 Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, Moorman MA, Simonetti DW, Craig S, Marshak DR: Multilineage potential of adult human mesenchymal stem cells. *Science* 1999;284:143–147.
- 22 Traktuev DO, Prater DN, Merfeld-Clauss S, Sanjeevaiah AR, Saadatzaheh MR, Murphy M, Johnstone BH, Ingram DA, March KL: Robust functional vascular network formation in vivo by cooperation of adipose progenitor and endothelial cells. *Circ Res* 2009;104:1410–1420.
- 23 Arciniegas E, Neves CY, Carrillo LM, Zambrano EA, Ramirez R: Endothelial-mesenchymal transition occurs during embryonic pulmonary artery development. *Endothelium* 2005;12:193–200.
- 24 Arciniegas E, Frid MG, Douglas IS, Stenmark KR: Perspectives on endothelial-to-mesenchymal transition: potential contribution to vascular remodeling in chronic pulmonary hypertension. *Am J Physiol Lung Cell Mol Physiol* 2007;293:L1–L8.
- 25 Barbera JA, Peinado VI: Vascular progenitor cells in chronic obstructive pulmonary disease. *Proc Am Thorac Soc* 2011;8:528–534.
- 26 Luan Y, Zhang ZH, Wei DE, Lu Y, Wang YB: Effects of autologous bone marrow mononuclear cells implantation in canine model of pulmonary hypertension. *Circ J* 2012;76:977–985.
- 27 Peinado VI, Ramirez J, Roca J, Rodriguez-Roisin R, Barbera JA: Identification of vascular progenitor cells in pulmonary arteries of patients with chronic obstructive pulmonary disease. *Am J Respir Cell Mol Biol* 2006;34:257–263.
- 28 Raoul W, Wagner-Ballon O, Saber G, Hulin A, Marcos E, Giraudier S, Vainchenker W, Adnot S, Eddahibi S, Maitre B: Effects of bone marrow-derived cells on monocrotaline- and hypoxia-induced pulmonary hypertension in mice. *Respir Res* 2007;8:8.
- 29 Zhu P, Huang L, Ge X, Yan F, Wu R, Ao Q: Transdifferentiation of pulmonary arteriolar endothelial cells into smooth muscle-like cells regulated by myocardium involved in hypoxia-induced pulmonary vascular remodelling. *Int J Exp Pathol* 2006;87:463–474.
- 30 Caramori G, Rigolin GM, Mazzoni F, Leprotti S, Campioni P, Papi A: Circulating endothelial stem cells are not decreased in pulmonary emphysema or COPD. *Thorax* 2010;65:554–555.
- 31 Fadini GP, Schiavon M, Cantini M, Baesso I, Faccio M, Miorin M, Tassinato M, de Kreutzenberg SV, Avogaro A, Agostini C: Circulating progenitor cells are reduced in patients with severe lung disease. *Stem Cells* 2006;24:1806–1813.
- 32 Huertas A, Testa U, Riccioni R, Petrucci E, Riti V, Savi D, Serra P, Bonsignore MR, Palange P: Bone marrow-derived progenitors are greatly reduced in patients with severe COPD and low-BMI. *Respir Physiol Neurobiol* 2010;170:23–31.
- 33 Palange P, Testa U, Huertas A, Calabro L, Antonucci R, Petrucci E, Pelosi E, Pasquini L, Satta A, Morici G, Vignola MA, Bonsignore MR: Circulating haemopoietic and endothelial progenitor cells are decreased in COPD. *Eur Respir J* 2006;27:529–541.
- 34 Hind M, Maden M: Is a regenerative approach viable for the treatment of COPD? *Br J Pharmacol* 2011;163:106–115.
- 35 Wang D, Morales JE, Calame DG, Alcorn JL, Wetsel RA: Transplantation of human embryonic stem cell-derived alveolar epithelial type II cells abrogates acute lung injury in mice. *Mol Ther* 2010;18:625–634.
- 36 Somers A, Jean JC, Sommer CA, Omari A, Ford CC, Mills JA, Ying L, Sommer AG, Jean JM, Smith BW, Lafyatis R, Demierre MF, Weiss DJ, French DL, Gadue P, Murphy GJ, Mostoslavsky G, Kotton DN: Generation of transgene-free lung disease-specific human induced pluripotent stem cells using a single excisable lentiviral stem cell cassette. *Stem Cells* 2010;28:1728–1740.
