


Mesenchymal Stem Cell Therapy of Pulmonary Fibrosis: Improvement with Target Combination

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Abstract

Although the clinical application of new drugs has been shown to be effective in slowing disease progression and improving the quality of life in patients with pulmonary fibrosis, the damaged lung tissue does not recover with these drugs. Thus, there is an urgent need to establish regenerative therapy, such as stem cell therapy or tissue engineering. Moreover, the clinical application of mesenchymal stem cell (MSC) therapy has been shown to be safe in humans with idiopathic pulmonary fibrosis (IPF). It seems that a combination of MSC transplantation and pharmaceutical therapy might have additional benefits; however, the experimental design for its efficacy is still lacking. In this review, we provide an overview of the mechanisms that were identified when IPF was treated with MSC transplantation or new drugs. To maximize the therapeutic effect, we suggest that MSC transplantation is combined with drug application for synergistic effects. This review provides clinicians and scientists with the most efficient medical options, in the hope that this will spur on future research and lead to an eventual cure for this disease.

Keywords

pulmonology, pharmacology, stem cell therapy

Introduction

Idiopathic pulmonary fibrosis (IPF) is a specific form of chronic and progressive fibrosing interstitial pneumonia of unknown cause¹. It occurs mainly in older adults (median age at diagnosis 66 years, range 55–75 years)², is limited to the lungs, and is characterized by fibroblast proliferation and extracellular matrix remodeling³, which results in progressively worsening dyspnea and pulmonary function, usually with poor prognosis. The diagnosis of IPF requires the histopathologic and/or radiologic pattern of usual interstitial pneumonitis on high-resolution computed tomography. Other known causes of interstitial lung disease (ILD) (e.g. connective tissue diseases, drug toxicity, and other environmental exposures) must be excluded first. IPF is a fatal lung disease, with median survival ranging from 3 to 5 years³. The majority of patients demonstrate a slow and gradual progression over many years, and some patients remain stable while others have an accelerated decline⁴. A small minority of IPF patients (approximately 5–10%) may suffer from acute exacerbation annually¹. The incidence of IPF is reported to be an estimated 4.6 to 16.3 cases per 100,000 worldwide. Based

on healthcare claims data from the United States, an estimated prevalence of between 14.0 and 42.7 per 100,000 persons was reported⁵.

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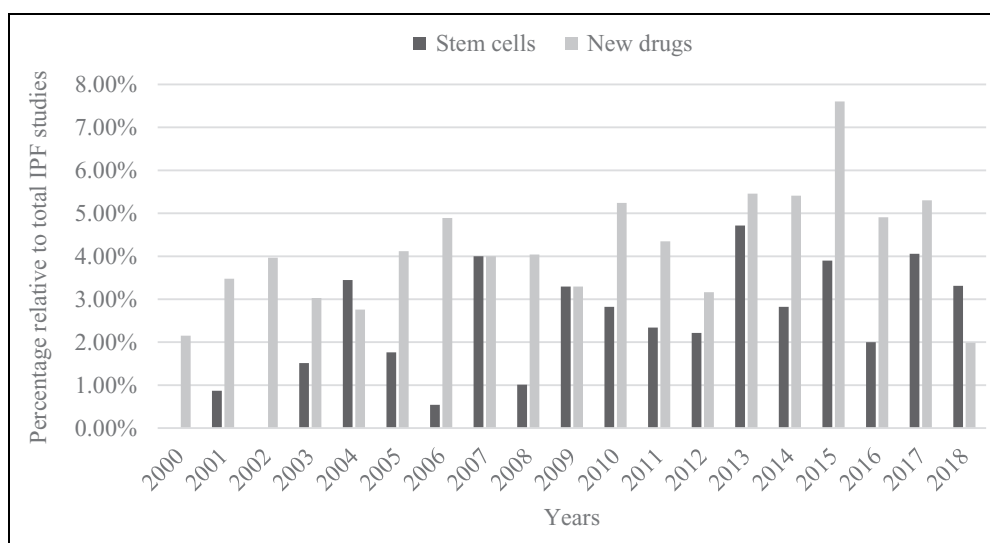


Fig. 1. Publications of new drugs or stem cells development and each percentage of total IPF studies.

The antifibrotic drugs nintedanib and pirfenidone have been shown to significantly reduce the decline in forced vital capacity (FVC), decrease acute exacerbation, and improve mortality rates in patients with IPF in several studies^{6–8}. Recently, they have been recommended conditionally in several guidelines for the treatment of IPF⁹; however, current medication can only slow the progression of IPF but does not reverse the fibrosis process. There is still a lack of effective options for treating IPF, although abundant possible targets from animal studies have shown their potential. Even though some off label drugs, such as inhaled NAC, could shorten the time of investigation through meta-analysis, the response is relatively limited compared with specific target therapy¹⁰. Previous studies have provided results for both stem cell and pharmacological treatments, but data on a combination of these are still lacking. In this review, we have summarized the experimental and clinical evidence and possible mechanisms of action to present the potential options for this intractable disease.

