Cell Therapy for Lung Disease: Current Status and Future Prospects

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Abstract

Purpose of Review Mesenchymal stromal cell (MSC)–based therapies provide a platform for new therapeutic strategies in lung diseases. This review provides an overview of the current status of the field, along with some of the challenges ahead including better understanding of MSC actions in different lung diseases, personalized approaches to select patients most likely to benefit, and the growing problem of stem cell tourism.

Recent Findings A newly evolving concept suggests that MSCs shape their immunomodulatory actions depending on the environment they encounter. Furthermore, in some models, it appears that dying or dead cells may contribute to the therapeutic efficacy by activating the host response.

Summary Despite many pre-clinical studies demonstrating that MSCs can be used to treat lung disorders, clinical trials have failed to show improved outcome. Understanding the complex interaction between MSCs and the host microenvironment is likely to be an important area for enhancing the efficacy of MSC-based cell therapies.

Keywords Mesenchymal stromal cells · MSC · Cell therapy · Microenvironment · Stem cell tourism

Introduction

Respiratory diseases remain a significant cause of morbidity and mortality worldwide, and for many of the severe lung diseases including idiopathic pulmonary fibrosis (IPF), chronic obstructive pulmonary disease (COPD), cystic fibrosis (CF), bronchopulmonary dysplasia (BPD), and acute respiratory distress syndrome (ARDS) there is currently no cure. Therefore, extensive efforts continue for development of new therapeutic strategies. The promising pre-clinical results using mesenchymal stromal cell (MSC)–based cell therapy in experimental lung disease models (reviewed in [1, 2]), in combination with successful clinical trials in other diseases such as graft-versus-host-disease, have resulted in an increased

interest and rapidly growing number of clinical trials using MSCs for a range of lung diseases and critical illnesses. MSCs are multipotent cells with immune-modulatory and regenerative properties, and in combination with their low or absent constitutive HLA class I and II expression, they are theoretically ideal candidates to be used in cell-based therapies, (reviewed in [3, 4]). In this review, we summarize the current status of the field of MSC therapies, highlight new discoveries about the role of the microenvironment in control of MSC therapeutic behaviors, and discuss the current challenges that need to be overcome in order to provide clinical benefit in patients with lung diseases.

MSC-Based Therapy in Lung Diseases: Where Do We Stand?

The enthusiasm for using MSCs as a cell-based therapy was raised by encouraging pre-clinical in vivo data leading to the first clinical trial using MSCs as therapy for hematologic malignancies by Lazarus et al. in 1995 [5] and has further translated into clinical trials for several different acute and chronic lung diseases. Searching on the ClinicalTrials.gov database for trials listed through November 27, 2019 using the keywords “lung diseases” and “mesenchymal stromal cell” or “stromal cell” or “mesenchymal stem cell”, or “pulmonary

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disease” and “mesenchymal stromal cell” or “stromal cell” or “mesenchymal stem cell” identified 77 human clinical trials, of which 68 are for patients with lung disease. So far, 19 of the studies have been completed with 11 of them having published results in the PubMed database, 3 are currently active trials, 22 are open and in the process of recruiting their first patient, 7 are not yet recruiting, 13 have unknown status, and 4 of the trials have been withdrawn or terminated [6] (Table 1). In this section, we will summarize and discuss some of the clinical studies that have been completed and highlight a few promising currently ongoing studies.

Acute Respiratory Distress Syndrome

Despite an increased understanding of the pathology and current advances in supportive care, morbidity and mortality remain high in patients with acute respiratory distress syndrome (ARDS) [7]. ARDS pathology is driven by an acute severe inflammatory response, and acknowledging that the general hypothesis is that MSCs mainly act as immunomodulatory cells via rapid-acting paracrine effects, ARDS could be an ideal target for MSC-based therapies. This assumption is further supported by pre-clinical studies demonstrating that MSC-based therapy is beneficial in experimental models of ARDS (reviewed in [8, 9]).

