Introduction

Pulmonary fibrosis is common in different inflammatory lung diseases, such as interstitial pneumonia, chronic obstructive pulmonary disease (COPD), and silicosis. The lungs have a very limited ability to regenerate, compared to other organs (1), and there is currently no effective clinical drug treatment for pulmonary fibrosis.

Idiopathic pulmonary fibrosis (IPF) is a life-threatening lung disease whose pathogenesis is associated with steady-state imbalance in pulmonary epithelial cells (2,3). Currently, there is no effective treatment for this end-stage lung fibrosis disease. However, some FDA-approved therapeutic agents, including pirfenidone and nintedanib, can clearly reduce the average decrease in the function of the lung in the IPF patients (3,4).

Current studies have investigated whether the pathogenesis of IPF is related to chronic epithelial injury that leads to abnormalities of the wound healing process, and both fibroblast proliferation and activation, rather than...
inflammation (2,3,5). The abnormal healing of wounds can cause a loss of the balance between the extracellular matrix formation and degradation (6,7). The IPF causes involve multiple mechanisms and factors, but the exact reason is still unknown. For example, smoking, environmental/occupational contaminants, microbial agents, chronic aspiration of gastroesophageal reflux and genetic abnormalities, are key factors that are associated with IPF (8). In addition, both the genetic predisposition and genome mutations are linked to the IPF pathology. Thus, mutations in the SFTPC gene, which codes for surfactant protein C (SP-C), the adenosine triphosphate binding cassette A3 gene, surfactant protein A2 (SP-A2) gene, telomerase gene or other genes, which play roles in host defense, adhesions between cells or repairing DNA, can contribute to the lung fibrosis (9,10). Other factors and enzymes are also important for the development of IPF. The telomerase activity, for instance, varies in different cell types and affects lung stem cells (11,12), while increased IPF telomerase activity in lung fibroblasts make these cells have certain anti-apoptotic functions (12).

IPF is still an unexplained chronic and progressive pulmonary fibrosis disease, with a poor prognosis. IPF patients have an average survival time of 3 to 5 years (13,14). The main reason for the failure of traditional therapies is due to a lack of proper understanding of the IPF pathogenesis. Both managing and treating the medical conditions associated with IPF comorbidities, including the COPD, gastro-esophageal reflux, obstructive sleep apnea and inhibiting the pathways which trigger the fibrogenic process, are the emphasis of the conventional therapeutic approaches (15).

The American Thoracic Society (ATS) has updated the IPF Clinical Practice Guide to include some changes in 2011 (16). These changes include an objection against the IPF therapy with prednisone combined with both N-acetylcysteine and azathioprine, which were found to increase the death rate by almost ten times. They also involve an opposition against the use of warfarin, imatinib, and ambisentan as well as sildenafil, macitentan and bosentan in the treatment of IPF patients (16). Meanwhile, the new ATS guideline has continued previous recommendation for N-acetylcysteine (conditional recommendation against monotherapy for IPF) and antacid therapy in patients without symptoms of gastro-esophageal reflux (conditional recommendation) (2,3,16).

Lung transplantation is currently a feasible scheme and successful curative therapy for some IPF patients with limited comorbidity symptoms (7). However, the lack of donor organs and limited patient suitability for transplantation make lung transplantation less applicable and, therefore, other therapeutic approaches have been investigated and tested in last decades (2,3,5,17,18). Recent discoveries of IPF mechanisms have helped in designing new treatment regimens. For example, new potential IPF targets include angiotensin receptor inhibitors, which hinder the proliferation of fibrotic fibroblasts induced by ANG II (19), NOX-4 antagonists (NADPH oxidase 4) that downregulate the reduction of O2 to reactive oxygen species (ROS) (20), and galectin-3 inhibitors that block TGF-β induced β-catenin activation and attenuate lung injury (21). These targets also involve FoxO3, which is an important integrator of pro-fibrotic signaling pathways in fibrotic lungs and, therefore, reconstitution of FoxO3 pharmacology is currently a novel therapeutic approach (22). In addition, the IPF-associated endothelial microparticles are other targets required for the fibrinolytic activity-mediated fibroblast invasion in fibrotic lungs (23).

