7 Stem Cell Based Therapy for Lung Disease Preclinical evidence for the role of stem/stromal cells Clinical application of stem/stromal cells in lung fibrosis

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7.1 Introduction

Interstitial lung fibrosis may develop as a consequence of occupational or drug exposures, lung injury, or as the end stage of chronic intersitital lung disease. The pathogenesis of lung fibrosis remains elusive and controversial, but prevailing hypotheses assume an ineffective wound healing response to alveolar epithelial cell injury [1, 2] Injury magnitude and susceptibility appears to be related to aging and genetic predisposition, with subsequent innate immune system and fibroblast activation [1, 3, 4].

In the right clinical picture, and in the absence of other known causes of lung fibrosis, the diagnosis of IPF may be made by typical radiologic findings on high-resolution computed tomography (subpleural and basilar predominance of honeycomb cysts and reticulation). In cases where the diagnosis is not clear, lung biopsy may be necessary. Histologically, IPF is identified by the presence of the usual interstitial pneumonia (UIP) pattern with extracellular matrix deposition, phenotypic alterations of fibroblasts and alveolar epithelial cells, formation of fibroblastic foci, and regional and temporal heterogeneity characterized by scattered areas of aberrant wound healing interspersed with normal lung parenchyma [1, 2, 5–13].

Evidence suggests that areas of fibrosis seen in the lungs of patients with IPF share features associated with normal aging lung, such as genomic instability, telomere attrition, mitochondrial dysfunction, cellular senescence, and immune dysregulation [9, 14, 15] (Fig. 7.1). Because of this overlap, connections between IPF and diseases of premature aging have been postulated.

Partly due to the inefficacy of immunomodulatory and immunosuppressive agents in the treatment of IPF, the role of the immune system in the pathogenesis of IPF remains poorly understood [16–21]. However, a link between IPF and immune dysregulation is suggested by the presence of highly activated and proliferative CD4+ cells and functional impairment of T-regulatory cells in patients with IPF [9, 22, 23]. Pathologic

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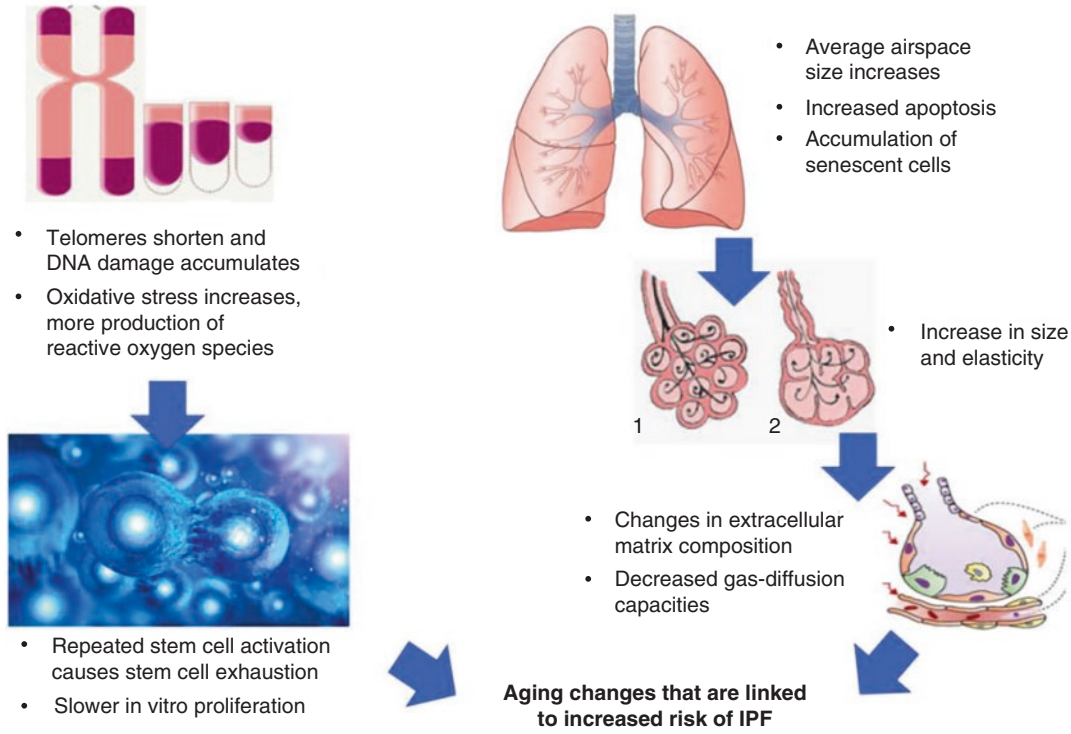

