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The Role of Mesenchymal Stromal Cells in Idiopathic Pulmonary Fibrosis

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Abstract

Idiopathic pulmonary fibrosis (IPF) is a rare and very severe respiratory disease with high morbidity and mortality. For now, no drug-based therapy has been proven unambiguously to reverse the fibrotic process, and no lasting treatment is available other than lung transplantation.

Mesenchymal stromal cells (MSC) are adult multipotent cells capable of differentiating into a number of different cell lineages, which can be isolated from bone marrow, adipose tissue, umbilical cord, amniotic membrane and other tissues, expanded in culture and, subsequently, administered by systemic or local routes into injured animals or ill patients. As a result of their proliferative potential, multipotency, immunomodulatory effects, migratory ability and immunoprivileged state (MSC express very low levels of histocompatibility complex), interest has accelerated as to their imaginable therapeutic applications. Here, we review the properties of MSC, the preclinical studies in animal models of bleomycin-induced lung fibrosis followed by the inoculation of MSC from different origins, and the ongoing human clinical trials using these cells as a therapeutic alternative for IPF.

Keywords

Idiopatic pulmonary fibrosis, Adult stem cells, Mesenchymal stromal cells

Introduction

Idiopathic pulmonary fibrosis (IPF) is a progressive disease with high morbidity and mortality with a 5-year survival of approximately 20% [1-3]. It is the most common of the idiopathic interstitial pneumonias and one of the most prevalent interstitial lung diseases (ILD). Anatomically it is characterized by scarring of the lung, generating a parenchymal fibrosis, which diminishes lung volumes causing progressive functional loss [4,5] and is associated with the histopathologic and/or radiologic pattern of usual interstitial pneumonia (UIP) [5]. The diagnosis requires exclusion of other known causes of ILD, the presence of a UIP pattern on high-resolution computed tomography (HRCT) in patients not subjected to surgical lung biopsy, and specific combinations of HRCT and surgical lung biopsy pattern in patients subjected to surgical lung biopsy [5]. The natural course of IPF is unpredictable with periods of stability followed by episodes of worsening that can result in respiratory failure and death [6].

IPF is unlikely to occur in adults younger than 50, nonetheless it arises in approximately 0.2% of those older than 75 years [7,8]. In Europe and North America the estimated incidence is 3-9 cases per 100,000 per year, and is increasing worldwide [9]. It is likely that IPF accounts for much of the increased ILD-related mortality reported worldwide between 1990 and 2013 [10].

The manifestation of IPF is believed to be linked to one or more factors, depending on the patient: age, genetic predisposition, and repeated damage to the alveolar epithelium. Cigarette smoking, environmental/occupational pollutants, microbial agents, chronic micro-aspiration secondary to gastroesophageal reflux have been cited as some of the insults that could trigger the fibrotic process [11-13].

Inflammation is involved in the pathogenesis of IPF since several cytokines, such as IL-4, IL-5, IL-13, IL-18, and MIP-1α, are at higher concentration in cellular cultures and bronchoalveolar lavage of patients with IPF, compared to normal individuals [14]. However, the leading point of view suggests that persistent micro-injuries to alveolar epithelial cells induce a fibrotic environment and that growth factors and other mediators secreted by the damaged epithelial cells, such as transforming growth factor (TGF)-β, platelet-derived growth factor (PDGF), connective-tissue growth factor, tumor necrosis factor (TNF)-α, fibroblast growth factor (FGF), insulin-like growth factor (IGF)-1 and Wnt-pathway components, among others, participate in the pathogenesis/progression of IPF, promoting fibroblast recruitment, proliferation, and differentiation to invasive myofibroblasts [15]. Consecutively, actively proliferating fibroblasts and myofibroblasts organize into fibroblastic foci and are responsible for the excessive collagen production that results in scarring of the lung and architectural deformation [15,16]. Additionally, matrix metalloproteinases (MMPs), such as MMP1, MMP2, MMP3, MMP7, MMP8, and MMP9 have also been implicated in IPF [17-19].

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Recent clinical trials have been testing new molecules with antifibrotic properties (e.g., pirfenidone and nintedanib) instead of just using anti-inflammatory and immunosuppressant drugs [20]. Nintedanib [21,22], an intracellular inhibitor of several tyrosine kinases that targets multiple growth factor receptors and pirfenidone [23-26], reduced the rate of forced vital capacity (FVC) decline, and are recommended in the treatment of patients with IPF [20]. However, no drug-based therapy has been proven unequivocally to reverse the fibrotic process, and no lasting option for therapy is available other than transplantation merely in a limited number of patients.

Stem cells are considered to represent one of the best hopes for regenerating permanently damaged tissue, and because mesenchymal stem (stromal) cells (MSC) are known to home to sites of injury, inhibit inflammation, and contribute to epithelial tissue repair, their use has been suggested as a potential therapy for the treatment of IPF.

Mesenchymal Stromal Cells (MSC)

Stem cells are undifferentiated cells that can continuously self-renew and also differentiate into multiple cell types [27]. Adult stem cells, which are derived from postnatal fully developed tissue, have a limited lifespan and a restricted differentiation potential [28].

MSC are adult multipotent cells capable of differentiating into a number of different cell lines that can be isolated from bone marrow (BM), adipose tissue (AT), umbilical cord (UC), amniotic membrane(AM) or other sources and expanded in culture and subsequently administered by systemic or local routes into injured animals. Because of their combination of proliferative potential, multipotency, immunomodulatory effects [29-31], migratory ability and immunoprivileged state (MSC do not express major histocompatibility factor II) [32], interest has grown regarding their potential therapeutic applications [33].

A growing body of preclinical literature supports the efficacy of MSC administration in a range of experimentally induced lung pathologies, including those used as models of acute lung injury, lung infections, asthma, bronchopulmonary dysplasia, bronchiolitis obliterans, chronic obstructive pulmonary disease, pulmonary ischemia-reperfusion injury, pulmonary hypertension [34], elastase-induced emphysema [35], asbestos-induced lung injury [36] and radiation-induced injury [37]. These studies show that MSC administration improves part or all of the model-specific disease endpoints.