Treatment Strategies in IPF

Compared with other diseases of the respiratory system, IPF is hard to predict and prognosticate. The main cause of IPF is still unclear, but it may involve genetic, environmental, or toxic components¹¹. Bleomycin (BLM) is commonly used to induce symptoms of IPF in animal models, as it produces an oxidative stress specific to alveolar epithelial cells¹². In these models, the immune cells and fibroblasts gradually infiltrate the lungs of BLM-treated mice. On the other hand, differentiation and proliferation of myofibroblasts from fibroblasts produce excessive extracellular matrix. Consequently, anti-inflammatories have been commonly used as a treatment option in previous studies; however, the treatment was stopped in one trial due to an increasing risk of death in IPF patients with no clinical benefit¹³. Other

inducers, such as transforming growth factor β (TGF- β) and fibroblast growth factor (FGF), have been used as targets for blockage by inhibitors or antibodies¹⁴. In recent years, there is evidence of an increasing trend on the use of stem cell therapy in IPF; its use almost comparable to studies on new drug development and compound studies (Fig 1). This trend suggests the potential benefit of stem cell treatment in IPF. The first successful stem cell transplantation was carried out 50 years ago¹⁵, but even now there are potential exciting applications that deserve our attention.

Developmental Drugs for Treatment of IPF

The two Food and Drug Administration (FDA)-approved pharmacological targets for treatment of IPF are TGF- β (pirfenidone) and PDGF/VEGF/FGF (nintedanib). The efficacy of pirfenidone is impressive, as it was found to significantly lower the relative risk of death compared to the placebo group at week 52¹⁶. The data collected from four phase III trials showed that the mean life expectancy was 8.72 years for patients given pirfenidone versus 6.24 years for those receiving best supportive care¹⁷. On the other hand, in a 52-week phase III trial involving 1,061 subjects, nintedanib was found to slow disease progression, but there was no statistically significant difference for the primary endpoint¹⁸. Although the preclinical studies are quite promising, the human trials did not show consistent results, not including the cases waiting for FDA approval. Potential developmental drugs provide additional possible targets for improving current therapy. Pamrevlumab, an anti-connective tissue growth factor antibody (FG3019), is under phase III trials (NCT01890265). Another drug that targets α v β 6 integrin has also completed a phase II trial involving IPF participants (NCT01371304). In short, new drug development for IPF is a highly risky and ineffective business.

Current Stem Cell Therapies for IPF

Despite the fact that MSC therapy has been used in the clinic for approximately 10 years, more than 75% of studies are still in phase II or earlier¹⁹. Lung diseases account for 23 of the 493 transplantation studies, according to <https://www.clinicaltrials.gov/>. More recently, an allogeneic bone marrow MSC (BMSC) transplantation study in IPF patients assigned three cohorts to a single IV infusion of 20, 100, or 200 × 10⁶ human BMSC²⁰. There is also a case report of a 56-year-old man with IPF who received human umbilical cord-derived mesenchymal stem cell (HUC-MS) transplantation with intravenous infusion²¹. Both studies, observed over 1 year, did not register any adverse events. The authors of a phase I trial of adipose-derived stem cell (ADSC) treatment for IPF reported on their experiences of acquiring FDA approval (NCT01385644). The protocol used three rounds of endobronchial infusion with Stromal vascular fraction (SVF), totaling 1.5 × 10⁶ cells, and the cell fate was traced by ^{99m}Tc-HMPAO²². These reports suggest that stem cell transplantation in IPF is initially safe and with some benefits, and the standardized protocol might encourage scientists to explore the potential for MSC therapy in IPF patients. Data from previous clinical studies suggest that the transplanted cell type is important for IPF treatment. Of the problems that may arise between the MSC source and the receiver during allograft, placenta-derived MSCs such as umbilical cord blood or amnion stem cell provides less complications.²³ It has been well-established that management of host rejection through HLA typing in placenta-derived MSCs can be performed easily in clinical applications.²⁴ The results with amniotic stem cells are the most abundant results in clinical studies. Although their primary endpoints were usually for safety and not efficacy, stem cells still hold promise for better outcomes than most known drugs²⁵.

