Mesenchymal stem cells for therapeutic applications in pulmonary medicine

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Abstract

Introduction: Mesenchymal stem cells (MSCs) of different biological sources are in Phase 1 clinical trials and are being considered for Phase 2 therapy of lung disorders, and lung (progenitor) cells derived from pluripotent stem cells (SCs) are under development in preclinical animal models.

Sources of data: PubMed.gov and ClinicalTrials.gov.

Areas of agreement: There is consensus about the therapeutic potential of transplanted SCs, mainly MSCs, primarily involves paracrine ‘bystander’ effects that confer protection of the epithelial and endothelial linings of the lung caused by inflammation and/or fibrosis and lead to increased survival in animal models. Clinical trials of Phase 1 indicate safety and suggest that the efficacy of SC therapy in patients with various lung diseases will require a higher dosage than previously evaluated.

Areas of controversy: A growing interest in the re-epithelialization and re-endothelialization of damaged lung tissue involves the putative pulmonary differentiation of exogenous MSCs. Currently, it is not clear whether or not the observed regeneration of distal airways/vasculature is derived from lung-resident and/or transplanted SCs.

Growing points: Important topics under investigation include optimization of the cell source with a decrease in cell population heterogeneity characterized by defined markers, route of delivery for effective treatment, potential dose and therapeutic protocol of SC application, development of quantitative assays and biomarkers of lung disease and repair, and the potential use of tissue engineered lung.
Areas timely for developing research: Ability of MSCs to differentiate into epithelial cells of the lung, use of autologous induced pluripotent SCs (iPSCs) derived from the patients, complete biochemical characterization of the secretome of SCs used for therapy, and the incorporation of simultaneous and/or subsequent treatment with drugs which also aid in lung repair and regeneration.

Cautionary note: Although safety of MSC-based cell therapy was proved in Phase 1, efficacy, long-term survival and preservation of lung respiratory function need to be further evaluated, cautioning against hastily translating SCs therapy from animal models of lung injury to clinical trials of patients with lung disorders.

Key words: stem cells, bioengineered lung, chronic lung diseases, transplantation, decellularized scaffold, recellularization, clinical applications, bystander effect, secretome

Introduction
A variety of exogenous and endogenous stem cells (SCs) are being considered for repairing, remodeling and/or regenerating damaged lungs. Specifically, mesenchymal stem cells (MSCs) isolated from different tissues are of particular interest as a source for pulmonary SC therapy because of their abundance, ease of isolation and characterization, multipotency and pleiotropic therapeutic effects, as well as the ability of MSCs to efficiently home to the sites of injury, and the beneficial role of the cell’s secretome. Studies in preclinical models of a variety of pediatric and adult pulmonary diseases, such as bronchopulmonary dysplasia (BPD), acute respiratory distress syndrome (ARDS), chronic lower respiratory disease (CLRD), cystic fibrosis (CF) and idiopathic pulmonary fibrosis (IPF) demonstrated MSC efficacy in attenuating lung injury and seem to mitigate the impaired epithelial repair and/or regeneration in these experimental models (Table 1). Sharing some common mechanisms of injury that damage/destroy airway epithelium and vascular endothelium followed by an inflammatory response, these lung diseases add to the burden of the health-care system. Current therapies are palliative only: they address the symptoms, but they are yet inefficient for rescuing or regenerating cellular function or even halting the pathological process. Therefore, SC therapies that promote lung repair and/or regeneration provide an alternative to current therapeutic approaches.

Therapeutic effect of SCs in experimental models of lung disease
Select preclinical animal models for the five major pathologies defined previously are listed in Table 1. These models include:

- BPD: Hyperoxia-induced lung injury in neonatal rodents, or double transgenic fas ligand (FASL)/Clara cell secretory protein (CCSP) rodent in combination with tetracycline exposure;
- ARDS: bacterial pneumonia in sheep, lipopolysaccharide (LPS)-induced inflammation in rodents or acute lung injury (ALI) by oleic acid intravenous infusion in rabbits;
- CLRD: cigarette smoke exposure in guinea pigs or rodents, LPS in combination with cigarette smoke and mild hypoxia in rodents, Bermuda grass allergen (BGA) asthma induction in felines or albumin sensitization for airway inflammation in rodents;
- CF: CF-transmembrane conductance regulator-knockout (CFTR-KO) rodents with or without secondary naphthalene-induced lung injury;