In 2014, MSC-based treatment entered clinical trials for ARDS patients. In this trial, allogeneic adipose-derived MSCs were administered to 12 ARDS patients (one single dose of MSCs (1×10^6 cells/kg) or saline IV) [10]. A subsequent phase 1 dose-escalation safety trial (START trial, NCT01775774) using a single dose of allogeneic bone marrow-derived MSCs at 1.0, 5.0, and 10.0×10^6 cells per kg of predicted bodyweight in 9 ARDS patients was conducted [11]. These two trials were early phase 1 studies with the primary outcome being safety and were both underpowered to detect significant differences in efficacy. Both trials showed that MSCs were well tolerated in ARDS patients. While no significant differences in efficacy were observed, in the START study changes observed in the Sequential Organ Failure Assessment (SOFA) score and Lung Injury score (LIS) with the high dose of 10.0×10^6 MSCs/kg were promising. This led to a randomized phase 2a safety trial (NCT02097641) in which patients with moderate to severe ARDS received a single dose of the higher concentration (10.0×10^6 MSCs/kg) of bone marrow-derived MSCs. Similar to the first trial, no safety issues were observed but no significant improvement in 28-day mortality was observed between the MSC treated group and the placebo group [12]. The authors reported that the patient group receiving the MSC treatment were more severely ill compared with the placebo group. However, there were no differences in SOFA between MSC- or placebo-treated groups in SOFA when assessed at either 3, 7, or 14 days. Also, an unexpected finding was that

many of the MSC batches had a very low post-thaw viability at the time of injection, which was speculated to have contributed to the lack of efficiency. However, as discussed further below, a new evolving concept suggests that dead or dying MSCs might be beneficial. More encouraging results were observed in a case study describing two patients with severe refractory ARDS who each received systemic administration of allogeneic bone marrow-derived MSCs (2×10^6 MSCs/kg) on a compassionate use basis [13]. In contrast to previous studies, rapid significant clinical improvements occurred with both patients surviving and eventually being discharged from the hospital. Moreover, in a recent industry-sponsored trial, the company Athersys Inc. recently (May 2019) published a press release in which they announced positive results at 28 days of follow-up in their randomized, placebo-controlled, phase 1/2 ARDS (MUST-ARDS) trial using IV administration of an adult stem cell product named MultiStem^R [14••]. MultiStem^R is composed of multipotent adult progenitor cells (MAPCs) obtained from adult bone marrow and expanded in adherence cultures. The MAPCs share some but not all attributes of MSCs utilized in the START study of from adult bone marrow, but they claim that cells are not the same as MSC. The key efficacy endpoint was met with a decreased mortality rate in the MultiStem^R group ($4/20 = 25\%$) compared with the placebo group ($4/10 = 40\%$). With these positive results, it will be exciting to learn the results of other ongoing clinical trials (Table 1).

Bronchopulmonary Dysplasia

Bronchopulmonary dysplasia (BPD) remains a major contributor to mortality and morbidity in infants born prematurely, and current strategies to prevent this disease have been only moderately successful. BPD is a multifactorial disease where none of the current treatment strategies has effectively decreased complications in BPD survivors. Over the past years, the interest in using MSC-based therapies to treat BPD has increased especially in response to findings in pre-clinical studies demonstrating positive benefits [15, 16]. Currently, there are several clinical trials for BPD registered as completed or active on ClinicalTrials.gov (Table 1). The first published trial using MSCs to treat infants with BPD was a phase 1, dose-escalation trial (NCT01297205) using umbilical cord blood-derived MSCs at a concentration of 1×10^7 or 2×10^7 cells per kg [17]. This study demonstrated that the treatment was well tolerated in patients with BPD and that the levels of IL-6, IL-8, MMP-9, TNF- α , and TGF- β in tracheal aspirates were significantly reduced compared with baseline values. A 2-year follow-up study of this trial (NCT01632475) was published in 2017 [18], revealing that although one infant in the MSC group died suffering from *Enterobacter cloacae* sepsis, the remaining 8 infants showed no sign of transplant-related adverse outcomes or tumorigenicity was observed [18]. In