Mechanisms of Stem Cell Therapy in IPF

The progression of IPF is a complex event consisting, at minimum, of epithelial-dependent fibroblast-activated processes and poor response to anti-inflammatories. These effects involve downstream pathways of epithelial mesenchymal transition (EMT) and an immune response³, as shown in Fig 2. Some EMT regulators like WISP-1 and BMP4 have been found to have a role in fibroblast differentiation or collagen production through Wnt and BMP pathways, respectively^{26,27}. In regard to an immune response, the PGE2 pathways can prevent Fas ligand-induced apoptosis in myofibroblasts and fibroblasts; however, due to the lower expression of PGE2, alveolar type II cells are still sensitive to apoptosis²⁸. The differences in expression might explain the apoptosis in alveolar epithelial cells but not in myofibroblasts or fibroblasts. Targeting of cell cycle-related proteins has also been reported as a potential treatment for IPF. S-phase kinase-associated protein 2 (Skp2) is an effector protein that enhances degradation of p27²⁹ and is essential for the progression of IPF. Increased

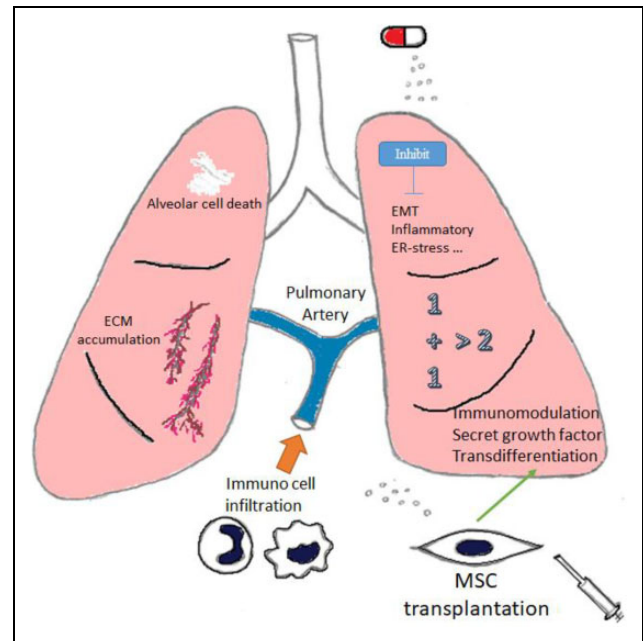


Fig. 2. Mechanism assuming that the fibrogenesis consists of alveolar cell apoptosis and immune cell infiltration (left). The transplanted stem cells circulate through the pulmonary artery (bottom) and act through immunomodulation, secretion of growth factor, and differentiation into alveolar-like cells (right).

expression of p27 with an antagonist for Skp2, SZL-P1-41, could inhibit BLM-induced pulmonary fibrosis³⁰. Additionally, treatment with BLM in fibroblasts also induces ER stress-related proteins, such as GRP78, CHOP, and ATF4, and enhances proliferation of fibroblasts through a PI3K/Akt pathway³¹. The activation of the PI3K/Akt pathway is another upstream regulator of EMT³² and makes the response more unpredictable and irreversible. Although there are few connections between each signaling pathway, the evidence has shown that the use of each inhibitor, such as LY294002 and Tauroursodeoxycholic acid (TUDCA), is efficient at ameliorating pulmonary fibrosis.

Regulation of Immune Response or Secretome

Bone marrow (BM)-derived MSCs are used more often in mechanism studies than in clinical studies³³, perhaps because the source is easier to obtain. It is thought that the injected cells could repair lung tissue with its immunomodulatory effects. From the immunological perspective, the BM-derived MSCs had lower levels of expression of the immunosuppressive molecules PDL-1 and CD1a than placenta-derived MSCs, and IFN- γ induction also showed lower potential to stimulate T cell proliferation in BM-derived MSCs³⁴. However, placenta-derived MSCs had a suppressive effect in cytokine-stimulated natural killer lymphocytes³⁵, though the cell type is still hard to ascertain. Furthermore, the process of IPF is usually accompanied by inflammation and profibrotic cytokines. To produce an

anti-inflammatory effect, MSCs reduce IL-1 β , IL-6, TNF- α , TGF- β , and vascular endothelial growth factor (VEGF) in BLM-induced lung injury³⁶. Although these studies did not find leftover stem cells in injured lungs, the secreted circulating mediators were taken into account to benefit the therapeutic effects for IPF³⁷.

Stem cell therapy also benefits from secretion of isolated exosomes that contain proteins and RNA from their own cell origin³⁸. These extracellular vesicles of between 10 and 100 nm in size, called exosomes, allow communication between distal cells and their cargos³⁹. The effects induced by exosomes may be associated with the modulation of the immune response and suppression of macrophage phenotype⁴⁰. To confirm this theory of stem cell secretion, Tan et al. purified exosomes from amniotic stem cells and administered them intranasally. They found that pulmonary capacity was recovered in the exosome-treated groups in BLM-induced pulmonary fibrosis⁴¹. Moreover, the specific delivery potential of exosomes has been reported to be due to the presence of membrane proteins⁴², making the use of exosomes instead of MSCs alone a better proposition. However, a contradictory result suggested that MSC-isolated microvesicles (MV) reduced pulmonary fibrosis, and that MSCs have a better treatment effect than MV⁴³. Unfortunately, these authors did not reveal the amount of MSCs used for isolating the MV, and the result is also lacking in dosage effectiveness. Nevertheless, these studies suggest that the treatment potential of MSC therapy is positive regardless of its presence in the lung tissue.