Fig. 7.1 Aged mesenchymal stem cells in aging lungs and IPF

features of the epithelium suggest that a dysregulation of progenitor cells may contribute to the IPF phenotype, with abnormal cell cycling resulting in dysfunctional repair [24].

Currently, pirfenidone and nintedanib are the only two FDA approved compounds for the treatment of IPF. Pirfenidone, an antifibrotic compound with an unknown mechanism of action, targets several molecules including transforming growth factor-β (TGF-β), tumor necrosis factor-α (TNF-α), and interleukin-6 [25]. Nintedanib, a tyrosine kinase inhibitor, targets vascular endothelial growth factor receptor (VEGFR), fibroblast growth factor receptor (FGFR), and platelet-derived growth factor receptor (PDGFR) [21]. Pirfenidone was shown to slow the rate of decline in forced vital capacity (FVC) and 6-min walking distance in patients with IPF, and may improve mortality in select patients [3]. Nintedanib was also shown to slow the rate of decline in FVC with a trend toward reduced mortality [4]. However, neither of these compounds has been shown to ameliorate respiratory symptoms or improve acute exacerbation rates, and lung function has continued to decline in all trials completed to date. In addition, though pirfenidone and nintedanib have been shown to slow the progression of IPF [26–28], both compounds are associated with significant side effects [28–30]. The only definitive treatment for IPF at this time is lung transplantation. Morbidity and mortality from IPF remains high and thus there is a pressing need for alternative therapeutic options for this complex and devastating disease.

Ongoing clinical trials of other potential therapeutic targets include mofiers of connective tissue growth factor, IL-4 and IL-13, galectin-3, lysophosphatidic acid, the phosphoinositide 3-kinase pathway, and finally, mesenchymal stromal cells (MSCs).

7.2 Animal Models of Pulmonary Fibrosis

Spontaneous pulmonary fibrosis does occur in nonhuman animals, including ferrets, dogs, horses, donkeys, and cats. While the fibrotic

lungs of these animals share many characteristics with the lungs of humans with IPF, current veterinary classifications of fibrotic lung disease are not equivalent. The field of comparative oncology has set the stage for collaborations utilizing spontaneous models of progressive fibrotic lung diseases of mutual interest to veterinary and human medicine. The results of these kinds of studies promise to enhance the understanding of common factors important to disease development in a variety of species and to refine treatments for both humans and animals. Moreover, they may provide insights into unanswered questions involving naturally occurring models of pulmonary fibrosis. However, further studies in veterinary models of lung fibrosis are needed to define their relation to human disease and their potential use as models for the development of effective treatments.

Because no reliable spontaneous animal model exists, understanding the pathogenesis of IPF and other fibrotic lung disorders has primarily relied on research using animal models of induced lung fibrosis. Unfortunately, although some of these animal models exhibit progressive disease, none fully recapitulates the histological pattern of UIP. Traditional animal models of lung fibrosis have generated important insights into the pathobiology of lung injury, inflammation, and fibroproliferation [8]. Although it is appreciated that the spontaneous development of fibrosis in other species (e.g., ferrets, donkey, sheep, cats, horses, and dogs) [9–11] can be instructive, the most tractable models for studies of pathogenesis involve rodents. Traditionally, preclinical trials have utilized mouse models of bleomycin (BLM)-induced pulmonary fibrosis and studies of BLM-induced fibrosis in aged male mice remain the most clinically relevant model for preclinical studies of IPF because young mice treated with BLM may show recovery from pulmonary fibrosis, an event not appreciated in human fibrotic lung disease.