Table 1 Clinical trials listed on ClinicalTrials.gov investigating MSCs for treatment of lung diseases including clinical phase and current trial status

NCT number	Status	Conditions	Phases	Enrollment	Study designs
NCT00683722	Completed	COPD	Phase 2	62	Randomized, parallel assignment, quadruple
NCT01175655	Completed	BO	Phase 1	10	Single group assignment, open label
NCT01297205	Completed	BPD	Phase 1	9	Single group assignment, open label
NCT01306513	Completed	Emphysema	Phase 1	10	Single group assignment, open label
NCT01385644	Completed	IPF	Phase 1	8	Non-randomized, single group assignment, open label
NCT01775774	Completed	ARDS	Phase 1	9	Single group assignment, open label
NCT01828957	Completed	BPD	Phase 2	69	Randomized, parallel assignment, quadruple
NCT01872624	Completed	Emphysema	N/A	10	Non-randomized, parallel assignment, open label
NCT01919827	Completed	IPF	Phase 1	17	Single group assignment, open label
NCT01977131	Completed	Silicosis	Phase 1/2	10	Single group assignment, single (participant)
NCT02013700	Completed	IPF	Phase 1	9	Randomized, parallel assignment, double
NCT02023788	Completed	BPD	Phase 1	8	Case-only, prospective
NCT02097641	Completed	ARDS	Phase 2	60	Randomized, parallel assignment, triple
NCT02277145	Completed	Post-radiotherapy pulmonary fibrosis	Phase 1	10	Single group assignment, open label
NCT02381366	Completed	BPD	Phase 1/2	12	Single group assignment, open label
NCT02594839	Completed	ILD	Phase 1/2	20	Randomized, parallel assignment, open label
NCT02625246	Completed	Bronchiectasis	Phase 1	6	Non-randomized, single group assignment, open label
NCT02668068	Completed	Pneumoconiosis	Phase 1	80	Randomized, parallel assignment, single (participant)
NCT02804945	Completed	ARDS	Phase 2	20	Single group assignment, open label
NCT01795950	Terminated	PAH	Phase 1	6	Non-randomized, single group assignment, open label
NCT01632475	Active, not recruiting	BPD	Phase 1	9	Case-only, prospective
NCT02161744	Active, not recruiting	COPD	Phase 1/2	9	Single group assignment, open label
NCT02192736	Active, not recruiting	Asthma	Phase 1/2	20	Single group assignment, open label
NCT01897987	Recruiting	BPD	Phase 2	70	Randomized, parallel assignment, triple
NCT02181712	Recruiting	BO	Phase 1	19	Non-randomized, single group assignment. Open label
NCT02348060	Recruiting	COPD	Phase 1	100	Cohort, prospective
NCT02859415	Recruiting	Esophageal neoplasms	Phase 1/2	100	Non-randomized, sequential assignment, open label
NCT02866721	Recruiting	CF	Phase 1	15	Single group assignment, open label
NCT02946658	Recruiting	Lung disease	Phase 1/2	100	Non-randomized, parallel assignment, single (participant)
NCT03042143	Recruiting	ARDS	Phase 1/2	75	Randomized, parallel assignment, quadruple
NCT03137199	Recruiting	Asthma	Phase 1	6	Non-randomized, sequential assignment, open label
NCT03378063	Recruiting	BPD	Early Phase 1	100	Non-randomized, parallel assignment, open label
NCT03392467	Recruiting	Severe BPD	Phase 2	60	Randomized, parallel assignment, quadruple
NCT03558334	Recruiting	BPD	Phase 1	12	Non-randomized, parallel assignment, open label
NCT03608592	Recruiting	ARDS	N/A	26	Single group assignment, open label
NCT03631420	Recruiting	BPD	Phase 1	9	Single group assignment, open label
NCT03774537	Recruiting	BPD	Phase 1/2	20	Non-randomized, parallel assignment, open label
NCT03857841	Recruiting	BPD	Phase 1	18	Randomized, sequential assignment, quadruple
NCT03873506	Recruiting	BPD	Phase 1	30	Single group assignment, open label
NCT03929120	Recruiting	ILD	Phase 1	10	Single group assignment, open label
NCT04003857	Recruiting	BPD	Phase 2	60	Randomized, parallel assignment, triple
NCT04047810	Recruiting	COPD	Phase 1	15	Single group assignment, open label
NCT04055415	Recruiting	PH	Phase 1/2	60	Randomized, parallel assignment, double
NCT04062136	Recruiting	BPD	Phase 1	10	Single group assignment, open label
NCT03909750	Enrolling by invitation	COPD	Phase 1	50	Non-randomized, parallel assignment, single (participant)
NCT02443961	Not yet recruiting	BPD	Phase 1	10	Single group assignment, open label