Evidence for Differentiation into Functional Alveolar Cells

The injected MSCs were found in the lung epithelium and expressed an epithelium-like phenotype⁴⁴; but researchers also found that their presence is not necessary for an effect, since the condition medium could also inhibit the effect of inflammatory cytokines and macrophage-produced TNF- α ⁴⁵. The anti-inflammatory molecule TSG-6 can be secreted and homed to the injury site of myocardiocytes by the embolized-MSCs in the lung⁴⁶. Evidence of MSC differentiation into lung epithelial cells was found in nude mice engrafted with amnion-derived stem cells⁴⁷, and the CXCR4/SDF1 axis is thought to be involved in stem cell plasticity. However, in immunocompetent mice, injection of allogenic amnion-derived stem cells did not find labeled cells in lung, brain, heart, spleen, liver, and kidney³⁷. It seems that the ability to engraft is determined by the innate immune responses. Although the cost is higher and time-scale longer, autogenic MSC transplantation is still considered better than allogenic MSC transplantation in IPF.

Influential Factors for Clinical Outcome

Data from a large-scale meta-analysis indicate that the most important factors for stem cell therapies are dosage and time

points⁴⁸. A double dosage of autologous stem cell transplantation produced a two-fold better outcome (10% vs 20%) in a study on humans⁴⁹. Ghadiri et al. summarized five clinical studies in IPF patients involving autogenic or allogenic MSC therapy; however, four of them are still under patient recruitment⁵⁰. The route of transplantation is also conflicting, since animal studies did not compare the efficiency between intravenous and intranasal transplantation. Interestingly, MSC transplantation via the intraperitoneal route is also effective for treating IPF, which suggests that direct contact of stem cells with lung tissue is not necessary⁵¹.

Comparison of Drugs and Stem Cells in Clinical Potential

Intravenous injection of ADSC on day 3, 6, and 9 after BLM-induction in mice has comparable therapeutic effects with the approved drug pirfenidone⁵². However, there are no beneficial effects when injected on day 14, 17, and 20, due to its diminished response of anti-inflammation and inhibited profibrotic genes⁵³. In current pharmacological studies, the targeting of drugs to specific organs or tissues is still under investigation. In the case of stem cell transplantation, tissue repair from migrating stem cells is a natural characteristic of MSCs¹⁹, which is also found in bleomycin-induced lung injury or infarcted myocardium^{44,54,55}. On the other hand, pharmacological applications have more detailed studies for absorption, distribution, metabolism, and excretion, and the mechanisms are clear in most clinical drugs. The manufacturing process of MSCs is determined by the donor and source (i.e. autogenic transplantation of MSCs is more expensive than allogenic). But the effect of engraftment in autogenic transplantation is more impressive than other known drugs and might apply to regenerative medicine. Advancements in MSC purification and modification might be a future goal when the limits of current medication are reached.

Problems and Future Breakthroughs

The ethical issues in stem cell transplantation include the potential of these cells to develop into a human being⁵⁶. However, MSCs lack such potential, so this issue is not relevant here. Other concerns that arise are the promotion of tumor growth and metastasis, and overestimating therapeutic potential⁵⁷. One of the problems we encountered in this review was characterization of the culturing method or media, since little is known about which characteristics would be changed from their original niche⁵⁸. Autologous transplantation is performed once a culture and expansion method, which is as similar to the natural niche as possible, is formalized⁵⁹. Considering the economic concerns, HLA-matched banking for allogenic transplantation and mass production is rational⁶⁰. Although it is not necessary to evaluate the effects of graft-versus-host disease, cases with

immunosuppressive agents should be evaluated with the total adding effect.

Conclusion

Recently, a convincing new drug has been discovered that slows the decline of Forced Vital Capacity (FVC) as well as prolonging survival, but it does not rebuild the normal structure of lung tissue. The final goal of therapy should be the recovery of pulmonary histological structure or retention of the cellular functions of each cell type. While there is no evidence suggesting MSC differentiates into alveolar epithelial cells, the theory that supplies remaining lung progenitor cell proliferation is still expectable. MSC transplantation is as effective as pharmaceutical therapies, but has relatively unclear mechanisms of action.

Here we have listed mechanisms sufficient for treating IPF, such as immunomodulatory effect, secretion of cytokines, and suppression of myofibroblast differentiation; however, MSCs neither act as an inhibitor of signaling pathways, nor as an agonist of receptors. To counter this disadvantage, a combination of drugs and MSCs might be of benefit. MSC treatment lacks the ability to improve myofibroblast apoptosis⁶¹, re-epithelization of mesenchymal type cells (or mesenchymal to epithelial transition, MET)⁶², and TGF- β inhibition⁶³. MSC-derived MV or exosomes might provide another option. Neutralizing antibodies or drugs approved for marketing can support such limitations. Rather than applying for new drug development trial approval at the Institutional Review Board (IRB), which can be both time-consuming and cost inefficient, use of approved drugs proves to be a better strategy. These suggestions provide more options for physicians and patients.