BLM is a chemotherapeutic antibiotic first identified as a pro-fibrotic agent after the development of pulmonary fibrosis in patients being treated for lymphoma. BLM has been studied in multiple species including mice, rats, sheep, guinea pigs, hamsters, dogs, and primates and in various modes of administration [20, 21], but the consensus view at this time is that the intratracheal murine BLM model is "the best-characterized animal model available for preclinical testing" of IPF [31, 32].

BLM acts by causing single- and double-strand DNA breaks thereby inducing apoptosis. BLM hydrolase, a BLM-inactivating enzyme, influences drug effects on a tissue-specific basis. Becuase the lungs maintain low levels of this enzyme, lung tissue is highly susceptible to BLM-induced injury. An overproduction of reactive oxygen species, due to chelation of metal ions and reaction of the formed pseudoenzyme with oxygen, leads to epithelial cell death (days 1–3), excessive inflammatory infiltrates (days 3–9, neutrophils found in the bronchoalveolar lavage fluid at day 3 and lymphocytes at day 6), and ultimately to fibroblast activation, extracellular matrix deposition, and development of fibrosis (days 10–21 with a peak around day 14). These changes are seen at both the molecular [23, 25, 26] and histologic [20, 23, 25, 27] levels.

The early molecular signature of BLM-induced injury appears to be most similar to the accelerated acute phase of IPF in humans [28]. Measurements of alveolar septal thickening, intra-alveolar fibrosis, increases in alveolar macrophages, and dilation of bronchioles and alveolar ducts demonstrates fairly uniform fibrosis [29]. Nevertheless, the BLM-induced lung fibrosis model is not perfectly representative of IPF. The rapidity of development of BLM-induced fibrosis, the marked inflammation preceding fibrosis, and the possibility of spontaneous resolution are signignificnt differences between the BLM model and human IPF.

C57BL/6J mice have been the predominant animal model, as this particular strain is highly susceptible to lung injury following intratracheal BLM administration [30, 33]. Conversely, the BALB/c or SV129 strains confer resistance to BLM-induced pulmonary fibrosis, presumably due to alterations in transforming growth factor (TGF)-β expression [33].

While BLM-induced lung injury has been studied via intratracheal, intraperitoneal, subcutaneous, intravenous, and inhalational delivery methods, the intratracheal route is most commonly

used because it best recapitulates the human phenotype which is limited to the lungs [20, 23, 26, 28, 29, 34–39]. Another issue identified in studies using the BLM mouse model is the wide range of dosing regimens used [40]. In mouse studies, weight-based dosing is most common [35, 36, 39] and slightly lower doses (2.0–2.5 U/kg) appear to provide the most effective model of lung fibrosis, while reducing sample loss due to high mortality [28]. With regards to the frequency of dosing, repetitive dosing in young mice was found to promote persistent fibrosis as evidenced by measures of hydroxyproline content and inflammatory cell infiltrates. In contrast, single-dose experiments have demonstrated spontaneous resolution in young mice [23, 41].

Animal models of IPF have yielded valuable insights, but studies using these models also have important limitations. Most studies of therapeutic interventions use a single dose of intratracheal BLM followed shortly thereafter by the administration of the therapy under investigation [35–38]. Because these thrapuetic agents are often administered within the first 1–7 days following BLM exposure, their observed effects may be due to prevention of the inflammatory cascade rather than reversal of fibrosis, thus limiting their applicability to human IPF [40]. More recent studies have begun to explore administration of drugs after 7 days [42, 43] and, to our knowledge, only two studies to date have evaluated repetitive BLM injury [44, 45]. Intriguingly, pirfenidone and nintedanib received approval to proceed to clinical trials based on preventive protocols or even therapeutic protocols targeting the inflammatory or the early-fibrotic phase of the BLM model [46–48].