Table 1 (continued)

NCT number	Status	Conditions	Phases	Enrollment	Study designs
NCT02985346	Not yet recruiting	IPH	Early Phase 1	100	Non-randomized, parallel assignment, open label
NCT03058068	Not yet recruiting	CF	Phase 1	18	Randomized, parallel assignment, double
NCT03601416	Not yet recruiting	BPD	Phase 2	57	Randomized, parallel assignment, open label
NCT03645525	Not yet recruiting	BPD	Phase 1/2	180	Randomized, parallel assignment, quadruple
NCT03818854	Not yet recruiting	RDS	Phase 2	120	Randomized, parallel assignment, triple
NCT04018729	Not yet recruiting	Severe COPD	Phase 2/3	34	Randomized, parallel assignment, double
NCT01207869	Unknown status	Severe BPD	Phase 1	10	Randomized, parallel assignment, double
NCT01373203	Unknown status	ALI	NA	20	Case-only
NCT01758055	Unknown status	Emphysema	Phase 1	12	Single group assignment, open label
NCT01902082	Unknown status	ARDS	Phase 1	20	Randomized, parallel assignment, triple
NCT02112500	Unknown status	RDS	Phase 2	10	Single group assignment, open label
NCT02135380	Unknown status	IPF	Phase 1/2	60	Randomized, parallel assignment, open label
NCT02175303	Unknown status	ALI	Phase 1/2	25	Non-randomized, single group assignment, open label
NCT02215811	Unknown status	ARDS	Phase 1	10	Single group assignment, open label
NCT02444455	Unknown status	ARDS	Phase 1/2	20	Single group assignment, open label
NCT02543073	Unknown status	BO	Phase 1/2	60	Non-randomized, parallel assignment, open label
NCT02645305	Unknown status	COPD	Phase 1/2	20	Single group assignment, open label
NCT02749448	Unknown status	Pulmonary disease	Phase 1	10	Single group assignment, open label
NCT02790762	Unknown status	Pneumoconiosis	Phase 1	10	Single group assignment, open label
NCT01559051	Withdrawn	COPD	Phase 1/2	0	Single group assignment, open label
NCT01849159	Withdrawn	Emphysema	Phase 1/2	0	Randomized, parallel assignment, open label
NCT02041000	Withdrawn	COPD	NA	0	Single group assignment, open label