In summary, we concluded that current MSC therapies have observed positive results in clinical and animal studies. Moreover, molecular targets involved in the process of fibrogenesis, such as cytokines or growth factors, can work alongside MSCs with synergistic effect. Here we discussed some of the known mechanisms involved and studies based on these mechanisms. Exploring possible combinations for making the best therapeutic strategies should be the next step in the pursuit of better outcomes in the treatment of IPF, bearing in mind that both a molecular approach and cell therapy have their own disadvantages. However, two heads are better than one as they say, so selecting the positive features from each approach might be the best way forward.

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Author Contribution

Hornig-Jyh Harn and Li-Ing Ho contributed equally to this study.

Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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References

1. Raghu G, Collard HR, Egan JJ, Martinez FJ, Behr J, Brown KK, Colby TV, Cordier JF, Flaherty KR, Lasky JA, Lynch DA, Ryu JH, Swigris JJ, Wells AU, Ancochea J, Bouros D, Carvalho C, Costabel U, Ebina M, Hansell DM, Johkoh T, Kim DS, et al. An official ATS/ERS/JRS/ALAT statement: idiopathic pulmonary fibrosis: evidence-based guidelines for diagnosis and management. *Am J Respir Crit Care Med.* 2011; 183(6):788–824.
2. King TE Jr, Pardo A, Selman M. Idiopathic pulmonary fibrosis. *Lancet.* 2011;378(9807):1949–1961.
3. Selman M, King TE, Pardo A. Idiopathic pulmonary fibrosis: prevailing and evolving hypotheses about its pathogenesis and implications for therapy. *Ann Intern Med.* 2001;134(2): 136–151.
4. Ley B, Collard HR, King TE Jr. Clinical course and prediction of survival in idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med.* 2011;183(4):431–440.
5. Raghu G, Weycker D, Edelsberg J, Bradford WZ, Oster G. Incidence and prevalence of idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med.* 2006;174(7):810–816.
6. Richeldi L, du Bois RM, Raghu G, Azuma A, Brown KK, Costabel U, Cottin V, Flaherty KR, Hansell DM, Inoue Y, Kim DS, Kolb M, Nicholson AG, Noble PW, Selman M, Taniguchi H, Brun M, Le Maulf F, Girard M, Stowasser S, Schlenker-Hereceg R, Disse B, et al. Efficacy and safety of nintedanib in idiopathic pulmonary fibrosis. *N Engl J Med.* 2014;370(22): 2071–2082.
7. Richeldi L, Costabel U, Selman M, Kim DS, Hansell DM, Nicholson AG, Brown KK, Flaherty KR, Noble PW, Raghu G, Brun M, Gupta A, Juhel N, Kluglich M, du Bois RM. Efficacy of a tyrosine kinase inhibitor in idiopathic pulmonary fibrosis. *N Engl J Med.* 2011;365(12):1079–1087.
8. Noble PW, Albera C, Bradford WZ, Costabel U, Glassberg MK, Kardatzke D, King TE Jr, Lancaster L, Sahn SA, Szwarzberg J, Valeyre D, du Bois RM. Pirfenidone in patients with idiopathic pulmonary fibrosis (capacity): two randomised trials. *Lancet.* 2011;377(9779):1760–1769.
9. Raghu G, Rochwerg B, Zhang Y, Garcia CA, Azuma A, Behr J, Brozek JL, Collard HR, Cunningham W, Homma S, Johkoh T, Martinez FJ, Myers J, Protzko SL, Richeldi L, Rind D, Selman M, Theodore A, Wells AU, Hoogsteden H, Schunemann HJ. 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.* 2015;192(2):e3–e19.
10. Loveman E, Copley VR, Colquitt JL, Scott DA, Clegg AJ, Jones J, O'Reilly KM, Singh S, Bausewein C, Wells A. The