In addition, most studies investigating BLM-induced pulmonary fibrosis have used young male mouse models, aged 8–12 weeks [28, 29, 34]. Young mice, however, have been shown to undergo spontaneous resolution of BLM-induced pulmonary fibrosis, a phenomenon not observed in aged mice [23, 41, 49]. Whether sex differences in mice parallel human IPF, which exhibits a tendency toward male predominance, has not been fully determined. However, the use of aged male mice may provide a more clinically relevant model of IPF [49]. Many of the hallmarks of aging, including genomic instability, telomere attrition, epigenetic alterations, deregulated cellular bioenergetics, and cellular senescence, are also seen in fibrotic lung disease [50–52]. Studies have shown that older mice are more susceptible than younger mice to pro-fibrotic stimuli including BLM [26]. This is of particular interest given that IPF is predominantly seen in older individuals. Transgenic deletion of senescence-related genes including RAGE and relaxin has been associated with spontaneous age-dependent development of lung fibrosis indicating a role for aging in disease susceptibility [31, 53, 54].

The preclinical efficacy of the majority of antifibrotic agents tested in animal models has utilized a single model, (most often BLM) and has measured histologic, not clinical, endpoints. In addition, the lack of blinding in most preclinical animal studies may contribute to evaluation bias in outcomes. Reproducibility issues arising from different experimental settings could also account for discrepancies in treatment effects and lack of generalizability. As with all studies, when using animal models, sample size must be balanced with the statistical power needed to generate robust data. Finally, insufficient reporting of experimental animal data or unpublished negative therapeutic results severely hamper the validity of experimental studies.

7.3 Rationale for Stem Cell Therapy

A stem cell is defined as an undifferentiated cell capable of self-renewal and multipotent differentiation potential. To achieve this remarkable task, stem cells undergo asymmetric cell division whereby one daughter cell is maintained as a self-renewing stem cell and the other becomes a precursor or progenitor cell capable of giving rise to futher differentiated cells. A progenitor cell shares the potential for differentiation into different tissue lineages, but has limited self-renewal capacity [6, 9].

Due to ethical issues and ecumenical directives, embryonic stem cells are not used in the research of human disease. Other stem cells used in research and disease treatment include tissue-specific stem cells, mesenchymal stem cells (MSCs), fetal stem cells, and cord blood stem cells. In addition, research has been directed at tissue-specific stem cells which reside in and give rise to mature parenchymal cells within a particular tissue or organ.

Mesenchymal stem cells (MSCs) have been shown to have immunomodulatory, antiproliferative, and anti-inflammatory effects. In addition, because of their migratory ability and immune-privileged state, much research has been aimed at understanding the therapeutic poetntial of MSCs. Among others, the therpuetic role of MSCs has been explored in cardiac ischemia, autoimmune disorders [10], severe graft-versus-host disease [11], chronic lung diseases [12–15], and acute lung injury [16–20].

While researchers, biologists, and bioethicists have rallied the medical community to improve our understanding of the biology and mechanism of action of stem cells, confusion over what exactly is a "stem cell" has led to a lack of standardization in production processes [7] and the indiscriminate commercialization of various cell products as purported treatments for patients [9].

7.4 The Role of Stem Cells in Lung Repair

Various stem/progenitor cells that function in lung repair reside in other areas of the body and are recruited in times of injury and inflammation. Cells from the bone marrow, blood, adipose tissue, placenta, and umbilical cord have been shown to structurally engraft in the airway [34, 49] as well as the pulmonary vasculature. In addition, evidence suggests that bone marrow-derived cells such as MSCs are recruited to areas of lung injury and exert their regenerative effects via a paracrine function [35, 41].

MSCs are multipotent and have a diverse but restricted differentiation capability. They appear to function in a paracrine manner with minimal engraftment, interact with the innate and adaptive immune systems [36, 37], and aid in lung repair and regeneration via secretion of cytokines and growth factors to restore alveolar epithelial and endothelial permeability [38–40, 42].

Other sources of stem cells have also been studied in lung disease. Endothelial progenitor cells (EPCs) appear to exert their therapeutic effects via direct differentiation and engraftment into the vasculature of the lung and secretion of factors that mobilize endothelial and progenitor cells and represent a promising source for pulmonary vascular regeneration [55]. Amniotic fluid stem cells (AFSCs), multipotent fetal-associated cells that can be easily and ethically obtained from amniocentesis specimens, have been shown to improve lung density and function in models of diaphragmatic hernia [56]. AFSCs have also been shown to integrate into areas of distal lung epitheleial injury with expedited repair and expression of NKX2-1 and SFPTC (markers of alveolar type 2 cell diffrernitation) [24, 49]. Human amnion epithelial cells (hAECs), found in the lining of the placenta, display low immunogenicity and also appear to possess regenerative and anti-inflammatory properties [57–60].