In the lung, at sites of injury, MSC contribute to tissue regeneration and repair [38-42]. Further, using Y chromosome fluorescence in situ hybridization, transplanted Y chromosome-positive MSC from male donors can be found at sites of lung injury in recipient female mice [39,42]. These male MSC appear to adopt epithelial cell morphology,

Table 1: Studies of animals treated with bleomycin and results after MSC therapy.

Study	Model	Cell Type	Delivery/Dose	Safety/Efficacy Results
Jun *et al.*, [54]	Mouse	Allogeneic LuMSC	i.v. 15 - 25 × 10^4	Reduced induced lung fibrosis
Kumamoto *et al.*, [71]	Mouse	BM-MSC	i.v. 5 × 10^5, 3 days after BLM	Reduced induced lung fibrosis
Ono *et al.*, [73]	Mouse	Xenogeneic cells (human) BM-MSC	i.v. 5 × 10^5, Cells were given 24 hours after BLM	Normal histopathology, Decreased TGF- β, Decreased lung collagen
Gao *et al.*, [74]	Rat	Xenogeneic cells (human) uMSC	i.v. 2.5 × 10^5, Cells were given 3 days after BLM	Increased TNF-α protein level when measured 13 days after MSC administration , Better with improvement ACE2
Lee *et al.*, [39]	Rat	BM-MSC	i.v. 1 × 10^6, 4 days after BLM	Decrease inflammation, Decreased TGF- β, Decreased BAL IL-6 No effect on lung collagen content, Improve the Ashcroft score, BAL total cell count and neutrophil count
Ortiz *et al.*, [42]	Mouse	AllogeneicBM-MSC	i.v. 5 × 10^5, Cells were given immediately after BLM or 7 days later	Decrease inflammation, Decreased MMP-2, MMP-9 and MMP-13 mRNA levels
Lee *et al.*, [72]	Mouse	Xenogeneic cells (human) AD-MSC	i.p. 3 × 10^5, Cells were given at weeks 8, 10, 12, and 14 at the same time as BLM	Decrease inflammation, Improved fibrosis, improvement in alveolar injury, Decreased TGF- β, Decreased IL-1 levels
Garcia *et al.*, [70]	Mouse	Xenogeneic cells (human) AFSC	i.v. 1 × 10^6, Cells were given two hours after BLM or 14 days later	Improved fibrosis, Improvement in alveolar injury, Decreased lung collagen
Zhao *et al.*, [69]	Rat	BM-MSC	i.t. 5 × 10^6, Cells were given 12 hours after BLM	Improved fibrosis, Normal histopathology, Improvement in alveolar injury, Decreased TGF-β
Huang *et al.*, [68]	Rat	BM-MSC	i.v. 2.5 × 10^6, Cells were given the same day as BLM and 7 days later	Improved fibrosis, Improvement in alveolar injury, better effects on hydroxyproline content and alveolitis and fibrosis scores
Moodley *et al.*, [40]	Mouse	Xenogeneic cells (human) uMSC	i.v. 1 × 10^6, Cells were given 24 hours after BLM	Decrease inflammation, Decreased TGF- β & TNF-α mRNA levels, No change in lung IL-1, IL-6 mRNA levels, Increased MMP-2, No change in MMP-9 or MMP-13, Decreased lung collagen
Moodley *et al.*, [75]	Mouse	Xenogeneic cells AM-MSC [vs BM-MSC]	i.v. 1 × 10^6, Cells were administered after 10 days of the first dose of BLM	Decreased TGF- β; TNF-α, IL-1 and IL-6 protein levels, No change in MMP-2 or MMP-13, Increased MMP-9 levels, Decreased lung collagen
Aguilar *et al.*, [76]	Mouse	BM-MSC	i.v. 5 × 10^5, Cells were administered after 8 hours after BLM	Lung collagen assessed at 14 days was decreased
Min *et al.*, [77]	Mouse	Xenogeneic cells (human) uMSC		No change in TNF-α, IL-1 or IL-6 levels, Increased MMP-2, reduced MMP-9, Decreased lung collagen
Ortiz *et al.*, [78]	Mouse	AllogeneicBM-MSC	i.v. 5 × 10^5, Cells were given immediately after BLM or 7 days later	No significant change in lung TNF-α mRNA level.
Gazdhar *et al.*, [79]	Rat	BM-MSC	i.t. 3 × 10^6, Cells were instilled after 7 days of bleomycin treatment	Reduced induced lung fibrosis
Cargnoni *et al.*, [41]	Mouse	Allogeneic & Xenogeneic cells (human) AM-MSC	i.v./i.t. 1 × 10^6, i.p. 4 × 10^6 Cells were administered after 15 min after BLM	Reduced induced lung fibrosis, Decrease in neutrophil infiltration

Abbreviations: BLM = bleomycin; MSC = mesenchymal stem cells; BM-MSC = bone marrow–derived mesenchymal stem cells; LuMSC = Lung MSC; AD-MSC = adipose tissue-derived MSC; AM-MSC = amniotic membrane MSC; uMSC = umbilical cord MSC; AFSC = Amniotic Fluid Stem Cells; BAL = bronchoalveolar lavage; TGF- β = transforming growth factor β; TNF-α = tumor necrosis factor-α; IL-1 = interleukin 1; IL-6 = interleukin 6; MMP-2 = matrix metalloproteinase-2; MMP-9 = matrix metalloproteinase-9; MMP-13 = matrix metalloproteinase-13; i.v. = intravenous; i.p. = intraperitoneal; i.t. = intratracheal

suggesting that they can contribute to tissue regeneration, either by fusion with resident epithelial cells or by mesenchymal-to-epithelial transition [42]. MSC cultured in airway growth media differentially express lung-specific epithelial markers, including club cell (Clara cell) secretory protein, surfactant protein-C, and thyroid transcription factor-1 [43,44].