- 37 Sommer CA, Stadtfeld M, Murphy GJ, Hochedlinger K, Kotton DN, Mostoslavsky G: Induced pluripotent stem cell generation using a single lentiviral stem cell cassette. *Stem Cells* 2009;27:543–549.
- 38 Sommer CA, Mostoslavsky G: The evolving field of induced pluripotency: recent progress and future challenges. *J Cell Physiol* 2013;228:267–275.
- 39 American Thoracic Society. Idiopathic pulmonary fibrosis: diagnosis and treatment. International consensus statement. American Thoracic Society (ATS), and the European Respiratory Society (ERS). *Am J Respir Crit Care Med* 2000;161:646–664.
- 40 The IPFnet strategy: creating a comprehensive approach in the treatment of idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med* 2010;181:527–528.
- 41 Wuyts WA, Agostini C, Antoniou K, Bouros D, Chambers R, Cottin V, Egan J, Lambrecht B, Lories R, Parfrey H, Prasse A, Robalo-Cordeiro C, Verbeke E, Verschakelen J, Wells A, Verleden G: The pathogenesis of pulmonary fibrosis: a moving target. *Eur Respir J* 2012, E-pub ahead of print.
- 42 Selman M, King TE, Pardo A: Idiopathic pulmonary fibrosis: prevailing and evolving hypotheses about its pathogenesis and implications for therapy. *Ann Intern Med* 2001;134:136–151.
- 43 Selman M, Pardo A: Idiopathic pulmonary fibrosis: misunderstandings between epithelial cells and fibroblasts? *Sarcoidosis Vasc Diffuse Lung Dis* 2004;21:165–172.
- 44 Tzouvelekis A, Kouliatsis G, Anevlavis S, Bouros D: Serum biomarkers in interstitial lung diseases. *Respir Res* 2005;6:78.
- 45 Tzouvelekis A, Harokopos V, Paparountas T, Oikonomou N, Chatziioannou A, Vilaras G, Tsiambas E, Karameris A, Bouros D, Aidinis V: Comparative expression profiling in pulmonary fibrosis suggests a role of hypoxia-inducible factor-1alpha in disease pathogenesis. *Am J Respir Crit Care Med* 2007;176:1108–1119.
- 46 Bouros D, Antoniou KM: Current and future therapeutic approaches in idiopathic pulmonary fibrosis. *Eur Respir J* 2005;26:693–702.
- 47 Raghu G: Improving the standard of care for patients with idiopathic pulmonary fibrosis requires participation in clinical trials. *Chest* 2009;136:330–333.
- 48 Ortiz LA, Gambelli F, McBride C, Gaupp D, Baddoo M, Kaminski N, Phinney DG: Mesenchymal stem cell engraftment in lung is enhanced in response to bleomycin exposure and ameliorates its fibrotic effects. *Proc Natl Acad Sci USA* 2003;100:8407–8411.
- 49 Rojas M, Xu J, Woods CR, Mora AL, Spears W, Roman J, Brigham KL: Bone marrow-derived mesenchymal stem cells in repair of the injured lung. *Am J Respir Cell Mol Biol* 2005;33:145–152.
- 50 Germano D, Blyszczuk P, Valaperti A, Kania G, Dirnhofer S, Landmesser U, Luscher TF, Hunziker L, Zulewski H, Eriksson U: Prominin-1/CD133+ lung epithelial progenitors protect from bleomycin-induced pulmonary fibrosis. *Am J Respir Crit Care Med* 2009;179:939–949.
- 51 Zhao F, Zhang YF, Liu YG, Zhou JJ, Li ZK, Wu CG, Qi HW: Therapeutic effects of bone marrow-derived mesenchymal stem cells engraftment on bleomycin-induced lung injury in rats. *Transplant Proc* 2008;40:1700–1705.

- 52 Moodley Y, Atienza D, Manuelpillai U, Samuel CS, Tchongue J, Ilancheran S, Boyd R, Trounson A: Human umbilical cord mesenchymal stem cells reduce fibrosis of bleomycin-induced lung injury. *Am J Pathol* 2009; 175:303–313.
- 53 Aguilar S, Scotton CJ, McNulty K, Nye E, Stamp G, Laurent G, Bonnet D, Janes SM: Bone marrow stem cells expressing keratinocyte growth factor via an inducible lentivirus protects against bleomycin-induced pulmonary fibrosis. *PLoS One* 2009;4:e8013.