7.5 Endogenous Stem Cells

Recent work has called into question the existence of stem cells in slowly renewing tissues like the lung [44, 45]. The epithelium lining pulmonary airways turns over slowly during the normal process of tissue maintenance and is replaced far more slowly than specialized post-mitotic cell types of the gut or the epidermis, a property that is reflected in the functional characteristics of airway progenitor cells in their resting versus proliferative states. Therefore, it is not surprising that progenitor cell hierarchies of the lung and other slowly renewing tissues do not fit the classical stem cell hierarchies described in tissues of rapid turnover [42]. In fact, Hu and colleagues, through use of in vivo injury models, have recently described the existence of a stem cell capable of renewing the endocrine pancreas, a tissue that until recently was thought to be maintained

solely through self-duplication of differentiated β cells [61]. This, and previous work in lung to be discussed in more detail below, illustrate a deviation from the classical stem cell hierarchy marked by lack of an obligate transit-amplifying progenitor cell in the steady state. Rather, slowly repairing tissues such as lung are maintained at steady state by an abundant facultative transit-amplifying progenitor that fulfils characteristics of a differentiated cell type in the quiescent state, yet retains proliferative capacity and the ability to generate daughter cells capable of generating other specialized lineages [61]. Therefore, the endogenous stem cell at steady state likely remains quiescent. However, studies utilizing in vivo injury models have revealed stem cells that can be functionally distinguished from facultative progenitors, based upon their resistance to environmental stimuli and spatial localization in the conducting airway.

7.6 Clara Cells

Within the normal lung, Clara cell proliferation maintains the facultative progenitor cell pool (self-renewal) and restores terminally differentiated cells of the conducting airway epithelium (ciliated cells). This vast reparative reservoir distinguishes lung epithelia from tissues such as the intestine that are maintained through proliferation and differentiation of tissue-specific stem cells. The unique features of lung epithelial maintenance and repair suggest that chronic lung disease could be treated through interventions that stabilize the Clara cell pool or by cell replacement strategies that restore this abundant cell type [62].

- Clara cells are non-ciliated secretory cells in the small airways and trachea. Their morphology and biochemical composition display amazing heterogeneity within the airway epithelium of a single species, among different species, and in response to injury.
- Clara cells have several lung protective functions. They detoxify xenobiotics and oxidant gasses, control the extent of inflammation, participate in mucociliary clearance of environmental agents, and proliferate/differentiate to maintain the ciliated cell population.
- Clara cells are secretory and the source of Clara cell secretory protein (CCSP) and contribute surfactant apoproteins A, B, and D, proteases, antimicrobial peptides, several cytokines and chemokines, and mucins to the extracellular fluid lining the airspaces.
- In humans, many forms of lung cancer may originate from Clara cells, including adenocarcinoma, the most frequently diagnosed form of lung cancer. Whether Clara cells have a similar etiologic function in mouse models of adenocarcinoma is more controversial [62].

7.7 Mesenchymal Stem Cells

MSCs, first desccibed by Friedenstein et al. in 1968, are a class of multipotent stem cells with self-proliferative and differentiation potential [40, 42]. MSCs may be isolated from bone marrow as well as other tissues including gingival, adipose, umbilical cord, and placenta. Isolation of MSCs requires that cells (1) exhibit fibroblastic morphology, clonogenicty, and plastic adherence when cultured in standard tissue culture conditions; (2) differentiate into adipocytes, osteoblasts, and chondrocytes in vitro; and (3) express certain cell surface markers such as CD44, Sca-1, CD29, and CD90 but not CD45, CD34, CD14, and CD11b [43–45].