- effectiveness and cost-effectiveness of treatments for idiopathic pulmonary fibrosis: systematic review, network meta-analysis and health economic evaluation. *BMC Pharmacol Toxicol.* 2014;15:63.
11. Wilson MS, Wynn TA. Pulmonary fibrosis: pathogenesis, etiology and regulation. *Mucosal Immunol.* 2009;2(2):103–121.
 12. Moeller A, Ask K, Warburton D, Gauldie J, Kolb M. The bleomycin animal model: a useful tool to investigate treatment options for idiopathic pulmonary fibrosis? *Int J Biochem Cell Biol.* 2008;40(3):362–382.
 13. Raghu G, Anstrom KJ, King TE Jr, Lasky JA, Martinez FJ. Prednisone, azathioprine, and n-acetylcysteine for pulmonary fibrosis. *N Engl J Med.* 2012;366(21):1968–1977.
 14. Rafii R, Juarez MM, Albertson TE, Chan AL. A review of current and novel therapies for idiopathic pulmonary fibrosis. *J Thorac Dis.* 2013;5(1):48–73.
 15. Kumar R, Sharma A, Pattnaik AK, Varadwaj PK. Stem cells: an overview with respect to cardiovascular and renal disease. *J Nat Sci Biol Med.* 2010;1(1):43–52.
 16. Nathan SD, Albera C, Bradford WZ, Costabel U, Glaspole I, Glassberg MK, Kardatzke DR, Daigl M, Kirchgaessler KU, Lancaster LH, Lederer DJ, Pereira CA, Swigris JJ, Valeyre D, Noble PW. Effect of pirfenidone on mortality: pooled analyses and meta-analyses of clinical trials in idiopathic pulmonary fibrosis. *Lancet Respir Med.* 2017;5(1):33–41.
 17. Fisher M, Nathan SD, Hill C, Marshall J, Dejonckheere F, Thureson PO, Maher TM. Predicting life expectancy for pirfenidone in idiopathic pulmonary fibrosis. *J Manag Care Spec Pharm.* 2017;23(3-b Suppl):S17–S24.
 18. Costabel U, Inoue Y, Richeldi L, Collard HR, Tschoepe I, Stowasser S, Azuma A. Efficacy of nintedanib in idiopathic pulmonary fibrosis across prespecified subgroups in impulsis. *Am J Respir Crit Care Med.* 2016;193(2):178–185.
 19. Squillaro T, Peluso G, Galderisi U. Clinical trials with mesenchymal stem cells: an update. *Cell Transplant.* 2016;25(5):829–848.
 20. Glassberg MK, Minkiewicz J, Toonkel RL, Simonet ES, Rubio GA, DiFede D, Shafazand S, Khan A, Pujol MV, LaRussa VF, Lancaster LH, Rosen GD, Fishman J, Mageto YN, Mendizabal A, Hare JM. Allogeneic human mesenchymal stem cells in patients with idiopathic pulmonary fibrosis via intravenous delivery (AETHER): a phase I safety clinical trial. *Chest.* 2017;151(5):971–981.
 21. Zhang C, Yin X, Zhang J, Ao Q, Gu Y, Liu Y. Clinical observation of umbilical cord mesenchymal stem cell treatment of severe idiopathic pulmonary fibrosis: a case report. *Exp Ther Med.* 2017;13(5):1922–1926.
 22. Tzouveleki A, Koliakos G, Ntoliou P, Baira I, Bouros E, Oikonomou A, Zissimopoulos A, Kolios G, Kakagia D, Paspaliaris V, Kotsianidis I, Froudarakis M, Bouros D. Stem cell therapy for idiopathic pulmonary fibrosis: a protocol proposal. *J Transl Med.* 2011;9:182.
 23. Feng M, Lu A, Gao H, Qian C, Zhang J, Lin T, Zhao Y. Safety of allogeneic umbilical cord blood stem cells therapy in patients with severe cerebral palsy: a retrospective study. *Stem Cells Int.* 2015;2015:325652.