More targets and therapeutic agents are under intensive investigation and/or development, including the FDA-approved pirfenidone and nintedineb, which can treat IPF by decreasing the decline of IPF lung functions and disease progression (4,16,24). Other effects of pirfenidone include preventing the deposition of hydroxyproline, procollagen I and III, inflammatory cells and transforming growth factor β (TGF-β) in different lung tissues (24). Similarly, nintedanib, which is a CDK4 kinase inhibitor that acts against three tyrosine kinase receptors; PDGFRα, VEGFR and FGFR1, can inhibit the progression of IPF by slowing down the declining rate of the forced vital capacity (FVC) in the lung (25). However, the ultimate cure for IPF remains to be seen, and other targets are still being sought.

### Types of cells used in the cell-based therapies of IPF

The cell-based strategies are extensively investigated to find treatments for IPF (26-30). Cell therapy for IPF is mainly achieved by replacing damaged cells with regenerated cells and/or the administered cell paracrine properties. In this section, we will discuss recent developments in the regenerative medicine field and its applications in the cell-based therapy of IPF. In addition, recent clinical trials in for IPF will be reviewed.

A variety of cells are used in the IPF treatment studies, including lung epithelial cells type II (31), lung mixed lung
epithelial cells (32), and different stem cell types, including lung stem cells, induced pluripotent stem cells (iPSCs), embryonic stem cells (ESCs), mesenchymal stem cells (MSCs), and adipose stem cells (ADSCs) (33-36). Notably, both endogenous alveolar epithelial and bone marrow-derived mesenchymal cells are most widely investigated for the IPF treatment (37). These different cell types will be discussed in the following subsections.

Alveolar epithelial cells (AECs)

In the alveolar epithelium of the peripheral lung, alveolar epithelial type I (AECI) cells enable gas exchange with lung blood capillaries, while alveolar epithelial type II (AECII) cells secrete pulmonary surfactants that reduce alveolar surface pressure (15,38,39). AECII cells can self-replicate and serve as adult stem cells that differentiate into AECI cells during normal homeostatic turnover and when the AECI cell number is reduced by injury (40). AECII cells are, therefore, the “caretaker” cells of the alveolar compartment, since they can protect the epithelium, initiate repair processes and maintain alveolar structure, if an injury occurs. A study showed that lung fibrogenesis was reversed after transplanting AECII cells in the rodent model of bleomycin-induced (BLM) lung fibrosis (31).

AECII cells used for transplantation could be isolated from the healthy lung (31) or produced in culture from differentiating adult stem cells (17). The use of freshly isolated AECII cells is more effective and safer since they do not have tumor forming potentials, while adult stem cells may undergo abnormalities in their chromosomes, leading to the formation of malignant tumors after transplantation in the lung (41). AECII cells-derived from human embryonic or bone marrow stem cells (BMSCs) could be also transplanted into a BLM induced IPF model (35,42). In addition, AECs can be produced from ESCs in culture. For example, the airway specific cells, including ciliated, Clara, basal and intermediate epithelial cells, can be formed when murine ECSs grow as embryonic bodies on surfaces coated with collagen. The culture medium of these cells is supplemented with specific growth factors before re-plating on an air liquid-interface culture (43,44). Despite their sources, transplantation of AECII cells into the lungs of BLM—induced IPF animal models can lead to a remarkable reduction of collagen contents, supporting the potential function of AECII cells in healing lung injuries (33). Remarkably, recent infusions of allogeneic adult lung spheroid cells (LSCs) into fibrosis animal models can inhibit the inflammation progression and fibrosis manifestation, suggesting intrinsic adult LSCs for lung therapy (45). A summary of key preclinical and clinical studies exploring the applications of AECII, LSCs and ESCs in IPF therapy is shown in Tables 1,2.

Mixed lung epithelial cells

Since the isolation of AEC cells from the lung or generation from differentiated stem cells is a complicated process, it was proposed that the use of mixed lung epithelial cells is easier, faster and efficient for the lung cell-based therapy than AEC alone (32). The lung epithelial cell mixture expresses surfactant protein C in culture and in vivo. The intra-tracheal delivery of lung epithelial cell mixture into a BLM-induced animal model of fibrosis was shown to improve lung fibrosis (32).

Stem cells

Stem cells have been studied for many years as a potential treatment for the chronic diseases. Stem cells, including ESCs, ADSCs, MSCs, bone marrow stem cell (BMSCs) and endogenous lung stem/progenitor cells, have two essential properties; a controlled and unlimited self-renewal capacity, and a differentiation ability into different specific cell lines. Adult stem cells from the bone marrow, umbilical cord, and adipose tissue are commonly used for the study and potential treatment of the chronic lung diseases such as IPF (37,49). Remarkably, stem cell-based tissue engineering aims to mimic the native stem cell niche and maintain stem cell function within the graft by providing appropriate microenvironmental cues in a controlled and reproducible fashion that will facilitate the application of stem cell therapy in human diseases, including IPF (50).