In each of these experimental models, a variety of SCs from various species have been used for therapy, mostly MSCs of different tissue origins, but also pluripotent (embryonic (ESC) and induced pluripotent SCs (iPSC)), endothelial progenitor cells (EPCs/ECFCs) or adult lung mixed lineage cell (LMDEC).
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<td>BPD&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Hyperoxia neonatal lung injury in rodent</td>
<td>rBMSC&lt;sup&gt;f&lt;/sup&gt;</td>
<td>IV&lt;sup&gt;′&lt;/sup&gt;</td>
<td>Protection of alveoli, reduced inflammation</td>
<td>Bystander paracrine effect on hypertension</td>
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<td>hECFC&lt;sup&gt;g&lt;/sup&gt;</td>
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<td>Protection of alveoli, vascular growth</td>
<td>Bystander paracrine effect</td>
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<td>rBMSC&lt;sup&gt;f&lt;/sup&gt;</td>
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<td>Increased survival, protection of alveoli, vascular growth, reduced pulmonary hypertension</td>
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<td>Not reported</td>
<td>Differentiation to lung resident SCs</td>
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<td>ARDs&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>LPS-induced inflammation in rodent</td>
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<td>mBMSC&lt;sup&gt;k&lt;/sup&gt;</td>
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<td>Decreased emphysema, decreased inflammation, improved pulmonary function, decreased level of metalloproteinases</td>
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<td>BGA asthma induction in felines</td>
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<td>CF&lt;sup&gt;d&lt;/sup&gt;</td>
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<td>CFTR-KO rodent</td>
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<td></td>
<td>mLMEDC&lt;sup&gt;p&lt;/sup&gt;</td>
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*Lung disease: *BPD, bronchopulmonary dysplasia; ARDS, acute respiratory distress syndrome; CLRD, chronic lower respiratory diseases; CF, cystic fibrosis; IPF, idiopathic pulmonary fibrosis. 
*Stem cell used for therapy: *rBMSC, rat bone marrow-derived MSC; hECFC, human endothelial colony forming cell; hUCBMSC, human umbilical cord blood-derived MSCs; hUCBHSC, human umbilical cord blood-derived hematopoietic SCs; hBMCSC, human bone marrow-derived MSCs; mLMEDC, mouse lung mixed culture derived epithelial cells; hESC-LC, human lung epithelial cells derived from ESCs. *Route of delivery: *IV, intravenous delivery of SCs; IT, intratracheal delivery of SCs; IP, intraperitoneal delivery of SCs; IN, intranasal delivery of SCs; BMT, bone marrow transplantation. 
*Therapeutic effect observed: *PaO2/FiO2, the ratio of partial pressure arterial oxygen to the fraction of inspired oxygen; ECM, extracellular matrix; IgE, immunoglobulin E; CFTR, CF-transmembrane conductance regulator. 
*Proposed mechanism: *VEGF, vascular endothelial growth factor; TGFbeta, transforming growth factor beta-1; IL, interleukin; SDF-1, stromal cell derived factor-1; CXC, family of chemokine receptors.
populations. The route of delivery of any of these cell types varies according to the study, e.g. intravenous, intratracheal, intraperitoneal, intranasal and conventional bone marrow transplantation. Though MSCs have been used in all delivery routes, other SCs such as ESCs and iPSCs have only been delivered intravenously and intratracheally to date. Remarkably, intravenous and intra-arterial infused MSCs are able to migrate and specifically home into the sites of lung injury. In all these models, the most common therapeutic effects observed included protection of alveoli, reduced inflammation, decreased fibrosis, improved lung mechanics, improved pulmonary function and increased survival of animals (Table 1).