Also, MSC secrete multiple trophic factors that suppress the immune system [45], inhibit apoptosis [46], stimulate mitosis [47] and differentiation [29-31], and enhance angiogenesis [46,48]. The benefits of MSC trophic factors secretion have been confirmed using co-cultures with endothelial cell populations [49], as well as conditioned media [50] from MSC. MSC are hypothesized to reduce inflammation primarily by release of soluble anti-inflammatory mediators and microvesicles without the need for engraftment or recapitulation of lung morphogenesis. Other mechanisms may be involved, and although significant deficiencies persist regarding our understanding of the disease-specific molecular mechanisms by which MSC mediate the regenerating outcomes, these studies provide a rational basis for the clinical application of MSC in human lung diseases.

Additionally, various populations of lung resident progenitor cells have been identified. Adult pulmonary tissue resident MSC demonstrates a phenotype and function similar to BM-MSC and have been identified in the side population of cells from both murine and human lung tissue [51,52] as well as bronchoalveolar lavage fluid from human lung allografts [53]. Depending on their microenvironment, the lung MSC demonstrate properties similar to other tissue MSC including multilineage differentiation, paracrine antiinflammatory properties, suppression of T cell proliferation as well as the ability to differentiate to myofibroblasts [52,54]. Lung MSC exhibit high telomerase activity which indicates the capacity for self-renewal [51,52,54]. These properties allow a small number of cells to contribute substantially to both tissue regeneration and to proliferative diseases [54-62]. However, in addition to their reparative properties, several studies indicate that MSC may be a critical factor in the development of dysfunctional lung remodeling in some diseases [63-67].

MSC for the Treatment of IPF: Preclinical Studies

MSC may support the restoration of the alveolar epithelium and reduce fibrosis through their anti-apoptotic and anti-scarring effects even in the absence of a substantial and sustained structural engraftment. The MSC effects on lung fibrosis have been investigated with syngeneic, allogeneic, or xenogeneic MSC administration in mouse or rat models of bleomycin-induced lung fibrosis, suggesting that MSC may be efficacious in the treatment of IPF (Table 1).

Of all bleomycin-induced lung fibrosis, improvement in fibrosis was reported in several studies [54,68-72], and a decrease in inflammation was reported in some others [39,40,42,72]. In addition, 3 studies reported near normalization of histopathological change [42,69,73] and four more showed an improvement in alveolar injury [68-70,72]. Moreover, one study reported less improvement with MSC alone than with MSC transfected with ACE2 [74].

On lung collagen, Moodley *et al.* [40] noted that the effect of MSC was significant 14 and 28 days after bleomycin injury (13 and 27 days after cell administration), but not after 7 days. Ortiz *et al.* [42] showed that the effect was meaningful when cells were given immediately, but not 7 days after bleomycin administration. In Moodley *et al.* [75], a significant decrease was seen when human amniotic membrane MSC (AM-MSC) were used, but not with human BM-MSC. Lung collagen content was not decreased by repeated AT-MSC administration at 8, 10, 12, and 14 weeks in a chronic injury model in which bleomycin was given every 2 weeks for 16 weeks [72]. Lung collagen assessed at 14 days was also not decreased by MSC administration twice, at 8 hours and 3 days after bleomycin injury [76].

MSC therapy decreased lung TGF-β levels [39,40,69,72,73,75], including the lung TGF-β protein [39,40,72,73,75] and lung TGF-β mRNA level [40,69]. Lung TNF-α protein level was decreased by amnion MSC but not by BM-MSC. Lung TNF-α mRNA level was also decreased by UC-MSC in [40]. In another study, MSC therapy increased TNF-α protein level when measured 13 days after MSC administration but not at earlier time points [74]. There was no change in TNF-α protein level in [77]. There was no significant change in lung TNF-α mRNA level with MSC therapy in [78]. By contrast, IL-1 levels were significantly decreased by stem cell therapy at all time points, up to 28 days [39]. In a study of Moodley *et al.* the effect was significant when using amnion MSC but not with BM-MSC [75]. There was no significant effect on lung IL-1 level in [77]. In another study [40], lung IL-1 mRNA level was assessed and was not affected by UC-MSC. Lung IL-6 protein level was decreased by MSC therapy in [75], but lung IL-6 mRNA level was not decreased in [40]. There was no significant change in lung IL-6 protein level with MSC therapy in [77] whereas BAL IL-6 was decreased in [39].

Lung MMP-2 and MMP-9 levels were also assessed [40,42,75,77]. In the study of Min *et al.* [77], MMP-2 was increased while MMP-9 was reduced. In one of the Moodley *et al.* studies [40] but not in the other [75], MMP-2 was increased. However, MMP-9 was increased by AM-MSC but not by BM-MSC in [75], but was not significantly affected by MSC therapy in [40]. Lung MMP-2 and MMP-9 mRNA level was decreased by immediate administration of MSC in [42]. Lung MMP-13 was not significantly affected in [40,75], but was significantly decreased by MSC in [42].

Regarding the timing of stem cell administration and chronicity of lung injury, most studies administered MSC within hours to a few days after bleomycin injury. Few studies administered MSC after 7

Table 2: Human Clinical Trials using MSC in IPF (https://clinicaltrials.gov)

Study name	Method of administration and type of transplanted cells	Phase	Status
Study of Autologous Mesenchymal Stem Cells to Treat Idiopathic Pulmonary Fibrosis (NCT01919827)	Endobronchial infusion of autologous MSC derived from bone marrow	1	Recruiting participants
Evaluate Safety and Efficacy of Intravenous Autologous AD-MSC for Treatment of Idiopathic Pulmonary Fibrosis (NCT02135380)	Intravenous autologous Stromal Vascular Fraction Intravenous autologous adipose-derived MSC	1 2	Recruiting participants
A Phase I Study to Evaluate the Potential Role of Mesenchymal Stem Cells in the Treatment of Idiopathic Pulmonary Fibrosis (NCT01385644) [94]	Intravenous infusion of allogenic placental MSC	1	Completed
Safety and Efficacy of Allogeneic Mesenchymal Stem Cells in Patients With Rapidly Progressive Interstitial Lung Disease (NCT02594839)	Intravenous infusion of allogenic bone marrow MSC	1	Recruiting participants
Allogeneic Human Cells (hMSC) in Patients With Idiopathic Pulmonary Fibrosis Via Intravenous Delivery (AETHER) (NCT02013700)	Intravenous allogeneic adult human MSC	1	Ongoing, but not recruiting participants