Mesenchymal stromal/stem cells

Adult mesenchymal stromal/stem cells (MSCs) are originally isolated from adult bone marrow stroma and exist in other tissues, such as the umbilical cord, amniotic fluid, epidermis and cord blood (51). MSCs are multipotent and, therefore, can differentiate into a wide range of cell lines (51,52). MSCs tend to target damaged tissues when systemically administrated via intravenous (IV) or intraperitoneal (IP) injection (53,54). Thus, intraperitoneally (IP) injected amniotic fluid stem cells, including MSCs, can migrate and be detected in different body organs such as the lung (55).

MSCs have potent anti-proliferative, anti-apoptotic,
immune-modulatory and anti-inflammatory properties, besides their multilineage capacity, which make them have a great therapeutic potential for different diseases (37,51,52). In addition, MSCs can modify the micro-environmental factors at the engraftment site that can enhance their therapeutic potential (37). Interestingly, the better understanding of the effect of extracellular environment on MSC paracrine activity, together with recent progress in bioengineering, can enhance the success of the clinical application of MSC therapy (56). Indeed, MSC therapy is a good candidate for different autoimmune diseases because of their immunomodulatory and anti-inflammatory competence (57,58). A summary of key preclinical and clinical studies exploring the applications of MSCs (from different sources) in IPF therapy is shown in Tables 3-6.

**MSCs derived from the bone marrow (BM-MSCs)**

The bone marrow is the major source of MSCs, and BM-MSCs have been intensively investigated in IPF treatment (34,81). Remarkably, the granulocyte colony-stimulating factor (G-CSF)-augmented BM-MSCs can result in an improvement of lung healing in the animal model of lung injury (82). Similarly, BM-MSC infusion can reverse the BLM-induced lung fibrosis (69). Therefore, BM-MSCs play a key role in the healing of different lung injuries and, consequently, can alleviate the fibrosis symptoms (83).

However, IPF patient-isolated BM-MSCs were recently shown to be senescent with alterations in mitochondrial functions and DNA damages (84).

A summary of key preclinical/clinical studies on IPF therapy using BM-MSCs is shown in Tables 3,4.