 24. In't Anker PS, Scherjon SA, Kleijburg-van der Keur C, de Groot-Swings GM, Claas FH, Fibbe WE, Kanhai HH. Isolation of mesenchymal stem cells of fetal or maternal origin from human placenta. *Stem Cells.* 2004;22(7):1338–1345.
 25. Geiger S, Hirsch D, Hermann FG. Cell therapy for lung disease. *Eur Respir Rev.* 2017;26(144)Pii: 170044.
 26. Konigshoff M, Kramer M, Balsara N, Wilhelm J, Amarie OV, Jahn A, Rose F, Fink L, Seeger W, Schaefer L, Gunther A, Eickelberg O. Wnt1-inducible signaling protein-1 mediates pulmonary fibrosis in mice and is upregulated in humans with idiopathic pulmonary fibrosis. *J Clin Invest.* 2009;119(4):772–787.
 27. Koli K, Myllarniemi M, Vuorinen K, Salmenkivi K, Ryyanen MJ, Kinnula VL, Keski-Oja J. Bone morphogenetic protein-4 inhibitor gremlin is overexpressed in idiopathic pulmonary fibrosis. *Am J Pathol.* 2006;169(1):61–71.
 28. Maher TM, Evans IC, Bottoms SE, Mercer PF, Thorley AJ, Nicholson AG, Laurent GJ, Tetley TD, Chambers RC, McAnulty RJ. Diminished prostaglandin e2 contributes to the apoptosis paradox in idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med.* 2010;182(1):73–82.
 29. Huang MH, Lin SZ, Lin PC, Chiou TW, Harn YW, Ho LI, Chan TM, Chou CW, Chuang CH, Su HL, Harn HJ. Brain tumor senescence might be mediated by downregulation of S-phase kinase-associated protein 2 via butylidenephthalide leading to decreased cell viability. *Tumour Biol.* 2014;35(5):4875–4884.
 30. Mikamo M, Kitagawa K, Sakai S, Uchida C, Ohhata T, Nishimoto K, Niida H, Suzuki S, Nakayama KI, Inui N, Suda T, Kitagawa M. Inhibiting skp2 e3 ligase suppresses bleomycin-induced pulmonary fibrosis. *Int J Mol Sci.* 2018;19(2):E474.
 31. Hsu HS, Liu CC, Lin JH, Hsu TW, Hsu JW, Su K, Hung SC. Involvement of ER stress, PI3K/AKT activation, and lung fibroblast proliferation in bleomycin-induced pulmonary fibrosis. *Sci Rep.* 2017;7(1):14272.
 32. Xu W, Yang Z, Lu N. A new role for the PI3K/AKT signaling pathway in the epithelial-mesenchymal transition. *Cell Adh Migr.* 2015;9(4):317–324.
 33. Li X, Yue S, Luo Z. Mesenchymal stem cells in idiopathic pulmonary fibrosis. *Oncotarget.* 2017;8(60):102600–102616.
 34. Fazekasova H, Lechler R, Langford K, Lombardi G. Placenta-derived MSCs are partially immunogenic and less immunomodulatory than bone marrow-derived MSCs. *J Tissue Eng Regen Med.* 2011;5(9):684–694.
 35. Liu KJ, Wang CJ, Chang CJ, Hu HI, Hsu PJ, Wu YC, Bai CH, Sytwu HK, Yen BL. Surface expression of HLA-g is involved in mediating immunomodulatory effects of placenta-derived multipotent cells (PDMCs) towards natural killer lymphocytes. *Cell Transplant.* 2011;20(11–12):1721–1730.
 36. Lee SH, Jang AS, Kim YE, Cha JY, Kim TH, Jung S, Park SK, Lee YK, Won JH, Kim YH, Park CS. Modulation of cytokine and nitric oxide by mesenchymal stem cell transfer in lung injury/fibrosis. *Respir Res.* 2010;11:16.