In addition to their capacity for multipotent differentiation and their ease of isolation, MSCs are characterized by a number of features that make them attractive subjects for research in regenerative medicine (Fig. 7.2). MSCs lack immunogenicity, home to areas of injured tissue, and have anti-inflammatory and immunomodulatory effects [26, 61]. Because MSCs have limited expression of MHC class I and II molecules, both autologous and allogeneic administration are easily achieved (Fig. 7.2) [40, 63]. In addition, MSCs can be genetically modified using viral vectors to enhance their therapeutic potential [64–66]. Because of these favorable characterstics, the therapeutic potential of MSCs has

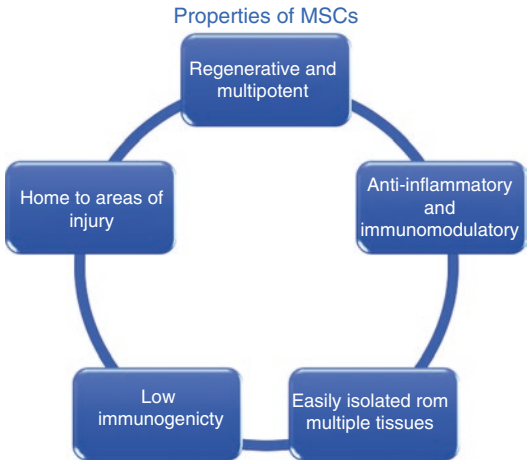

Fig. 7.2 Properties of MSCs

been investigated not only for lung diseases [61] but also for a wide variety of other conditions, including hepatic failure [55], myocardial infarction [67], diabetes [68], sepsis [56], and acute renal failure [24].

7.8 MSCs in the Treatment of IPF

A number of preclinical studies have examined the therapeutic potential of stem and progenitor cell populations in animal models of pulmonary fibrosis and MSCs have been studied in human phase I clinical trials [2]. In addition to those discussed above, a number of additional features suggest that MSCs may be beneficial in the treatment of IPF. MSCs inhibit cytotoxic T-cells and natural killer cells, are known to secrete growth factors including KGF, HGF, VEGF, and Ang-1, and play an important role in the repair of alveolar epithelium through mitochondrial transfer [51, 69].

Because epithelial injury underlies the pathogenesis of pulmonary fibrosis, the delivery of exogenous stem or progenitor cells capable of participating in alveolar reepithelialization may have therapeutic potential to break the cycle of aberrant epithelial–mesenchymal crosstalk and halt disease progression. It is possible that, due to changes in the lung microenvironment and the continued presence of injurious stimuli, exogenous progenitor cells, like endogenous cells, may simply participate in the characteristic pathological repair process. On the other hand, intrinsic factors are thought to play a role in alveolar epithelial cell injury, and preclinical studies suggest that stem cells can exert profound effects through the secretion of soluble mediators [31, 53, 54].

Systematic reviews reveal numerous preclinical studies of MSCs in the treatment of BLM-induced lung injury [5]. To date, these studies suggest that MSCs are effective in improving histopathology, Ashcroft scores of lung fibrosis, lung collagen deposition, and survival in animal models of BLM-induced lung injury. However, most of these studies used young animals and examined the initial inflammatory phase rather than the chronic fibrotic phase. As previously discussed, spontaneous reversal of BLM-induced lung fibrosis may occur spontaneously in young mice [70], but does not occur in aged mouse models [70, 71]. A more recent study utilizing an aged mouse model of BLM-induced lung fibrosis found that treatment with adipose-derived MSCs may promote a systemic acute repair phenotype to prevent fibrosis in multiple organs and enhance wound healing by modulating pro-fibotic factors such as miR-199 and its downstream target, CAV1 [1].

Preclinical studies have shown MSCs to be efficacious in the treatment and prevention of lung fibrosis [53, 72]. Nonetheless, concerns remain regarding the activity of MSCs within a pro-fibrotic microenvironment [73–76]. While some preclinical studies suggest that MSCs might promote fibrosis, to date, no human studies have found a similar pro-fibrotic effect [37, 43, 63, 70, 74, 75, 77–85].

7.9 Clinical Trials of MSCs for the Treatment of IPF

Twenty years ago, Lazarus conducted the first clinical trial using bone marrow cell injection in patients with hematologic malignancies [52]. Since then, numerous clinical trials have been conducted to test the feasibility and efficacy of MSC-based therapy, and more than 2000 patients have recieved allogeneic or autologous MSCs for the treatment of various diseases [52].