**Mechanisms involved in SC-based therapies of preclinical models of lung disease**

**Direct bystander paracrine mechanism**

Several lines of evidence suggest that MSCs, via a paracrine mechanism (‘bystander effects’), protect the lung from injury. Some of the growth factors already identified as having a protective role in lung injury include hepatocyte growth factor (HGF), epithelial growth factor (EGF), keratinocyte growth factor (KGF), vascular endothelial growth factor (VEGF), angiopoietin-1 and adiponectin. An important tool in studying the beneficial paracrine effects of MSCs is the analysis of the cells’ secreted factors (secretome) in the ‘conditioned medium’ (CM). For example, media conditioned by adipose and bone marrow MSCs prevented alveolar epithelial cell apoptosis, accelerated alveolar epithelial cell wound healing and preserved lung vascular function in a BPD and/or CLRD animal model. The identification of specific soluble factors in the CM may lead to the discovery of repair molecules that each by itself or in combination could yield new therapeutic options. In vivo, the paracrine effect was also inferred from the efficacy of intraperitoneally administered MSCs in preventing oxygen-induced neonatal lung injury (Table 1). Interestingly, a single intravenous injection of MSC-derived CM had a more pronounced effect on attenuating alveolar injury and preventing lung fibrosis than iv injected MSCs. A single intratracheal instillation of MSCs or CM protected newborn rats against oxygen-induced alveolar and vascular injury with a persistent therapeutic benefit for up to 3 months. Likewise, CM derived from human umbilical cord MSCs and pericytes promoted alveolar growth and lung function in hyperoxia-exposed rats with persistent therapeutic benefits for up to 6 months and without adverse effect on lung structure or without appearance of tumors. Interestingly, injection of CM of hyperoxia-primed cells exerted a more potent therapeutic effect on lung architecture and prevention of pulmonary hypertension in this rat model of BPD than media from non-primed cells. Thus, rather than replacing injured cells and/or differentiating into lung cells, the beneficial effects of MSCs may be caused by the release of growth factors such as VEGF, transforming growth factor beta-1 (TGFβ1) or cytokines such as tumor necrosis factor alpha (TNFα), interleukin-1 (IL-1) or interleukin-6 (IL-6) that protect resident lung cells from injury or immunomodulate the function of inflammatory cells, respectively. Indeed, there is accumulating evidence that SCs, and in particular MSCs that interact with inflammatory cells, immunomodulate the lung’s response to injury. When instilled intratracheally or intraperitoneally, MSCs can re-direct alveolar macrophages from an M1 (pro-inflammatory) to an M2 (protective) phenotype and thus mitigate pulmonary remodeling in a model of ALI induced by Escherichia coli-derived LPS. Furthermore, the secretory ability of MSCs can be manipulated by ex vivo pre-conditioning the cells (24 h exposure to hyperoxia) prior to intraperitoneal administration to augment the therapeutic effect in a rodent model of BPD. The similarity in the therapeutic effects of the transplanted cells and their secretome is further highlighted in studies in which either MSCs or MSC-CM administration increased the number of resident bronchoalveolar SCs (BASCs) in neonatal mice exposed to hyperoxia. This study offers also new therapeutic perspectives, i.e. protection of resident lung progenitor cells rather than exogenous transplantation of SCs, MSCs and others, to achieve a therapeutic effect. Interestingly, in support of these paracrine mechanism observations, when MSCs
were delivered intravenously in a murine sepsis model, without significant engraftment, a multitude of genes involved in epithelial and endothelial protection were upregulated in several organs including the lungs. Taken together, these findings suggest that the most likely mechanisms behind the therapeutic effects observed following MSC therapy include paracrine signaling, and protection/activation of endogenous progenitors, rather than acting via a direct/effective ‘differentiation’ of adult MSCs into pulmonary epithelial/vascular cells (see below).