- 54 Kumamoto M, Nishiwaki T, Matsuo N, Kimura H, Matsushima K: Minimally cultured bone marrow mesenchymal stem cells ameliorate fibrotic lung injury. *Eur Respir J* 2009;34:740–748.
- 55 Lee SH, Jang AS, Kim YE, Cha JY, Kim TH, Jung S, Park SK, Lee YK, Won JH, Kim YH, Park CS: Modulation of cytokine and nitric oxide by mesenchymal stem cell transfer in lung injury/fibrosis. *Respir Res* 2010;11:16.
- 56 Cargnoni A, Gibelli L, Tosini A, Signoroni PB, Nassuato C, Arienti D, Lombardi G, Albertini A, Wengler GS, Parolini O: Transplantation of allogeneic and xenogeneic placenta-derived cells reduces bleomycin-induced lung fibrosis. *Cell Transplant* 2009;18:405–422.
- 57 Spitalieri P, Quitadamo MC, Orlandi A, Guerra L, Giardina E, Casavola V, Novelli G, Saltini C, Sangiuolo F: Rescue of murine silica-induced lung injury and fibrosis by human embryonic stem cells. *Eur Respir J* 2012;39:446–457.
- 58 Sueblinvong V, Loi R, Eisenhauer PL, Bernstein IM, Suratt BT, Spees JL, Weiss DJ: Derivation of lung epithelium from human cord blood-derived mesenchymal stem cells. *Am J Respir Crit Care Med* 2008;177:701–711.
- 59 Adcock IM, Caramori G, Barnes PJ: Chronic obstructive pulmonary disease and lung cancer: new molecular insights. *Respiration* 2011; 81:265–284.
- 60 Barnes PJ: Small airways in COPD. *N Engl J Med* 2004;350:2635–2637.
- 61 Barnes PJ: Emerging pharmacotherapies for COPD. *Chest* 2008;134:1278–1286.
- 62 Chilosi M, Poletti V, Rossi A: The pathogenesis of COPD and IPF: distinct horns of the same devil? *Respir Res* 2012;13:3.
- 63 Shigemura N, Okumura M, Mizuno S, Imanishi Y, Nakamura T, Sawa Y: Autologous transplantation of adipose tissue-derived stromal cells ameliorates pulmonary emphysema. *Am J Transplant* 2006;6:2592–2600.
- 64 Shigemura N, Okumura M, Mizuno S, Imanishi Y, Matsuyama A, Shiono H, Nakamura T, Sawa Y: Lung tissue engineering technique with adipose stromal cells improves surgical outcome for pulmonary emphysema. *Am J Respir Crit Care Med* 2006;174:1199–1205.
- 65 Zhen G, Liu H, Gu N, Zhang H, Xu Y, Zhang Z: Mesenchymal stem cells transplantation protects against rat pulmonary emphysema. *Front Biosci* 2008;13:3415–3422.
- 66 Zhen G, Xue Z, Zhao J, Gu N, Tang Z, Xu Y, Zhang Z: Mesenchymal stem cell transplantation increases expression of vascular endothelial growth factor in papain-induced emphysematous lungs and inhibits apoptosis of lung cells. *Cytotherapy* 2010;12:605–614.
- 67 Katsha AM, Ohkouchi S, Xin H, Kanehira M, Sun R, Nukiwa T, Saijo Y: Paracrine factors of multipotent stromal cells ameliorate lung injury in an elastase-induced emphysema model. *Mol Ther* 2011;19:196–203.
- 68 Schweitzer KS, Johnstone BH, Garrison J, Rush NI, Cooper S, Traktuev DO, Feng D, Adamowicz JJ, Van DM, Fisher AJ, Kamocki K, Brown MB, Presson RG Jr, Broxmeyer HE, March KL, Petrache I: Adipose stem cell treatment in mice attenuates lung and systemic injury induced by cigarette smoking. *Am J Respir Crit Care Med* 2011;183:215–225.
- 69 Sala E, Villena C, Balaguer C, Rios A, Fernandez-Palomeque C, Cosio BG, Garcia J, Noguera A, Agusti A: Abnormal levels of circulating endothelial progenitor cells during exacerbations of COPD. *Lung* 2010;188:331–338.