**MSCs derived from the umbilical cord and placenta**

MSCs derived from the aborted fetuses, umbilical cord, or discarded test-tube human embryos have high stem cell plasticity/phenotype, with low immunogenicity in...
<table>
<thead>
<tr>
<th>Type/source of MSCs</th>
<th>Delivery route/dose</th>
<th>Efficacy results</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human placental MSCs</td>
<td>IV route. A dose of $1 \times 10^5$ cells per mouse</td>
<td>Activation of MyD88 and TGF-β signalling decreased collagen deposition, pro-fibrotic cytokines production</td>
<td>(59)</td>
</tr>
<tr>
<td>Murine placenta-MSCs, human placenta-MSCs</td>
<td>IV or Intratracheal route, with a dose of $1 \times 10^5$ cells/mouse, Or IP route with a dose of $4 \times 10^5$ cells/mouse</td>
<td>A decreased level of BLM-induced lung fibrosis and reduced neutrophil infiltration</td>
<td>(60)</td>
</tr>
<tr>
<td>Human umbilical-MSCs</td>
<td>IV route. A dose of $1 \times 10^6$ cells per mouse</td>
<td>Treated mice show reduced fibrosis and inflammation, and decreased TIMP expression and lung cytokine production, and increased MMP expression</td>
<td>(61)</td>
</tr>
<tr>
<td>Lung resident-MSCs</td>
<td>IV route. A dose of $0.15 \times 10^6$ or $0.25 \times 10^6$ cells per mouse</td>
<td>Treated animals have a decreased infiltration of lymphocyte and granulocyte, and display a reduced pulmonary damage and mitigation of the PAH development</td>
<td>(62)</td>
</tr>
<tr>
<td>BM-MSCs, Amnion-MSCs, or human amniotic epithelial cells (hAECs)</td>
<td>IV. A dose of $1 \times 10^6$ cells per mouse, 2 repeated doses at 0 and 7 days of treatments</td>
<td>All types of cells used in the treatments show a wide range of anti-inflammatory effects. Compared to the other used cell types, amnion-MSC treatments are more effective and decrease both fibrosis and TGF-β, but cause enhanced MMP-9 activity, GM-CSF secretion and IL-1RA induction</td>
<td>(63)</td>
</tr>
<tr>
<td>Human BM-MSCs (overexpressing microRNAs let-7d or miR-154)</td>
<td>IV route. A dose of $5 \times 10^4$ cells per mouse</td>
<td>B-MSCs (overexpressing let-7d) administration leads to a decrease in both collagen deposition and CD45-positive cells, and a shift in animal weight loss as well as</td>
<td>(64)</td>
</tr>
<tr>
<td>BM-MSCs (transfected with HGF)</td>
<td>Intratracheal. A dose of $3 \times 10^6$ cells per rat</td>
<td>Treated rats show downregulated collagen deposition and decreased fibrosis in Ashcroft score</td>
<td>(65)</td>
</tr>
<tr>
<td>BM-MSCs</td>
<td>IV route. A dose of $5 \times 10^6$ cells per mouse</td>
<td>Treated mice after BLM instillation show decreased collagen deposition and inflammation</td>
<td>(66)</td>
</tr>
<tr>
<td>BM-MSCs</td>
<td>IV route. A dose of $5 \times 10^5$ cells per mouse</td>
<td>Protection of treated lung tissue after BLM instillation, with inhibiting the pro-inflammatory cytokines IL-1 and TNF-α</td>
<td>(67)</td>
</tr>
<tr>
<td>BM-MSCs</td>
<td>IV route. A dose of $2.5 \times 10^6$ cells per rat</td>
<td>Treated rats after BLM instillation show decreased oxidative stress and collagen deposition</td>
<td>(68)</td>
</tr>
<tr>
<td>BM-MSCs</td>
<td>IV route. A dose of $5 \times 10^5$ cells per mouse</td>
<td>Treated mice after BLM instillation have suppressed inflammation and reduced reparative growth factor production</td>
<td>(69)</td>
</tr>
<tr>
<td>BM-MSCs</td>
<td>IV route. A dose of $10^6$ cells per rat</td>
<td>Treated rats after BLM instillation show a decrease of pulmonary inflammation and some fibrosis factors (e.g., TGF-β, IL-1β, VEGF, TNF-α, IL-6, and NOS)</td>
<td>(70)</td>
</tr>
<tr>
<td>BM-MSCs (human)</td>
<td>IV route. A dose of $5 \times 10^5$ cells per mouse</td>
<td>Treated mice after BLM instillation have a reduced endoplasmic reticulum stress and oxidative stress, and downregulated TGF-β1 production by alveolar cells</td>
<td>(71)</td>
</tr>
<tr>
<td>BM-MSCs (human)</td>
<td>IV route. A dose of $5 \times 10^6$ cells per mouse</td>
<td>Low levels of BM-MSCs engraft in BLM-induced fibrosis in immunodeficient NOD/SCID and NOD/SCID/β2 microglobulin (β2M) null mice</td>
<td>(72)</td>
</tr>
<tr>
<td>Hypoxia-preconditioned BM-MSCs</td>
<td>Intratracheal route. A dose of $5 \times 10^6$ cells per mouse</td>
<td>Treated mice after BLM instillation show decreased fibrosis and inflammation and improvement of lung function</td>
<td>(73)</td>
</tr>
<tr>
<td>knockdown BM-MSCs</td>
<td>IV route. A dose of $5 \times 10^4$ cells/g body weight</td>
<td>Treated mice after BLM instillation show low levels of interleukin-1b and apoptosis, decreased fibrosis, and upregulated HGF levels</td>
<td>(74)</td>
</tr>
</tbody>
</table>

IPF, Idiopathic pulmonary fibrosis; MSCs, mesenchymal stem cells; BM, human bone; PAH, pulmonary arterial hypertension.
culture and in vivo (85). The umbilical cord-derived MSCs (uMSCs) are less readily available compared to ESCs, while the placenta-derived MSCs can engraft in the lung and other solid organs after xenotransplantation. Moodley and colleagues studied the therapeutic effects of uMSCs in BLM-induced lung injury and found that these cells can inhibit lung inflammation and fibrosis by up-regulating anti-inflammatory modulators but downregulating the cytokine expression (61). The systemically administered uMSCs are present in the injured lung after 2 weeks and may not exactly match with the recipient phenotype to avoid the graft-versus host reaction (61).

The effect of transplanted placenta-derived MSCs on lung fibrosis was also studies using murine models.