An important goal for the design of clinical trials is to determine the most efficacious and safest SC-based approach: cell-based therapy vs. cell free, i.e. injection of CM, and/or use of single defined bioactive molecules vs. identification and determination of the most promising combination of such molecules providing lung protection. In addition to the growth factors and cytokines mentioned above, SCs release small membrane vesicles (i.e. exosomes), which act as nanocarriers, containing a combination of bioactive molecules and microRNA (miRNAs). MiRNAs are small, non-coding RNA molecules that are involved in transcriptional regulation of gene expression and as such are interesting therapeutic targets, e.g. in the prevention of BPD by silencing specific genes with deleterious effects during lung injury. For example, MSC-derived exosomes attenuated lung macrophage influx, decreased pro-inflammatory cytokine levels in the bronchoalveolar lavage and prevented pulmonary vascular remodeling in hypoxia-induced pulmonary hypertension in mice. With the exosomes removed, the CM showed no therapeutic effect whatsoever in this model. Another mechanism of lung protection by MSCs, similar to exosome release, is the mitochondrial transfer to alveolar epithelium as seen in an experimental model of ALI. The mitochondrial transfer resulted in an increased intra-alveolar ATP concentration, which aided in inflation-induced alveolar surfactant production. These pleiotropic effects open exciting therapeutic avenues in particular for complex lung diseases and identification of the lung protective molecules in the CM provide targets for the discovery of novel drug entities. A limitation for the exploitation of exosomes as therapeutic tools remains the challenge of their isolation, characterization, quality control and the large-scale manufacturing of exosomes/microvesicles from both pluripotent and multipotent SCs.

**Differentiation of transplanted SCs into the pulmonary epithelium**

According to some recent reports, adult MSCs from different species and tissue origins such as bone marrow, adipose and cord blood infrequently differentiate into cells of various non-hematopoietic tissues such as lung epithelium in vitro. For example, when exposed to lung-specific differentiation media, human umbilical cord blood-derived MSCs (hUCBMSCs) differentiated into alveolar epithelium in vitro. A few studies reported occasional in vivo differentiation of transplanted rat bone marrow-derived MSCs (rBMSCs) or human umbilical cord blood-derived hematopoietic SCs (hUCBHSs) into an alveolar type-II phenotype with a minor contribution to epithelium repair. One of the confounding complications in utilizing SCs as exogenous sources for lung therapy is the fact that different cell populations, such as MSCs or hematopoietic SCs used in the literature, may have vastly dissimilar differentiation potential. Furthermore, specific subsets of MSCs such as CCSP positive rather than negative phenotypes were able to more efficiently engraft into naphthalene-injured rodent lungs following intratracheal instillation. There seems to be a growing set of observations that suggest the MSCs are responsible for some of the observed epithelium regeneration; however, these findings are currently controversial since engraftment efficacy of MSCs, delivered intratracheally and systemically, has been reported to be very low. The prevalence of therapeutic effects in spite of the low engraftment efficiency supports the notion that paracrine mechanisms account for the majority if not all of the therapeutic effects of transplanted MSCs.

**Safety of SCs in clinical trials of lung disease**

Clinical trials using MSCs for treating a variety of diseases has indicated safety, but at the same time also stressed the need for more comprehensive clinical trials.
to the safety profile of MSC therapy. The summary of representative clinical trials of SCs therapy in lung diseases (Table 2) indicates no serious side effects or dose-limiting toxicity, similar to that of the placebo-treated groups. Clinical efficacy, albeit not a primary outcome during Phase 1 studies, was reported as being marginal or none based on respiratory severity score, oxygenation index and both tracheal and systemic inflammatory markers (Table 2). The lack of clinical efficacy taken together with the good safety profile raises the possibility that the initially chosen administered 1 × 10⁶ cells/kg dose is far too low, requiring escalation of the number of administered cells in future studies. For example, the recently completed Phase 2 study of treating chronic obstructive pulmonary disorder (COPD) patients with allogeneic MSCs reported a good safety profile of a high dose (100 × 10⁶ cells) administered four times once a month, but did not show any significant clinical improvement. Given that an even higher dose of cells may be required to exhibit beneficial therapeutic effects, it will be interesting to follow the outcomes of the AETHER trial, which aims to use allogeneic human MSCs for treatment of IPF by escalating the dose up to 200 × 10⁶ cells/infusion (NCT02013700). The clinical studies consistently show anti-inflammatory effects of transplanted MSCs regardless of the delivery route; however, clinical studies must be performed to address pulmonary airway epithelium regeneration.