[68,79] or 10 [75] days, and 1 study, Lee *et al.* examined chronic lung injury with bleomycin injection twice weekly for 16 weeks and MSC administration at weeks 8, 10, 12, and 14 [72]. In this latter study MSC administration had no effect on lung collagen content, but interestingly it improved the Ashcroft score, BAL total cell count and neutrophil count, and lung TGF-β levels. In the study of Huang *et al.*, immediate administration of MSC resulted in significantly better effects on hydroxyproline content and alveolitis and fibrosis scores [68].

Considering the origin of the cells, Ortiz *et al.* [42,78] used allogeneic BALB/c mice BM-MSC in C57BL/6 mice. In [42], allogeneic cell administration reduced the extent of inflammation and lung collagen deposition when administered immediately after lung injury, but not after 7 days. However, MSC were unable to decrease totalBAL cell count or lung TNF-αmRNA levels [42]. There were no studies comparing allogeneic and syngeneic cells. Xenogeneic cells, human cells in mice or rats, were used *in vivo* in many studies [40,41,70,72-75,77], and were able to improve lung collagen content in some of them [40,70,73,75,77]. In summary, improvement in severity and extent of fibrosis 14 days after bleomycin injury and MSC administration was observed with allogeneic cells, whereas treatment with xenogeneic cells resulted in improvement in severity but not extent of fibrosis.

Finally, although the precise mechanism of action of MSC remains elusive [80], in several successful clinical trials [81,82], paracrine factors secretion has been proposed as a mechanism of action. Several paracrine factors released by MSC may alter the microenvironment and may contribute to a beneficial effect in IPF [78,81]. In addition to paracrine signaling, MSC may initiate an effect via cell fusion, cell to cell interactions [83], differentiation, and promotion of neovascularization. Each of these mechanisms alone or in combination may contribute to beneficial effects [84].

MSC for the Treatment of IPF: Human Clinical Data

As of March 2016, there are 5 recruiting, active or completed clinical trials registered on https://clinicaltrials.gov/ (U.S. National Institutes of Health) trying to evaluate the safety and efficacy of MSC therapy in IPF (Table 2). For the time being, the only completed is a small open-label, single center, non-randomized study with 8 patients with moderately severe IPF (FVC ≥ 50% and DLCO ≥ 25%) [85]. These IPF patients received either 1×10^6 ($n = 4$) or 2×10^6 ($n = 4$) unrelated-donor, placenta-derived MSC/kg via a large peripheral vein and were followed for 6 months with lung function (FVC and DLCO), 6-min walk distance (6MWD) and CT scan of the chest. Eight patients with median FVC 60% and DLCO 34.5% predicted, were treated. Both dose schedules were well tolerated with only minor and transient acute adverse effects. MSC infusion was associated with a transient (1% (0-2%)) fall in SaO2 after 15 min. At 6 months FVC, DLCO, 6MWD and CT fibrosis score were unchanged compared with baseline, supporting a good safety profile after 6 months follow-up [85].

There are other studies supposedly not registered in this database, like the one published by Tzouvelekis *et al.* [86], a phase Ib, prospective, non-randomized clinical trial, designed to study the safety of 3 endobronchial infusions, at monthly intervals, of 0.5 million autologous adipose derived stromal cells-stromal vascular fraction (ADSC-SVF) per kg/body weight per infusion (40 million cells per infusion) in 14 IPF patients of mild to moderate disease severity (FVC > 50% predicted value and DLCO > 35% of predicted value). The incidence of treatment emergent adverse events within 12 months was the primary end-point and alterations of functional, exercise capacity and quality of life parameters at serial time points (baseline, 6 and 12 months after first infusion) were exploratory secondary end-points. No cases of serious or clinically meaningful adverse events including short-term infusion toxicities as well as long-term ectopic tissue formation were recorded in all patients. Also, detailed safety monitoring through several time-points indicated that cell-treated patients did not deteriorate in both functional parameters and indicators of quality of life [86].

For the time being, these studies demonstrate a precious safety profile, but nevertheless lack the requirements of a double-blind prospective randomized controlled trial to scientifically and clinically assess the efficacy of a treatment.

Conclusions and Considerations for Future

In conclusion, MSC are a promising alternative for the therapy of IPF, particularly for their suitability to easily be isolated, expanded to big numbers in culture and their immunomodulatory and regenerative properties. However, a number of questions have still no answers: what is the most efficacious source of MSC? Are allogeneic cells as safe and efficacious as autologous MSC? Do MSC retain efficacy after passage? Are MSC most effective in the lung when administered intra-tracheally, intravenously or by some other method? [87].

In this sense, some authors argue that the somehow contradictory results found in the preclinical studies are probably dependent on the native tissue from which these cells are derived. Tissue-specific MSC may retain characteristics of their original tissue source in terms of their differentiation capability and specific cytokine gene expression profile [88]. According to Li *et al.* [89], AD-MSC as compared to BM-MSC have biological benefits in the proliferative capacity, secreted proteins (FGF, interferon-γ, and IGF-1) and immunomodulatory effects. On the other hand BM-MSC seem to have advantages in osteogenic and chondrogenic differentiation potential and other secreted proteins (stem cell-derived factor-1 and hepatocyte growth factor [HGF]) [89].