**Table 4** Summary of key IPF clinical human study results using MSC cells

<table>
<thead>
<tr>
<th>Type/source of MSCs</th>
<th>Delivery route/dose</th>
<th>Efficacy results</th>
<th>Safety results</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placental MSCs (allogeneic)</td>
<td>IV route. A dose of 1 &amp; 2×10⁶ cells/kg. One dose</td>
<td>No worsening of IPF and no deterioration in lung function that is stable in treated patients</td>
<td>Transient, but minor, acute adverse events</td>
<td>(75)</td>
</tr>
<tr>
<td>BM-MSCs (allogenic)</td>
<td>IV route. A dose of 20×10⁶ (n=3) 100×10⁶ (n=3) &amp; 200×10⁶ cells (n=3). One dose</td>
<td>Exploratory results with: 5.4% mean decline in % predicted DLCO and 3.0% mean decline in % predicted FVC in treated patients</td>
<td>No serious adverse events, but IPF progression has 2 non-treatment related deaths</td>
<td>(76)</td>
</tr>
</tbody>
</table>

IPF, Idiopathic pulmonary fibrosis; MSCs, mesenchymal stem cells; BM, human bone.

**Table 5** Summary of key IPF preclinical study results using ADSCs

<table>
<thead>
<tr>
<th>Type/source of ADSCs</th>
<th>Delivery route/dose</th>
<th>Efficacy results</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADSCs</td>
<td>IV route. A dose of 2.5×10⁴ or 2.5×10⁵ cells per mouse</td>
<td>Treated mice show a decreased lung fibrosis and inflammation in a dose-dependent manner</td>
<td>(77)</td>
</tr>
<tr>
<td>ADSCs (human)</td>
<td>IP route. A dose of 3×10⁵ cells per mouse</td>
<td>Treated mice show a decreased lung fibrosis, inflammatory cell infiltration and epithelial cell hyperplasia, associated with inhibited TGF-β expression and epithelial cell apoptosis</td>
<td>(36)</td>
</tr>
<tr>
<td>ADSCs (young vs. old donor)</td>
<td>IV route. A dose of 5×10⁵ cells per mouse</td>
<td>Treated old mice (&gt;22 weeks old) with young ADSCs display a greater reduction in fibrosis, oxidative stress, MMP-2 activity, and apoptosis markers than mice treated with old ADSCs</td>
<td>(78)</td>
</tr>
</tbody>
</table>

IPF, Idiopathic pulmonary fibrosis; ADSCs, adipose-derived MSCs.

**Table 6** Summary of key IPF clinical human study results using ADSCs

<table>
<thead>
<tr>
<th>Type/source of ADSCs</th>
<th>Delivery route/dose</th>
<th>Efficacy results</th>
<th>Safety results</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADSCs-SVF</td>
<td>Endobronchial route. A dose of 0.5×10⁶ cells/kg of body weight in 10 cc. Three dosages for 3 months</td>
<td>All patients are alive (at least for 2 years after treatments, with median overall survival of 32 months, and median overall progression-free survival of 26 months)</td>
<td>No formation of ectopic tissues and no difference in adverse events compared to the placebo effect</td>
<td>(79)</td>
</tr>
<tr>
<td>ADSCs-SVF</td>
<td>Endobronchial route. A dose of 0.5×10⁶ cells/kg of body weight in 10 cc. Three dosages (for 3 months)</td>
<td>No deterioration in the functional parameters and life quality indicators in the treated patients</td>
<td>No formation of ectopic tissues and no difference in adverse events compared to the placebo effect</td>
<td>(80)</td>
</tr>
</tbody>
</table>

IPF, Idiopathic pulmonary fibrosis; ADSCs, adipose-derived MSCs.
Transplantation of allogeneic and xenogeneic placenta-derived MSCs notably reduces BLM-induced lung fibrosis by suppression the infiltration of neutrophils, and act as a potential treatment for lung fibrosis (60). Placenta-derived MSCs are plastic and have immunomodulation properties and are, therefore, important for lung repair and regeneration like other MSCs (86). A summary of key studies on IPF therapy using placental/umbilical cord MSCs is shown in Tables 3,4.