Early clinical studies of MSCs in patients with IPF have shown promising safety profiles [30, 78, 86]. A phase Ib study of endobronchially administered autologous adipose-derived MSCs showed not only acceptable safety outcomes but also improvements in quality of life parameters [78]. Longitudinal outcomes of this study also demonstrated an acceptable safety profile with a 100% survival rate at 2 years after the first administration of MSCs and a median overall progression-free survival of 26 months [87].

Studies of intravenously administered placental-derived MSCs [79, 83] have found that administration of up to 2×10^6 cells per kilogram is safe in subjects with moderately severe IPF [83]. Importantly, only minor and transient alterations in peri-infusion hemodynamics and gas exchange were reported, ameliorating concerns regarding the potential embolization of stem cells to an already compromised pulmonary vasculature. At 6 months, there were no observable declines in forced vital capacity (FVC), diffusing lung capacity for carbon monoxide (DLCO), six-minute walk test (6MWT), or CT fibrosis score [88].

The AETHER trial also showed favorable safety outcomes for the single-dose intravenous delivery of up to 2×10^8 allogeneic MSCs in patients with IPF [79]. Although this study was underpowered for the detection of significant changes in functional indices, the mean decline in % predicted FVC and DLCO were below the thresholds for disease progression [1, 46].

At this time, the only study actively recruiting IPF patients for treatment with stem cells is a Phase 1/2 clinical trial (NCT02745184) taking place at two sites in China. Researchers intend to isolate autologous lung stem cells, MSCs from the patient's own bronchi, expand them in the laboratory, and then deliver the expanded cell population via a single injection directly into an area affected by IPF. Safety parameters will be monitored for 1 year and efficacy will be measured by changes in lung function and exercise ability tests.

Looking ahead, ReCell, an FDA-approved phase 1b trial, is planned but has not yet begun enrollment. In this multidose, randomized, double-blind trial, 10×10^6 MSCs will be delivered intravenously to patients with IPF.

A team of scientists from the UNC School of Medicine and North Carolina State University demonstrated that they could harvest lung stem cells from people using a noninvasive office procedure. They snipped tiny, seed-sized samples of airway tissue using a bronchoscope, this method involves far less risk to the patient than does a standard, chest-penetrating surgical biopsy of lung tissue. Cheng and his colleagues cultured lung spheroid cells from these tiny tissue samples until they multiplied to the thousands and were enough to be therapeutically injected.

Once the stem cells were harvested they were able to multiply these lung cells in the lab. The results yielded enough cells sufficient for human therapy. In 2017 these researchers were working with the FDA for preparation of clinical trials in patients with IPF. Cheng, Lobo, and their teams are now planning an initial study of therapeutic lung spheroid cells in a small group of IPF patients.

Currently this study is still undergoing data collection.

7.10 Moving Forward

Stem cells have been used in medicine since the 1950s when bone marrow transplants were first used to treat leukemia. Congressional involvement in stem cell policy started as early as 1974. The first major amendment related to the use of federal funds for research involving embryonic stem (ES) cells occurred in 1996. In 2016 President Obama signed into effect, the 21st Century Cures Act, which includes provisions intended to assure timely regulatory review of regenerative therapies, including cell therapies enabled by stem cell therapy.

While preclinical trials suggest that MSCs may be effective in the treatment of IPF, and early clinical trials support their safety, currently the data to support their efficacy for the treatment of IPF is insufficient. Despite this lack of evidence, cell-based therapies are being aggressively marketed to vulnerable patient populations. A review carried out in November 2018 of the FDA web-

site lists over 1000 stem cell-related businesses registered. These clinics offer unproven, experimental treatments for a wide variety of conditions [89, 90].

In the case of IPF, desperate patients and their physicians continue to succumb to an onslaught of marketing and branding of as yet unproven "stem cell" treatments. Unfortunately, these businesses are also almost wholly unregulated [91]. A review of unapproved stem cell interventions by Turner and Knoepfler and the harm arising from the misuse of unproven treatments support increased government oversight in the interest of patient safety [92]. A sense of urgency exists to establish solid evidence regarding the efficacy of stem cell therapies for the treatment of chronic lung disease.

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