- 70 Takahashi T, Suzuki S, Kubo H, Yamaya M, Kurosawa S, Kato M: Impaired endothelial progenitor cell mobilization and colony-forming capacity in chronic obstructive pulmonary disease. *Respirology* 2011;16:680–687.
- 71 Marsboom G, Pokreisz P, Gheysens O, Vermeersch P, Gillijns H, Pellen M, Liu X, Colleen D, Janssens S: Sustained endothelial progenitor cell dysfunction after chronic hypoxia-induced pulmonary hypertension. *Stem Cells* 2008;26:1017–1026.
- 72 Massaro GD, Massaro D: Postnatal treatment with retinoic acid increases the number of pulmonary alveoli in rats. *Am J Physiol* 1996; 270:L305–L310.
- 73 McGowan S, Jackson SK, Jenkins-Moore M, Dai HH, Chambon P, Snyder JM: Mice bearing deletions of retinoic acid receptors demonstrate reduced lung elastin and alveolar numbers. *Am J Respir Cell Mol Biol* 2000;23: 162–167.
- 74 Roth MD, Connett JE, D'Armiento JM, Foronjy RF, Friedman PJ, Goldin JG, Louis TA, Mao JT, Muindi JR, O'Connor GT, Ramsdell JW, Ries AL, Scharf SM, Schluger NW, Scurba FC, Skeans MA, Walter RE, Wendt CH, Wise RA: Feasibility of retinoids for the treatment of emphysema study. *Chest* 2006;130: 1334–1345.
- 75 Mao JT, Goldin JG, Derman J, Ibrahim G, Brown MS, Emerick A, McNitt-Gray MF, Gjertson DW, Estrada F, Tashkin DP, Roth MD: A pilot study of all-trans-retinoic acid for the treatment of human emphysema. *Am J Respir Crit Care Med* 2002;165:718–723.
- 76 Burke W: Genomics as a probe for disease biology. *N Engl J Med* 2003;349:969–974.
- 77 Stern RC: The diagnosis of cystic fibrosis. *N Engl J Med* 1997;336:487–491.
- 78 Pasyk S, Molinski S, Yu W, Eckford PD, Bear CE: Identification and validation of hits from high throughput screens for CFTR modulators. *Curr Pharm Des* 2012;18:628–641.
- 79 Pickering SJ, Minger SL, Patel M, Taylor H, Black C, Burns CJ, Ekonomou A, Braude PR: Generation of a human embryonic stem cell line encoding the cystic fibrosis mutation deltaF508, using preimplantation genetic diagnosis. *Reprod Biomed Online* 2005;10:390–397.
- 80 Wong AP, Keating A, Lu WY, Duchesneau P, Wang X, Sacher A, Hu J, Waddell TK: Identification of a bone marrow-derived epithelial-like population capable of repopulating injured mouse airway epithelium. *J Clin Invest* 2009;119:336–348.
- 81 Wong AP, Bear CE, Chin S, Pasceri P, Thompson TO, Huan LJ, Ratjen F, Ellis J, Rossant J: Directed differentiation of human pluripotent stem cells into mature airway epithelia expressing functional CFTRTR protein. *Nat Biotechnol* 2012;30:876–882.
- 82 Mou H, Zhao R, Sherwood R, Ahfeldt T, Lapey A, Wain J, Sicilian L, Izvolsky K, Musunuru K, Cowan C, Rajagopal J: Generation of multipotent lung and airway progenitors from mouse ESCs and patient-specific cystic fibrosis iPSCs. *Cell Stem Cell* 2012;10:385–397.
- 83 Barnes PJ: New drugs for asthma. *Nat Rev Drug Discov* 2004;3:831–844.
- 84 Barnes PJ: Intrinsic asthma: not so different from allergic asthma but driven by superantigens? *Clin Exp Allergy* 2009;39:1145–1151.
- 85 Barnes PJ: New therapies for asthma: is there any progress? *Trends Pharmacol Sci* 2010;31: 335–343.
- 86 Bonfield TL, Kolozs M, Lennon DP, Zuchowski B, Yang SE, Caplan AI: Human mesenchymal stem cells suppress chronic airway inflammation in the murine ovalbumin asthma model. *Am J Physiol Lung Cell Mol Physiol* 2010;299:L760–L770.