Adipose tissue-derived mesenchymal stromal/stem cells (ADSCs)

The adipose tissue contains pluripotent cells that can act as alternative sources of stem cells to BM-MSCs since they can give rise to many cell lineages (87). ADSCs can be isolated easily from patients through liposuction, show good results in cell therapy, and produce many bioactive factors, including the hepatocyte growth factor (HGF) and interleukin (IL-1, IL-6, IL-8) receptor antagonists (88,89). ADSC therapy can improve the detrimental effect of repeating the intra-tracheal instillations of BLM (36). Mechanistically, the ADSC therapy results in a decreased AEC and Clara cell hyperplasia, and a reduction of the thickening of septum, enlargement of alveoli and inflammatory cell infiltrations (36). The elevation of both apoptosis and TGF-β levels is also suppressed (36). Furthermore, ADSCs administration can ameliorate the renal function in the acute pyelonephritis animal model (89).

A contradictory effect of ADSCs on lung fibrosis is found when administrating intravenously in rat model of fibrosis since they could not decrease the BLM-induced lung injury (90). In contrast to many other studies, Uji and co-workers infused the isolated ADSCs to rats after long time (14 days) of the BLM instillation that probably leads to the inefficiency of administrated ADSCs in the BLM-induced lung injury (90). Other reasons that are proposed for the treatment failure include the animal age, stage of fibrotic lung injury (90). Other reasons that are proposed for the treatment failure include the animal age, stage of fibrotic lung injury (90). Other reasons that are proposed for the treatment failure include the animal age, stage of fibrotic lung injury (90). In contrast to many other studies, Uji and co-workers infused the isolated ADSCs to rats after long time (14 days) of the BLM instillation that probably leads to the inefficiency of administrated ADSCs in the BLM-induced lung injury (90). Other reasons that are proposed for the treatment failure include the animal age, stage of fibrotic lung injury (90).

The iPSCs

A new approach to investigate the applications of iPSCs in IPF treatment is using IPF-specific cells isolated from IPF patients (37,92). Deriving lung tissue-specific stem cells from patients is a key step for establishing human disease models and transplanting lung epithelial cells. The iPSCs are successfully produced from many lung disease patients. However, the use of iPSCs in developing human lung disease models effectively faces many challenges, including the development of effective research techniques that allow converting iPSCs into lung progenitor cells and then into different differentiated lung epithelial cells. However, some studies have shown progress in generating multipotent airway and lung progenitor cells from human patient-specific cystic fibrosis iPSCs. For example, lung progenitor cells are generated by mimicking the developmental environment in which the signaling interactions and events occur in the developing murine lungs (92). The generated human disease-specific lung progenitor cells can form respiratory epithelial cells when engrafting into immune-deficient mice subcutaneously (92). Generating iPSCs from diseased human lungs is, therefore, particularly important, and can create a remarkable platform for the treatment of different lung diseases in humans. A summary of key preclinical and clinical studies on IPF therapy using iPSCs is shown in Table 7.

Endogenous lung tissue-specific stem cells

Several studies have identified adult stem cell types in anatomic locations of the lung (95,96). Moreover, resident multipotent lung stem cells were isolated and well-characterized from adult mouse lung (97,98). Lung resident stem cells can produce different types of cytokines, growth factors and surfactant proteins, which are specific biomarker of the lung (35,38,39,96,99-101).

Resident MSCs can regulate tissue repair and/or regeneration, and different pathophysiological processes, including inflammation, fibrogenesis, angiogenesis and tumorigenesis in different tissue types. Lung mesenchymal stem cells (L-MSCs) are functionally distinct from other MSCs and are specifically equipped for the pulmonary environment and may play roles in treating chronic lung diseases (102,103). In contrast to the well-investigated effects of exogenously administered MSCs, little is known about the healing effects of endogenous L-MSCs. However, some studies suggest that endogenous stem/progenitor
cells in the lung, including L-MSCs, contribute to both the maintenance of epithelium and repair/regeneration of injured lungs in vitro and in vivo (103).

L-MSCs have several characteristics such as mesenchymal signature, and multi-lineage differentiation capacity to other tissue such as to myo-fibroblasts bone, fat, bone and cartilage (95,102), and Clara, AECI and AECII cells (104), as well as endothelial cells (105) in culture. However, L-MSC differentiation into these different types of cells in vivo is still under question, and the emphasis in L-MSC research has largely shifted to their paracrine effects (106). This probably explains the relatively limited studies that attempted to investigate the use of L-MSCs in the treatment of chronic lung disease (107,108). More research is, therefore, still needed to validate the potential of L-MSC—based therapies for fibrosis and other lung diseases.

Circulating endothelial progenitors (EPCs)

EPCs have some vascular remodeling and lung tissue-specific repairing properties (109). The association between IPF and the abnormal vascular remodeling is well-established (110).