Additional precautions and considerations of long-term adverse consequences in the infant population upon transplantation of MSCs in infants suffering from lung diseases, such as BPD, will require dedicated Phase 1 clinical trials. For example, intratracheal delivery of MSCs decreased the severity of the disease in preterm newborns suffering from BPD, based on decreased levels of IL-6, interleukin-8 (IL-8), matrix metalloproteinase-9 (MMP-9), TNFα and TGFβ1 in tracheal aspirates 1 week post transplantation. On the other hand, for adults, the major route of administration is intravenous infusion; currently, no clinical trials of intratracheal delivery of cells for adult patients are listed (http://clinicaltrials.gov). Although the lungs are the first major organ that the cells encounter after intravenous infusion, as previously mentioned the current cell dosage has yet to elicit a measurable therapeutic effect. In summary, the clinical findings of treating lung diseases with MSCs are promising at best—no acute toxicity, but no efficacy, yet. To claim success/efficacy, a plethora of additional parameters will have to be investigated and optimized with an emphasis on the route of delivery (i.e. intratracheal, endobronchial, intravenous, etc.). A complex combination and balance between the pathophysiology, type/origin of the SCs, dose, regimen and route of administration has to be empirically determined in large animal models of lung diseases and further refined in Phase 1–3 clinical trials before cell therapy will be introduced in pulmonary clinical practice.

Areas of agreement

The cells most commonly used for clinical trials have been MSCs from various allogeneic and autologous origins. The source of cells is an important consideration. Autologous cell therapies may avoid immunological complications and allow the use of minimally manipulated cells. Given the immune-protective properties of MSCs, allogeneic cell therapy is feasible and may facilitate the logistics of cell therapy. Current clinical efforts with MSCs proved the acute tolerability of MSCs from various autologous and allogeneic origins and confirmed anti-inflammatory, immunoprotective ‘bystander’ effects, regardless of the route of administration. There is an agreement that Phase 2 studies will provide information on optimal cell dosage and schedule for transplantation.

Areas of disagreement

A major area of controversy is whether or not the MSCs of various tissue origins are capable of transdifferentiating into airway epithelial cells in vivo providing tissue regeneration. In addition, the necessity of this regeneration to originate from the exogenous cells versus endogenous lung SCs is up for debate.

Future challenges

The timing and cell dosage of current cell-based therapeutic approaches are among the key other factors yet
Table 2: Representative list of completed and published clinical trials using cell therapy for lung diseases