- 87 Cho KS, Roh HJ: Immunomodulatory effects of adipose-derived stem cells in airway allergic diseases. *Curr Stem Cell Res Ther* 2010;5: 111–115.
- 88 Nemeth K, Keane-Myers A, Brown JM, Metcalfe DD, Gorham JD, Bundoc VG, Hodges MG, Jelinek I, Madala S, Karpati S, Mezey E: Bone marrow stromal cells use TGF-beta to suppress allergic responses in a mouse model of ragweed-induced asthma. *Proc Natl Acad Sci USA* 2010;107:5652–5657.
- 89 Park HK, Cho KS, Park HY, Shin DH, Kim YK, Jung JS, Park SK, Roh HJ: Adipose-derived stromal cells inhibit allergic airway inflammation in mice. *Stem Cells Dev* 2010;19: 1811–1818.

- 90 Goodwin M, Sueblinvong V, Eisenhauer P, Ziats NP, LeClair L, Poynter ME, Steele C, Rincon M, Weiss DJ: Bone marrow-derived mesenchymal stromal cells inhibit Th2-mediated allergic airways inflammation in mice. *Stem Cells* 2011;29:1137–1148.
- 91 Zhu JH, Wang XX, Zhang FR, Shang YP, Tao QM, Zhu JH, Chen JZ: Safety and efficacy of autologous endothelial progenitor cells transplantation in children with idiopathic pulmonary arterial hypertension: open-label pilot study. *Pediatr Transplant* 2008;12:650–655.
- 92 Perin EC, Silva GV, Henry TD, Cabreira-Hansen MG, Moore WH, Coulter SA, Herlihy JP, Fernandes MR, Cheong BY, Flamm SD, Traverse JH, Zheng Y, Smith D, Shaw S, Westbrook L, Olson R, Patel D, Gahremanpour A, Canales J, Vaughn WK, Willerson JT: A randomized study of transcatheter injection of autologous bone marrow mononuclear cells and cell function analysis in ischemic heart failure (FOCUS-HF). *Am Heart J* 2011;161:1078–1087.
- 93 Perin EC, Willerson JT, Pepine CJ, Henry TD, Ellis SG, Zhao DX, Silva GV, Lai D, Thomas JD, Kronenberg MW, Martin AD, Anderson RD, Traverse JH, Penn MS, Anwaruddin S, Hatzopoulos AK, Gee AP, Taylor DA, Cogle CR, Smith D, Westbrook L, Chen J, Handberg E, Olson RE, Geither C, Bowman S, Francescon J, Baraniuk S, Piller LB, Simpson LM, Loghin C, Aguilar D, Richman S, Zierold C, Bettencourt J, Sayre SL, Vojvodic RW, Skarlatos SI, Gordon DJ, Ebert RF, Kwak M, Moye LA, Simari RD: Effect of transcatheter delivery of autologous bone marrow mononuclear cells on functional capacity, left ventricular function, and perfusion in chronic heart failure: the FOCUS-CCTRN trial. *JAMA* 2012;307:1717–1726.
- 94 Traverse JH, Henry TD, Vaughan DE, Ellis SG, Pepine CJ, Willerson JT, Zhao DX, Simpson LM, Penn MS, Byrne BJ, Perin EC, Gee AP, Hatzopoulos AK, McKenna DH, Forder JR, Taylor DA, Cogle CR, Baraniuk S, Olson RE, Jorgenson BC, Sayre SL, Vojvodic RW, Gordon DJ, Skarlatos SI, Moye LA, Simari RD: LateTIME: a phase-II, randomized, double-blinded, placebo-controlled, pilot trial evaluating the safety and effect of administration of bone marrow mononuclear cells 2 to 3 weeks after acute myocardial infarction. *Tex Heart Inst J* 2010;37:412–420.
- 95 Traverse JH, Henry TD, Ellis SG, Pepine CJ, Willerson JT, Zhao DX, Forder JR, Byrne BJ, Hatzopoulos AK, Penn MS, Perin EC, Baran KW, Chambers J, Lambert C, Raveendran G, Simon DI, Vaughan DE, Simpson LM, Gee AP, Taylor DA, Cogle CR, Thomas JD, Silva GV, Jorgenson BC, Olson RE, Bowman S, Francescon J, Geither C, Handberg E, Smith DX, Baraniuk S, Piller LB, Loghin C, Aguilar D, Richman S, Zierold C, Bettencourt J, Sayre SL, Vojvodic RW, Skarlatos SI, Gordon DJ, Ebert RF, Kwak M, Moye LA, Simari RD: Effect of intracoronary delivery of autologous bone marrow mononuclear cells 2 to 3 weeks following acute myocardial infarction on left ventricular function: the LateTIME randomized trial. *JAMA* 2011;306:2110–2119.