37. Murphy S, Lim R, Dickinson H, Acharya R, Rosli S, Jenkin G, Wallace E. Human amnion epithelial cells prevent bleomycin-induced lung injury and preserve lung function. *Cell Transplant*. 2011;20(6):909–923.
38. Bai L, Shao H, Wang H, Zhang Z, Su C, Dong L, Yu B, Chen X, Li X, Zhang X. Effects of mesenchymal stem cell-derived exosomes on experimental autoimmune uveitis. *Sci Rep*. 2017;7(1):4323.
39. Phinney DG, Di Giuseppe M, Njah J, Sala E, Shiva S, St Croix CM, Stolz DB, Watkins SC, Di YP, Leikauf GD, Kolls J, Riches DW, Deiuliis G, Kaminski N, Boregowda SV, McKenna DH, Ortiz LA. Mesenchymal stem cells use extracellular vesicles to outsource mitophagy and shuttle micRNAs. *Nat Commun*. 2015;6:8472.
40. Willis GR, Fernandez-Gonzalez A, Anastas J, Vitali SH, Liu X, Ericsson M, Kwong A, Mitsialis SA, Kourembanas S. Mesenchymal stromal cell exosomes ameliorate experimental bronchopulmonary dysplasia and restore lung function through macrophage immunomodulation. *Am J Respir Crit Care Med*. 2018;197(1):104–116.
41. Tan JL, Lau SN, Leaw B, Nguyen HPT, Salamonsen LA, Saad MI, Chan ST, Zhu D, Krause M, Kim C, Sievert W, Wallace EM, Lim R. Amnion epithelial cell-derived exosomes restrict lung injury and enhance endogenous lung repair. *Stem Cells Transl Med*. 2018;7(2):180–196.
42. Sung BH, Ketova T, Hoshino D, Zijlstra A, Weaver AM. Directional cell movement through tissues is controlled by exosome secretion. *Nat Commun*. 2015;6:7164.
43. Choi M, Ban T, Rhim T. Therapeutic use of stem cell transplantation for cell replacement or cytoprotective effect of microvesicle released from mesenchymal stem cell. *Mol Cells*. 2014;37(2):133–139.
44. Ortiz LA, Gambelli F, McBride C, Gaupp D, Baddoo M, Kaminski N, Phinney DG. Mesenchymal stem cell engraftment in lung is enhanced in response to bleomycin exposure and ameliorates its fibrotic effects. *Proc Natl Acad Sci U S A*. 2003;100(14):8407–8411.
45. Ortiz LA, Dutreil M, Fattman C, Pandey AC, Torres G, Go K, Phinney DG. Interleukin 1 receptor antagonist mediates the antiinflammatory and antifibrotic effect of mesenchymal stem cells during lung injury. *Proc Natl Acad Sci U S A*. 2007;104(26):11002–11007.
46. Lee RH, Pulin AA, Seo MJ, Kota DJ, Ylostalo J, Larson BL, Semprun-Prieto L, Delafontaine P, Prockop DJ. 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*. 2009;5(1):54–63.
47. Carraro G, Perin L, Sedrakyan S, Giuliani S, Tiozzo C, Lee J, Turcatel G, De Langhe SP, Driscoll B, Bellusci S, Minoo P, Atala A, De Filippo RE, Warburton D. Human amniotic fluid stem cells can integrate and differentiate into epithelial lung lineages. *Stem Cells*. 2008;26(11):2902–2911.
48. Wang Z, Wang L, Su X, Pu J, Jiang M, He B. Rational transplant timing and dose of mesenchymal stromal cells in patients with acute myocardial infarction: a meta-analysis of randomized controlled trials. *Stem Cell Res Ther*. 2017;8(1):21.
49. Attal M, Harousseau JL, Facon T, Guilhot F, Doyen C, Fuzibet JG, Monconduit M, Hulin C, Caillot D, Bouabdallah R, Voillat L, Sotto JJ, Grosbois B, Bataille R. Single versus double autologous stem-cell transplantation for multiple myeloma. *N Engl J Med*. 2003;349(26):2495–2502.
50. Ghadiri M, Young PM, Traini D. Cell-based therapies for the treatment of idiopathic pulmonary fibrosis (IPF) disease. *Expert Opin Biol Ther*. 2016;16(3):375–387.
51. Cargnoni A, Gibelli L, Tosini A, Signoroni PB, Nassuato C, Arienti D, Lombardi G, Albertini A, Wengler GS, Parolini O. Transplantation of allogeneic and xenogeneic placenta-derived cells reduces bleomycin-induced lung fibrosis. *Cell Transplant*. 2009;18(4):405–422.
52. Reddy M, Fonseca L, Gowda S, Chougule B, Hari A, Totey S. Human adipose-derived mesenchymal stem cells attenuate early stage of bleomycin induced pulmonary fibrosis: comparison with pirfenidone. *Int J Stem Cells*. 2016;9(2):192–206.
53. Vosdoganes P, Wallace EM, Chan ST, Acharya R, Moss TJ, Lim R. Human amnion epithelial cells repair established lung injury. *Cell Transplant*. 2013;22(8):1337–1349.
54. Strauer BE, Brehm M, Zeus T, Kosterling M, Hernandez A, Sorg RV, Kogler G, Wernet P. Repair of infarcted myocardium by autologous intracoronary mononuclear bone marrow cell transplantation in humans. *Circulation*. 2002;106(15):1913–1918.
55. Rojas M, Xu J, Woods CR, Mora AL, Spears W, Roman J, Brigham KL. Bone marrow-derived mesenchymal stem cells in repair of the injured lung. *Am J Respir Cell Mol Biol*. 2005;33(2):145–152.
56. King NM, Perrin J. Ethical issues in stem cell research and therapy. *Stem Cell Res Ther*. 2014;5(4):85.
57. Volarevic V, Markovic BS, Gazdic M, Volarevic A, Jovicic N, Arsenijevic N, Armstrong L, Djonov V, Lako M, Stojkovic M. Ethical and safety issues of stem cell-based therapy. *Int J Med Sci*. 2018;15(1):36–45.
58. Boulais PE, Frenette PS. Making sense of hematopoietic stem cell niches. *Blood*. 2015;125(17):2621–2629.
59. Lodi D, Iannitti T, Palmieri B. Stem cells in clinical practice: applications and warnings. *J Exp Clin Cancer Res*. 2011;30:9.
60. Nakatsuji N, Nakajima F, Tokunaga K. HLA-haplotype banking and IPS cells. *Nat Biotechnol*. 2008;26(7):739–740.
61. Moodley YP, Misso NL, Scaffidi AK, Fogel-Petrovic M, McNulty RJ, Laurent GJ, Thompson PJ, Knight DA. Inverse effects of interleukin-6 on apoptosis of fibroblasts from pulmonary fibrosis and normal lungs. *Am J Respir Cell Mol Biol*. 2003;29(4):490–498.
62. Chuang HM, Su HL, Li C, Lin SZ, Yen SY, Huang MH, Ho LI, Chiou TW, Harn HJ. The role of butylidenephthalide in targeting the microenvironment which contributes to liver fibrosis amelioration. *Front Pharmacol*. 2016;7:112.
63. Stahnke T, Kowtharapu BS, Stachs O, Schmitz KP, Wurm J, Wree A, Guthoff RF, Hovakimyan M. Suppression of TGF-beta pathway by pirfenidone decreases extracellular matrix deposition in ocular fibroblasts in vitro. *PLoS One*. 2017;12(2):e0172592.