Profiles of paracrine factor secretion and gene expression of BM-MSC and MSC derived from perinatal tissues (UC-MSC and AM-MSC), have revealed that UC and AM are noticeably different from BM. Although MSC from all sources were found to express similar surface markers, UC-MSC and AM-MSC revealed higher potential of immunomodulatory capacity than BM-MSC (higher levels of HGF, MCP-1, and M-CSF in supernatants). Cytokine IL-1ra was the only factor associated with anti-inflammatory and anti-fibrotic effects of MSC which was secreted significantly higher by BM-MSC compared to perinatal MSC [90]. Perinatal MSC may also be of meaningful value in view of the fact that the treatment of aged patients, as it the case of IPF, may require an alternative source of stem cells, other than their own autologous MSC [91].

Regarding the administration route, in an animal model of neonatal hyperoxic lung injury that induces up-regulation of genes associated with the inflammatory and fibrosis response and cell death, the local intra-tracheal transplantation of human UC-MSC was more effective than the systemic intravenous infusion, with respect to delivery efficiency and therapeutic efficacy [92]. In any case, both of these inoculation routes seem to be better than the intraperitoneal route [93].

There are many alternatives when talking about the "timing" of MSC administration in animal models. Most of the bleomycin preclinicial protocols discussed above are actually testing the prophylactic or preventive effect of these cells, since they are administered at the same time as, or soon after, the damage is caused. In contrast, IPF in humans is most of the times diagnosed when the disease is already in the advanced stage, consequently the genuine regenerative potential of these cells could be at stake unless we are very stringent during the patient selection process, mostly defining very well the magnitude or grade of the disease in order to be counted in the clinical study.

Hence, biological variations between MSC from different pedigrees (BM-MSC, AD-MSC, UC-MSC, AM-MSC, etc.), inoculation routes (intravenous, intra-tracheal, etc.) and extent of the disease ("timing") should be thoroughly considered when choosing a specific clinical application for these cells. And, either way, is still early to draw conclusions on the best source of MSC and the best way to administer, although, possibly, intra-tracheal instillation of MSC obtained from perinatal tissues may have some advantages.