The development of lung vasculature is closely related to the release of some specific factors, including the endothelial-derived angiogenic factors, that promote the alveolization by stimulating the proliferation of lung specific epithelial stem/progenitor cells. Restoring the endothelial cell function and maintenance of lung homeostasis, therefore, make EPCs important cell types in lung development, morphogenesis and repair/regeneration after injury. In addition, defects in lung EPCs can lead to loss of their capacity for repairing the damaged endothelial cells and maintaining the vascular integrity that can lead to several lung diseases. For example, EPC defects may contribute to the lung injury, leading to developing many profibrogenic events and, therefore, EPC transplantation may inhibit lung fibrosis (111). Indeed, in the clinical context of IPF, the EPC defects can be overcome by increasing the expression levels of the vascular endothelial growth factor (VEGF) (111). Furthermore, EPCs were suggested to contribute directly to angiogenesis, probably by secreting specific angiogenesis-promoting growth factors (112).

Table 7 Summary of key IPF preclinical study results using iPSCs

<table>
<thead>
<tr>
<th>Types/source of iPSCs</th>
<th>Delivery route/dose</th>
<th>Efficacy results</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>iPSCs</td>
<td>IV route. A dose of 2×10^5 cells per mouse</td>
<td>Treated mice after BLM instillation show an inhibition of EMT, inflammatory responses, and TGF-b1/Smad2/3 signaling pathway</td>
<td>(93)</td>
</tr>
<tr>
<td>iPSC conditioned medium</td>
<td>IV route. A dose of 2×10^6 cells per mouse</td>
<td>Treated mice after BLM instillation show an inhibition of collagen deposition, neutrophil infiltration and myeloperoxidase activity, as well as rescued pulmonary function</td>
<td>(94)</td>
</tr>
<tr>
<td>iPSCs derived to AECII cells</td>
<td>Intratracheal route. A dose of 5×10^5 cells per mouse</td>
<td>Treated mice after BLM instillation have decreased collagen deposition and lung inflammation</td>
<td>(35)</td>
</tr>
</tbody>
</table>

IPSCs, induced pluripotent stem cells; IPF, Idiopathic pulmonary fibrosis; TGF, transforming growth factor; AECII, alveolar epithelial type II cells; BLM, bleomycin; EMT, epithelial-mesenchymal transition.
increasing the delivered cell number at the injured sites and reducing the pulmonary first-pass effect (116,117).

**The effective dose for cell therapy**

Enough cell number reaching the target organ/sties is required for the success of cell-based therapy. The most efficient cell dose for a successful cell therapy is the minimum number of cells that is required to achieve significantly successful and safe outcomes, which varies between different studies. Several attempts have been made to quantitatively evaluate the safety and efficiency of different doses of stem cell types such as MSCs. For example, the IV dose of allogeneic BM-MSCs administered in patients with the first acute myocardial infarction is safe and efficient at the range of 0.5, 1.6 and 5 million cells per kilogram (kg) (118). Notably, among different parameters analyzed for the dose-dependent effect in this study, the premature ventricular contraction exhibited a clear dose-responsiveness (118).

Some preclinical studies used stem cells at doses in a range of 5×10³ to 5×10⁶ cells/kg for IPF therapy (69,119). In murine studies, the effective dose for cell-based therapy is normally 1×10⁶ cells per 30 g mouse, which is equivalent to 2.3×10⁷ cells per average human. Deciding the effective cell dose for cell-based therapy is critical for both the success and safety of clinical trials in humans.

**Timing of the delivery**

The timing of stem cell delivery is a major influencing factor for the success of the cell-based therapy of different human diseases, including IPF (120). For example, in different IPF-inducing strategies and experiments, cells have been transplanted in the IPF model at different time courses; either immediately (66), 15 min (60), 6 hr (69), 8 hr (81), 24 hr (61), 3 days (121), 4 days (65), or even 1 to 2 months (50) after IPF inductions. However, the early administration of cells within 24 hr of lung injury shows the most promising results in healing fibrotic lesions (53). This is probably due to the immunomodulatory ability of the transplanted cells that can reduce the inflammation and lung epithelial damage, leading to the amelioration of the IPF (53).

Transplanted cells, however, do not show a significant effect on the subsequent collagen deposition and fibrosis prevention, but have some aberrant actions when transplanting few days after the injury (53). In contrast, transplanting AECII cells into the damaged lungs 3, 7, or 15 days after BLM instillation can result in a reduced disposition of collagen in the cell matrix, and lead to a reduction in the IPF severity (31). The findings of this study indicate that AEC II cell therapy can reverse the IPF even after the formation of fibrotic lesions. However, when MSCs are administered at later time points of the fibrosis development, the engrafted cells apparently differentiate into the interstitial tissue cells (the tissue and space around the alveoli), and probably contribute to the fibrosis development (113).