- 96 Traverse JH, Henry TD, Pepine CJ, Willerson JT, Zhao DX, Ellis SG, Forder JR, Anderson RD, Hatzopoulos AK, Penn MS, Perin EC, Chambers J, Baran KW, Raveendran G, Lambert C, Lerman A, Simon DI, Vaughan DE, Lai D, Gee AP, Taylor DA, Cogle CR, Thomas JD, Olson RE, Bowman S, Francescon J, Geither C, Handberg E, Kappenman C, Westbrook L, Piller LB, Simpson LM, Baraniuk S, Loghin C, Aguilar D, Richman S, Zierold C, Spoon DB, Bettencourt J, Sayre SL, Vojvodic RW, Skarlatos SI, Gordon DJ, Ebert RF, Kwak M, Moye LA, Simari RD: Effect of the use and timing of bone marrow mononuclear cell delivery on left ventricular function after acute myocardial infarction: the TIME randomized trial. *JAMA* 2012;308:2380–2389.
- 97 Hare JM, Traverse JH, Henry TD, Dib N, Strumpf RK, Schulman SP, Gerstenblith G, DeMaria AN, Denktas AE, Gammon RS, Hermiller JB Jr, Reisman MA, Schaer GL, Sherman W: A randomized, double-blind, placebo-controlled, dose-escalation study of intravenous adult human mesenchymal stem cells (prochymal) after acute myocardial infarction. *J Am Coll Cardiol* 2009;54:2277–2286.
- 98 Ribeiro-Paes JT, Bilaqui A, Greco OT, Ruiz MA, Marcelino MY, Stessuk T, de Faria CA, Lago MR: Unicentric study of cell therapy in chronic obstructive pulmonary disease/pulmonary emphysema. *Int J Chron Obstruct Pulmon Dis* 2011;6:63–71.
- 99 Global Strategy for the Diagnosis, Management and Prevention of Chronic Obstructive Pulmonary Disease 2009. Global Initiative for Chronic Obstructive Lung Disease (GOLD), 2011, www.goldcopd.com.
- 100 Tzouvelekis A, Koliakos G, Ntoliou P, Baira I, Bouros E, Oikonomou A, Zissimopoulos A, Kolios G, Kakagia D, Paspaliaris V, Kotsianidis I, Froudarakis M, Bouros D: Stem cell therapy for idiopathic pulmonary fibrosis: a protocol proposal. *J Transl Med* 2011;9:182.
- 101 Jones CP, Rankin SM: Bone marrow-derived stem cells and respiratory disease. *Chest* 2011;140:205–211.

Erratum

The authors of the article entitled 'Stem Cell Treatment for Chronic Lung Diseases' [Respiration 2013;85:179–192] wish to publish the following corrections.

On page 182, right column, first paragraph, the following text should be published after the last sentence:

In particular, Germano et al. [50] intratracheally injected, in the BLM-model of lung fibrosis, BM-derived pulmonary progenitor cells that were different from MSCs since they expressed Proliferating Cell Nuclear Antigen (PCNA) and hematopoietic CD45 marker, whereas no expression of typical mesenchymal markers was observed. Intriguingly a reduction of histologic lesions, collagen deposition and BALF inflammatory cells was reported. The latter therapeutic effect was mainly attributed to the production of nitric oxide and was associated with engraftment and differentiation into alveolar type II epithelial cells.

On page 183, table 1, the third line should read as follows:

Table 1. Experimental data of stem cell therapy in animal models of pulmonary fibrosis

Study year	Type of stem cells	Animal model	Route of administration	Outcome	Potential mechanisms of action
Germano et al. [50] 2009	Mouse Lung BM-derived Proliferating Cell Nuclear Antigen (PCNA)/CD133 ⁺ cells	BLM	Intra-tracheal	Decreased systemic inflammatory cytokines (IL-1 β , IFN- γ , IL-6, IL-8, MIP-1 α), BALF inflammatory cells and histologic lesions	Nitric oxide production; alveolar epithelium differentiation