References

1. King TE Jr, Schwarz MI, Brown K, Tooze JA, Colby TV, et al. (2001) Idiopathic pulmonary fibrosis: relationship between histopathologic features and mortality. Am J Respir Crit Care Med 164: 1025-1032.
2. Flaherty KR,Toews GB, Travis WD, Colby TV, Kazerooni EA, et al. (2002) Clinical significance of histological classification of idiopathic interstitial pneumonia. Eur Respir J 19: 275-283.
3. Bjoraker JA, Ryu JH, Edwin MK, Myers JL, Tazelaar HD, et al., Prognostic significance of histopathologic subsets in idiopathic pulmonary fibrosis. Am J Respir Crit Care Med, 1998. 157(1): 199-203.
4. Spagnolo P, Sverzellati N, Rossi G, Cavazza A, Tzouvelekis A, et al. (2015) Idiopathic pulmonary fibrosis: an update. Ann Med 47: 15-27.
5. Raghu G, Collard HR, Egan JJ, Martinez FJ, Behr J, et al. (2011) An official ATS/ERS/JRS/ALAT statement: idiopathic pulmonary fibrosis: evidence-based guidelines for diagnosis and management. Am J Respir Crit Care Med 183: 788-824.
6. Ley B, Collard HR, King TE Jr (2011) Clinical course and prediction of survival in idiopathic pulmonary fibrosis. Am J Respir Crit Care Med 183: 431-440.
7. Raghu G, Chen SY, Yeh WS, Maroni B, Li Q, et al. (2014) Idiopathic pulmonary fibrosis in US Medicare beneficiaries aged 65 years and older: incidence, prevalence, and survival, 2001-11. Lancet Respir Med 2: 566-572.
8. Raghu G,Weycker D, Edelsberg J, Bradford WZ, Oster G (2006) Incidence and prevalence of idiopathic pulmonary fibrosis. Am J Respir Crit Care Med 174: 810-816.
9. Hutchinson J, Fogarty A, Hubbard R, McKeever T (2015) Global incidence and mortality of idiopathic pulmonary fibrosis: a systematic review. Eur Respir J 46: 795-806.
10. GBD 2013 Mortality and Causes of Death Collaborators (2015) Global, regional, and national age-sex specific all-cause and cause-specific mortality for 240 causes of death, 1990-2013: a systematic analysis for the Global Burden of Disease Study 2013. Lancet 385: 117-171.
11. Spagnolo P, Grunewald J, du Bois RM (2014) Genetic determinants of pulmonary fibrosis: evolving concepts. Lancet Respir Med 2: 416-428.
12. Olson AL,Swigris JJ (2012) Idiopathic pulmonary fibrosis: diagnosis and epidemiology. Clin Chest Med 33: 41-50.
13. Iwai K, Mori T, Yamada N, Yamaguchi M, Hosoda Y (1994) Idiopathicpulmonary fibrosis. Epidemiologic approaches to occupational exposure. Am J Respir Crit Care Med 150: 670-675.
14. Bringardner BD,Baran CP, Eubank TD, Marsh CB (2008) The role of inflammation in the pathogenesis of idiopathic pulmonary fibrosis. Antioxid Redox Signal 10: 287-301.
15. Selman, M. and A. Pardo, Revealing the pathogenic and aging-related mechanisms of the enigmatic idiopathic pulmonary fibrosis. an integral model. Am J Respir Crit Care Med, 2014. 189(10): 1161-72.
16. Spagnolo P, Rossi G, Cavazza A (2014) Pathogenesis of idiopathic pulmonary fibrosis and its clinical implications. Expert Rev ClinImmunol 10: 1005-1017.
17. Pardo A, Selman M (2012) Role of matrix metaloproteases in idiopathic pulmonary fibrosis. Fibrogenesis Tissue Repair 5: S9.
18. Dancer RC, Wood AM, Thickett DR (2011) Metalloproteinases in idiopathic pulmonary fibrosis. Eur Respir J 38: 1461-1467.
19. Sbardella D, Fasciglione GF, Gioia M, Ciaccio C, Tundo GR, et al. (2012) Human matrix metalloproteinases: an ubiquitarian class of enzymes involved in several pathological processes. Mol Aspects Med 33: 119-208.
20. Raghu G, Rochwerg B, Zhang Y, Garcia CA, Azuma A, et al. (2015) An Official ATS/ERS/JRS/ALAT Clinical Practice Guideline: Treatment of Idiopathic Pulmonary Fibrosis. An Update of the 2011 Clinical Practice Guideline. Am J Respir Crit Care Med 192: e3-19.
21. Richeldi L, du Bois RM, Raghu G, Azuma A, Brown KK, et al. (2014) Efficacy and safety of nintedanib in idiopathic pulmonary fibrosis. N Engl J Med 370: 2071-2082.
22. Richeldi L, Costabel U, Selman M, Kim DS, Hansell DM, et al. (2011) Efficacy of a tyrosine kinase inhibitor in idiopathic pulmonary fibrosis. N Engl J Med 365: 1079-1087.
23. King TE Jr, Bradford WZ, Castro-Bernardini S, Fagan EA, Glaspole I, et al. (2014) A phase 3 trial of pirfenidone in patients with idiopathic pulmonary fibrosis. N Engl J Med 370: 2083-2092.
24. Noble PW, Albera C, Bradford WZ, Costabel U, Glassberg MK, et al. (2011) Pirfenidone in patients with idiopathic pulmonary fibrosis (CAPACITY): two randomised trials. Lancet 377: 1760-1769.
25. Taniguchi H, Ebina M, Kondoh Y, Ogura T, Azuma A, et al. (2010) Pirfenidone in idiopathic pulmonary fibrosis. Eur Respir J 35: 821-829.
26. Azuma A, Nukiwa T, Tsuboi E, Suga M, Abe S, et al. (2005) Double-blind, placebo-controlled trial of pirfenidone in patients with idiopathic pulmonary fibrosis. Am J Respir Crit Care Med 171: 1040-1047.
27. Wagers AJ,Weissman IL (2004) Plasticity of adult stem cells. Cell 116: 639-648.
28. Blanpain C, Fuchs E (2014) Stem cell plasticity. Plasticity of epithelial stem cells in tissue regeneration. Science 344: 1242281.
29. Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, et al. (1999) Multilineage potential of adult human mesenchymal stem cells. Science 284: 143-147.
30. Hocking AM, Gibran NS (2010) Mesenchymal stem cells: paracrine signaling and differentiation during cutaneous wound repair. Exp Cell Res 316: 2213-2219.
31. Haynesworth SE,Goshima J, Goldberg VM, Caplan AI (1992) Characterization of cells with osteogenic potential from human marrow. Bone 13: 81-88.
32. Le Blanc K, Frassoni F, Ball L, Locatelli F, Roelofs H, et al. (2008) Mesenchymal stem cells for treatment of steroid-resistant, severe, acute graft-versus-host disease: a phase II study. Lancet 371: 1579-1586.
33. Kotton DN (2012) Next-generation regeneration: the hope and hype of lung stem cell research. Am J RespirCrit Care Med 185: 1255-1260.
34. Weiss DJ, Kolls JK, Ortiz LA, Panoskaltsis-Mortari A, Prockop DJ (2008) Stem cells and cell therapies in lung biology and lung diseases. Proc Am ThoracSoc 5: 637-667.
35. Ishizawa K, Kubo H, Yamada M, Kobayashi S, Numasaki M, et al. (2004) Bone marrow-derived cells contribute to lung regeneration after elastase-induced pulmonary emphysema. FEBS Lett 556: 249-252.
36. Spees JL, Pociask DA, Sullivan DE, Whitney MJ, Lasky JA, et al. (2007) Engraftment of bone marrow progenitor cells in a rat model of asbestos-induced pulmonary fibrosis. Am J Respir Crit Care Med 176: 385-394.
37. Westbury CB, Yarnold JR (2012) Radiation fibrosis--current clinical and therapeutic perspectives. Clin Oncol (R CollRadiol) 24: 657-672.
38. Rojas M, Xu J, Woods CR, Mora AL, Spears W, et al. (2005) Bone marrow-derived mesenchymal stem cells in repair of the injured lung. Am J Respir Cell Mol Biol 33: 145-152.
39. Lee SH, Jang AS, Kim YE, Cha JY, Kim TH, et al. (2010) Modulation of cytokine and nitric oxide by mesenchymal stem cell transfer in lung injury/fibrosis. Respir Res 11: 16.
40. Moodley Y, Atienza D, Manuelpillai U, Samuel CS, Tchongue J, et al. (2009) Human umbilical cord mesenchymal stem cells reduce fibrosis of bleomycin-induced lung injury. Am J Pathol 175: 303-313.
41. Cargnoni A, Gibelli L, Tosini A, Signoroni PB, Nassuato C, et al. (2009) Transplantation of allogeneic and xenogeneic placenta-derived cells reduces bleomycin-induced lung fibrosis. Cell Transplant 18: 405-422.
42. Ortiz LA, Gambelli F, McBride C, Gaupp D, Baddoo M, et al. (2003) Mesenchymal stem cell engraftment in lung is enhanced in response to bleomycin exposure and ameliorates its fibrotic effects. Proc Natl Acad Sci U S A 100: 8407-8411.
43. Ma N, Gai H, Mei J, Ding FB, Bao CR, et al. (2011) Bone marrow mesenchymal stem cells can differentiate into type II alveolar epithelial cells in vitro. Cell BiolInt 35: 1261-1266.
44. Sueblinvong V, Loi R, Eisenhauer PL, Bernstein IM, Suratt BT, et al. (2008) Derivation of lung epithelium from human cord blood-derived mesenchymal stem cells. Am J Respir Crit Care Med 177: 701-711.
45. Le Blanc K, Rasmusson I, Sundberg B, Götherström C, Hassan M, et al. (2004) Treatment of severe acute graft-versus-host disease with third party haploidentical mesenchymal stem cells. Lancet 363: 1439-1441.
46. Hung, SC, Pochampally RR, Chen SC, Hsu SC, Prockop DJ et al. (2009) Angiogenic effects of human multipotent stromal cell conditioned medium activate the PI3K-Akt pathway in hypoxic endothelial cells to inhibit apoptosis, increase survival, and stimulate angiogenesis. Stem Cells, 2007. 25: 2363-70.
47. Fierro FA, Kalomoiris S, Sondergaard CS, Nolta JA (2011) Effects on proliferation and differentiation of multipotent bone marrow stromal cells engineered to express growth factors for combined cell and gene therapy. Stem Cells 29: 1727-1737.
48. Caplan AI, Dennis JE (2006) Mesenchymal stem cells as trophic mediators. J Cell Biochem 98: 1076-1084.
49. Buschmann J, Welti M, Hemmi S, Neuenschwander P, Baltes C, et al. (2011) Three-dimensional co-cultures of osteoblasts and endothelial cells in DegraPol foam: histological and high-field magnetic resonance imaging analyses of pre-engineered capillary networks in bone grafts. Tissue Eng Part A 17: 291-299.
50. Chen L,Tredget EE, Wu PY, Wu Y (2008) Paracrine factors of mesenchymal stem cells recruit macrophages and endothelial lineage cells and enhance wound healing. PLoS One 3: e1886.