**Current IPF clinical trials**

There is currently no effective cure for IPF, but there are some promising preclinical and in vitro data. Stem cell-based therapeutic approaches for human diseases, including IPF, are generally at early experimental phases, and far from mature clinical practices. Several clinical trials on the stem cell-based therapy of IPF are still ongoing (3,122). The major objectives in these clinical trials are the efficacy, safety and tolerability of cell-based therapies in humans. The risk profile in these trials includes the teratoma risk (tumor formation), and risks associated with cell handling methods and the culture/storage protocols, as well as other risks related to the surgical procedures, immunosuppression, comorbidities and allergic immune responses.

Since stem cells are potential candidates for the malignant transformation, the risk of tumor formation for stem cells used in these clinical trials could be high. For example, a patient who received transplanted stem cells has developed a donor-derived multifocal brain tumor 4 years after transplantation, highlighting the potential risk of tumor formation in transplanted stem cells in humans (123).

A major objective of current clinical trials is the efficacy of stem cell therapeutic approaches. Some important questions should be addressed to evaluate the efficacy of a stem cell-based therapeutic approach, including deciding the most appropriate delivery route that enables an efficient recruitment of stem cells to the lung and the appropriate method to induce the functional differentiation of recruited stem cells into lung epithelium to achieve successful therapeutic effects. These questions also include both the ideal dose and time for administration and addressing them properly can lead to an efficient cell-based therapy.

MSCs are the most commonly used stem cells in current clinical trials, because of their low immunogenicity and risk of teratoma, and lack of potential ethical problems (124,125).
Human placenta- and BM-derived MSCs are, particularly, popular in IPF clinical trials (126,127). However, most IPF clinical trials have not been completed yet, and are still in Phase I and II.

Other treatment methods used in clinical trials have revolutionized the IPF management since 2014 when two new anti-fibrotic agents, Nintedanib and Pirfenidone, have emerged. Both Nintedanib and Pirfenidone have shown an ability for delivering a significant reduction in the progression of chronic IPF and approved for the IPF treatment (128,129). The tyrosine kinase inhibitor Nintedanib can slow the IPF progress by both decreasing the declining rate of the FVC and moderating the impairment of lung functions in IPF patients subjected to clinical trials (129). However, there are still many difficulties that surround the clinical end-point selections in IPF clinical trials (128).

Conclusions, current challenges and future prospects

Few cell-based therapies have been used in clinics, including BMSC transplantation, which shows a success in replacing the diseased blood system of patients (130), and skin-derived stem cells used for treating patients with severe burns (131). These cell-based therapy also include cord blood stem cells that are used for both cancerous blood disorders like Fanconi anemia in children (132), and genetic blood diseases such as CFTR.

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The susceptibility to the development of IPF is associated with the genetic polymorphism of certain genes (140-142). Hence, combining cell-based therapy and gene therapy may offer a new strategy for IPF intervention. This has been proposed by developing genetically modified stem cells using viral vectors targeting IPF disease (81). Lung fibrosis such as cystic fibrosis, is indeed an ideal gene therapy target, compared to other lung diseases for several reasons, including the ease of access to the lung, and both CFTR gene cloning and characterization (143).

However, this kind of therapy still have several barriers and challenges (143).

Genetically modified stem cells can be delivered specifically to the injured lung sites and, therefore, can deliver certain genes to be expressed in these lung sites. Progress toward identifying the appropriate specific stem cell population for gene therapy in the airway, and the appropriate gene vector that provides a sustained expression is still an ongoing challenge. One major challenge is the generation of vector-specific tolerance in human patients through the modification of the response of the host immune system to the gene therapy vector that will facilitate the administration of the gene therapy vector.

Other major factors for the success of the gene therapy are their safety and effectiveness that are currently under intensive investigation.

Another future direction is utilizing the cytokine effects on targeted cells to improve the cell-based therapy. This will be largely based on our understanding of the functional roles of cytokines and signaling molecules in enhancing the efficacy of both immune cells and cells responsible for healing the damaged lung, which are well investigated (144). The efficient cell-based therapy approach is, therefore, largely dependent on identifying the appropriate cytokines.
for cell treatments.

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None.

Footnote

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