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<th>Lung disease</th>
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<th>Clinical study design</th>
<th>Clinical observations</th>
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<tr>
<td>BPD&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Allogenic hUCBMSCs</td>
<td>Unicentric, non-randomized, open-label, dose escalation. Untreated matched-comparison group</td>
<td>IT&lt;sup&gt;g&lt;/sup&gt; • the dose divided in two and each portion administered to each lung • &lt;5 min • 1 x 10&lt;sup&gt;7&lt;/sup&gt; and 2 x 10&lt;sup&gt;7&lt;/sup&gt; cells/kg</td>
<td>Respiratory severity score, tracheal aspirate fluid (HGF, TGF-β1, MMP-9, IL-1, IL-6, IL-8, IL-10, TNF-α, VEGF)</td>
<td>Significantly lower BPD severity. No significant long-term (7d) change in respiratory severity score. Significantly reduced IL-6, IL-8, MMP-9, TNF-α, TGF-β1&lt;br&gt;No serious event, no dose-limiting toxicity&lt;br&gt;Safe therapy, lower risk of death or developing moderate/severe BPD.</td>
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<tr>
<td>ARDS&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Allogeneic adipose-derived MSCs</td>
<td>Unicentric randomized, placebo-controlled</td>
<td>IV&lt;sup&gt;f&lt;/sup&gt; • Single dose • 1 h infusion • 1 x 10&lt;sup&gt;6&lt;/sup&gt; cells/kg</td>
<td>Oxygenation index, IL-6, IL-8, SP-D</td>
<td>Marginal/Weak&lt;br&gt;No serious events&lt;br&gt;Safe therapy. No significant clinical effect.</td>
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<td>CLRD&lt;sup&gt;c&lt;/sup&gt; Advanced COPD (Stage IV dyspnea)</td>
<td>Autologous bone marrow MNCs</td>
<td>Unicentric non-randomized, open-label, prospective (12 months)</td>
<td>IV&lt;sup&gt;f&lt;/sup&gt; • single dose • 20 min infusion</td>
<td>Lung functions, oxygenation indices</td>
<td>Marginal/None&lt;br&gt;No serious events&lt;br&gt;Safe therapy. No significant clinical effect.</td>
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<td>Moderate to severe COPD (Stage II or III)</td>
<td>Allogenic MSCs</td>
<td>Multi-center, randomized, double-blinded, placebo-controlled, prospective (2 years)</td>
<td>IV&lt;sup&gt;f&lt;/sup&gt; • 100 x 10&lt;sup&gt;6&lt;/sup&gt; cells/infusion • infusion/30 days x 4 times • Max. 30 min infusion</td>
<td>Lung functions, quality-of-life questionnaire, exacerbations ratio and systemic inflammation markers (TNF-α, IFN-γ, IL-2, TGF-β, IL-4, IL-5, IL-10, C-reactive protein)</td>
<td>Marginal/None&lt;br&gt;No serious events&lt;br&gt;Safe therapy. No significant clinical effect.</td>
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The development of autologous iPSCs has created a new cell source for clinical therapies of lung disorders, such as hyperoxia-induced lung injury, without the risk of rejection or ethical concerns. However, the mechanism of reprogramming is as yet not fully understood, the efficacy of directed reprogramming is still rather low and the risk of tumorigenicity of these cells still exists, therefore delaying progress towards clinical trials. As an exciting new development, efficient derivatization of MSCs from iPSCs could avoid the innate heterogeneity of cells such as BMSCs and allow for a more homogeneous population to be used for therapy. Some tissue engineering approaches may help to improve the long-term survival and efficacy of MSC therapy by encapsulation in an injectable hydrogel. Alternatively, recent focus has been on bioengineering of the whole lung through the use of SCs to recreate the epithelium and endothelium in ex vivo decellularized lung scaffolds, which in turn could serve as a replacement for transplantation to aid pulmonary function. Other challenges include specification of the molecules within the MSC-CM which have therapeutic properties and/or pharmaceutical design of specific micro and/or nanocarriers such as exosomes that may lead to targeted pharmacological therapies for lung disorders.

Conclusions

The multipotency, self-renewal properties and the therapeutic potential of the secretome of MSCs make cell-based therapies appealing for providing both lung injury therapy and stimulating the regeneration of damaged or diseased lung tissue, as a replacement of or supplement to current pharmaceutical regimen. Preclinical studies have demonstrated the proof of concept that SC-based therapies can attenuate tissue injury and improve survival in animal models of experimental lung diseases. Phase1 clinical trials have proven safety of MSC therapy for certain lung diseases. Currently, cell survival and engraftment are two of the main obstacles to fully leveraging the therapeutic potential of transplanted SCs. Transplantation of exogenous MSCs and or their secretome may provide the key cues/signals necessary for stimulating endogenous lung progenitors, speculating a synergy.
between exogenous and endogenous SCs activities, which should be further exploited for therapeutic purposes. Transplanted MSCs or their CM/exosomes may provide a therapeutic effect by secreting trophic factors, bioactive molecules and also by immune modulation. In addition, they may cooperate with and/or activate lung progenitors such as the BSCs located at the bronchoalveolar duct junction to aid in the regeneration process. Understanding the specific requirements of the micro-environmental cues and signals in the lung SC niche, which foster interactions between exogenous and endogenous lung SCs, will provide novel targets that might be exploited for a variety of lung disorders. Moreover, the paracrine effects of SC-based therapies have opened unexpected therapeutic options through the possibility of identifying individual molecules or discovering novel mechanisms and targets including exosomes and their contents. The promise for lung disease therapy may not lie in the SCs only, but rather in the vast array of therapeutic mediators they harbor in their secretome.

**Conflict of Interest statement**

The authors have no potential conflicts of interest.

**References**