51. Summer R, Fitzsimmons K, Dwyer D, Murphy J, Fine A (2007) Isolation of an adult mouse lung mesenchymal progenitor cell population. Am J Respir Cell Mol Biol 37: 152-159.

52. Martin J, Helm K, Ruegg P, Varella-Garcia M, Burnham E, et al. (2008) Adult lung side population cells have mesenchymal stem cell potential. Cytotherapy 10: 140-151.

53. Lama VN, Smith L, Badri L, Flint A, Andrei AC, et al. (2007) Evidence for tissue-resident mesenchymal stem cells in human adult lung from studies of transplanted allografts. J Clin Invest 117: 989-996.

54. Jun D, Garat C, West J, Thorn N, Chow K, et al. (2011) The pathology of bleomycin-induced fibrosis is associated with loss of resident lung mesenchymal stem cells that regulate effector T-cell proliferation. Stem Cells 29: 725-735.

55. Fatima S, Zhou S, Sorrentino BP (2012) Abcg2 expression marks tissue-specific stem cells in multiple organs in a mouse progeny tracking model. Stem Cells 30: 210-221.

56. Benvenuto F, Ferrari S, Gerdoni E, Gualandi F, Frassoni F, et al. (2007) Human mesenchymal stem cells promote survival of T cells in a quiescent state. Stem Cells 25: 1753-1760.

57. Tsai MS, Hwang SM, Chen KD, Lee YS, Hsu LW, et al. (2007) Functional network analysis of the transcriptomes of mesenchymal stem cells derived from amniotic fluid, amniotic membrane, cord blood, and bone marrow. Stem Cells 25: 2511-2523.

58. Aslam M, Baveja R, Liang OD, Fernandez-Gonzalez A, Lee C, et al. (2009) Bone marrow stromal cells attenuate lung injury in a murine model of neonatal chronic lung disease. Am J Respir Crit Care Med 180: 1122-1130.

59. McQualter JL, Brouard N, Williams B, Baird BN, Sims-Lucas S, et al. (2009) Endogenous fibroblastic progenitor cells in the adult mouse lung are highly enriched in the sca-1 positive cell fraction. Stem Cells 27: 623-633.

60. Chateauvieux S, Ichanté JL, Delorme B, Frouin V, Piétu G, et al. (2007) Molecular profile of mouse stromal mesenchymal stem cells. Physiol Genomics 29: 128-138.

61. Volckaert T, Dill E, Campbell A, Tiozzo C, Majka S, et al. (2011) Parabronchial smooth muscle constitutes an airway epithelial stem cell niche in the mouse lung after injury. J Clin Invest 121: 4409-4419.

62. Lee RH, Pulin AA, Seo MJ, Kota DJ, Ylostalo J, et al. (2009) Intravenous hMSCs improve myocardial infarction in mice because cells embolized in lung are activated to secrete the anti-inflammatory protein TSG-6. Cell Stem Cell 5: 54-63.

63. Bentley JK, Popova AP, Bozyk PD, Linn MJ, Baek AE, et al. (2010) Ovalbumin sensitization and challenge increases the number of lung cells possessing a mesenchymal stromal cell phenotype. Respir Res 11: 127.

64. Brewster CE, Howarth PH, Djukanovic R, Wilson J, Holgate ST, et al. (1990) Myofibroblasts and subepithelial fibrosis in bronchial asthma. Am J Respir Cell Mol Biol 3: 507-511.

65. Todd JL, Palmer SM (2011) Bronchiolitis obliterans syndrome: the final frontier for lung transplantation. Chest 140: 502-508.

66. Walker N, Badri L, Wettlaufer S, Flint A, Sajjan U, et al. (2011) Resident tissue-specific mesenchymal progenitor cells contribute to fibrogenesis in human lung allografts. Am J Pathol 178: 2461-2469.

67. Popova AP, Bozyk PD, Bentley JK, Linn MJ, Goldsmith AM, et al. (2010) Isolation of tracheal aspirate mesenchymal stromal cells predicts bronchopulmonary dysplasia. Pediatrics 126: e1127-1133.

68. Huang, K, Bozyk PD, Bently JK, ynn MJ, Goldsmith AM, et al. (2015) Conversion of bone marrow mesenchymal stem cells into type II alveolar epithelial cells reduces pulmonary fibrosis by decreasing oxidative stress in rats. Mol Med Rep 11: 1685-92.

69. Zhao F, Zhang YF, Liu YG, Zhou JJ, Li ZK, et al. (2008) Therapeutic effects of bone marrow-derived mesenchymal stem cells engraftment on bleomycin-induced lung injury in rats. Transplant Proc 40: 1700-1705.

70. Garcia O, Carraro G, Turcatel G, Hall M, Sedrakyan S, et al. (2013) Amniotic fluid stem cells inhibit the progression of bleomycin-induced pulmonary fibrosis via CCL2 modulation in bronchoalveolar lavage. PLoS One 8: e71679.

71. Kumamoto M, Nishiwaki T, Matsuo N, Kimura H, Matsushima K (2009) Minimally cultured bone marrow mesenchymal stem cells ameliorate fibrotic lung injury. Eur Respir J 34: 740-748.

72. Lee SH, Lee EJ, Lee SY, Kim JH, Shim JJ, et al. (2014) The effect of adipose stem cell therapy on pulmonary fibrosis induced by repetitive intratracheal bleomycin in mice. Exp Lung Res 40: 117-125.

73. Ono M, Ohkouchi S, Kanehira M, Tode N, Kobayashi M, et al. (2015) Mesenchymal stem cells correct inappropriate epithelial-mesenchyme relation in pulmonary fibrosis using stanniocalcin-1. Mol Ther 23: 549-60.

74. Gao F, Li Q, Hou L, Li Z, Min F, et al. (2014) Mesenchymal stem cell-based angiotensin-converting enzyme 2 in treatment of acute lung injury rat induced by bleomycin. Exp Lung Res 40: 392-403.

75. Moodley Y, Vaghjiani V, Chan J, Baltic S, Ryan M, et al. (2013) Anti-inflammatory effects of adult stem cells in sustained lung injury: a comparative study. PLoS One 8: e69299.

76. Aguilar S, Scotton CJ, McNulty K, Nye E, Stamp G, et al. (2009) Bone marrow stem cells expressing keratinocyte growth factor via an inducible lentivirus protects against bleomycin-induced pulmonary fibrosis. PLoS One 4: e8013.

77. Min F, Gao F, Li Q, Liu Z (2015) Therapeutic effect of human umbilical cord mesenchymal stem cells modified by angiotensin-converting enzyme 2 gene on bleomycin-induced lung fibrosis injury. Mol Med Rep 11: 2387-2396.

78. Ortiz LA, Ditreil M, Fattman C, Pandey AC, Torres G, et al. (2007) Interleukin 1 receptor antagonist mediates the antiinflammatory and antifibrotic effect of mesenchymal stem cells during lung injury. Proceedings of the National Academy of Sciences of the United States of America 104: 11002-11007.

79. Gazdhar A, Susuri N, Hostettler K, Gugger M, Knudsen L, et al. (2013) HGF Expressing Stem Cells in Usual Interstitial Pneumonia Originate from the Bone Marrow and Are Antifibrotic. PLoS One 8: e65453.

80. McNulty K, Janes SM (2012) Stem cells and pulmonary fibrosis: cause or cure? Proc Am Thorac Soc 9: 164-171.

81. Williams AR, Hare JM (2011) Mesenchymal stem cells: biology, pathophysiology, translational findings, and therapeutic implications for cardiac disease. Circ Res 109: 923-940.

82. Elnakish MT, Hassan F, Dakhlallah D, Marsh CB, Alhaider IA, et al. (2012) Mesenchymal stem cells for cardiac regeneration: translation to bedside reality. Stem Cells Int 2012: 646038.

83. Islam MN, Das SR, Emin MT, Wei M, Sun L, et al. (2012) Mitochondrial transfer from bone-marrow-derived stromal cells to pulmonary alveoli protects against acute lung injury. Nat Med 18: 759-765.

84. Toonkel RL, Hare JM, Matthay MA, Glassberg MK (2013) Mesenchymal stem cells and idiopathic pulmonary fibrosis. Potential for clinical testing. Am J Respir Crit Care Med 188: 133-140.

85. Chambers DC, Enever D, Ilic N, Sparks L, Whitelaw K, et al. (2014) A phase 1b study of placenta-derived mesenchymal stromal cells in patients with idiopathic pulmonary fibrosis. Respirology 19: 1013-1018.

86. Tzouvelekis A, Paspaliaris V, Koliakos G, Ntolios P, Bouros E, et al. (2013) A prospective, non-randomized, no placebo-controlled, phase Ib clinical trial to study the safety of the adipose derived stromal cells-stromal vascular fraction in idiopathic pulmonary fibrosis. J Transl Med 11: 171.

87. Glassberg MK, Toonkel RL (2014) Moving stem cell therapy to patients with idiopathic pulmonary fibrosis. Respirology 19: 950-951.

88. Ock SA, Baregundi Subbarao R, Lee YM, Lee JH, Jeon RH, et al. (2016) Comparison of Immunomodulation Properties of Porcine Mesenchymal Stromal/Stem Cells Derived from the Bone Marrow, Adipose Tissue, and Dermal Skin Tissue. Stem Cells Int 2016: 9581350.

89. Li CY, Wu XY, Tong JB, Yang XX, Zhao JL, et al. (2015) Comparative analysis of human mesenchymal stem cells from bone marrow and adipose tissue under xeno-free conditions for cell therapy. Stem Cell Res Ther 6: 55.

90. Wegmeyer H, Bröske AM, Leddin M, Kuentzer K, Nisslbeck AK, et al. (2013) Mesenchymal stromal cell characteristics vary depending on their origin. Stem Cells Dev 22: 2606-2618.

91. Kalaszczynska I, Ferdyn K (2015) Wharton's jelly derived mesenchymal stem cells: future of regenerative medicine? Recent findings and clinical significance. Biomed Res Int 2015: 430847.

92. Sung DK, Chang YS, Ahn SY, Sung SI, Yoo HS, et al. (2015) Optimal Route for Human Umbilical Cord Blood-Derived Mesenchymal Stem Cell Transplantation to Protect Against Neonatal Hyperoxic Lung Injury: Gene Expression Profiles and Histopathology. PLoS One 10: e0135574.

93. Chang YS, Oh W, Choi SJ, Sung DK, Kim SY, et al. (2009) Human umbilical cord blood-derived mesenchymal stem cells attenuate hyperoxia-induced lung injury in neonatal rats. Cell Transplant 18: 869-886.

94. Ilić N, Atkinson K, (2014) Manufacturing and use of human placenta-derived mesenchymal stromal cells for phase I clinical trials: establishment and evaluation of a protocol. Vojnosanit Pregl 71(7): 651-9.