NCT clinical trial registry number in [ClinicalTrials.gov](https://clinicaltrials.gov) database, BPD bronchopulmonary dysplasia, IPF idiopathic pulmonary fibrosis, ARDS acute respiratory distress syndrome, PAH pulmonary arterial hypertension, COPD chronic obstructive pulmonary disease, BO bronchiolitis obliterans, CF cystic fibrosis, ILD interstitial lung disease, PH pulmonary hypertension, IPH idiopathic pulmonary hemosiderosis, RDS respiratory distress syndrome, ALI acute lung injury, NA not applicable

addition, this follow-up study reported decreased need for supplemental oxygen at discharge compared with the control group; however, larger trials are needed in order to prove efficacy. The same investigators are currently running a 5-year follow-up study of the [NCT01297205](#) trial ([NCT02023788](#)) as well as a phase 2 study ([NCT01828957](#)). Moreover, there is one additional clinical trial listed on [ClinicalTrials.gov](#) as completed ([NCT02381366](#)); however, there are no results yet published on the PubMed database from this trial. Taken together, the enthusiasm for MSC-based strategies for treatment of premature infants with BPD is currently increasing and at present, there are 9 clinical trials recruiting patients (Table 1).

Chronic Obstructive Pulmonary Disease and Pulmonary Emphysema

Chronic obstructive pulmonary disease (COPD) is a progressive life-threatening disease, which has a massive impact on public health and is currently increasing in prevalence. COPD is a complex pathology with several clinical phenotypes involving different degrees of inflammation, tissue damage, and structural changes. Current treatments are not curative and primarily consist of strategies to delay disease progression [19–22]. In pre-clinical (rodent) models (reviewed in [23]), systemic MSC administration has been variably demonstrated to improve inflammation and histologic lung structure. These promising data led to clinical trials for COPD and emphysema. The first trial using MSCs to treat COPD ([NCT00683722](#)) was performed in 2013 [24]. This investigation demonstrated the safety of using non-HLA-matched bone marrow-derived MSCs in an older patient population with moderate-to-severe COPD. Additional studies have since been performed using both systemic and direct airway delivery approaches and have demonstrated similar results of safety of MSC administration to COPD patients [25, 26, 27]. However, none of these studies has demonstrated efficacy for treating COPD. Concerns with MSC have been whether or not the cells survive in vivo after administration to patients, and whether they could access sites of injury. In a recently published phase 1 study (Australian clinical trials registry number 12614000731695), MSCs were radiolabeled with indium-111 prior infusion. As in previous trials, all COPD patients tolerated the MSC infusions well. Interestingly, within 30 min the pre-labeled cells were detected in the lungs where they remained detectable for 24 h. Moreover, the amount of positive labeling correlated with the diffusing capacity of the lung for carbon monoxide as well as with the baseline FEV1 [27]. This study demonstrates that the third-party MSCs survive and diffuse throughout the lung to the same extent as carbon monoxide gas. Currently, there are four trials listed on [ClinicalTrials.gov](#) as active or recruiting participants with COPD (Table 1). These studies will hopefully reveal if COPD is suitable for

MSC-based therapy either as the main treatment or in combination with other relevant therapies.

Idiopathic Pulmonary Fibrosis

Idiopathic pulmonary fibrosis (IPF) is a life-threatening, progressive fibrotic lung disease of still poorly understood etiology. It has a patchy pathology with areas of massive deposition of extracellular matrix components, leading to reduced lung function. Currently, there are no curative treatments and in severe stages of the disease lung transplantation remain the only option [28]. MSC-based strategies have been extensively investigated in pre-clinical bleomycin-induced and other models of fibrotic lung injuries (reviewed in [29]). Despite the lack of convincing pre-clinical data on established lung fibrosis, early-phase clinical trials have been completed (Table 1). In 2013, Tzouveleakis et al. demonstrated in a prospective, non-randomized, no placebo-controlled phase 1 study that adipose-derived MSC were safe to give to IPF patients with no adverse events detected [30]. In a dose-escalation phase 1 study ([NCT01385644](#)), Chambers et al. demonstrated that it was safe to inject placenta-derived MSCs at either 1×10^6 or 2×10^6 cells/kg; however, this study was not powered to detect efficacy [31]. In 2017, Glassberg et al. published the results from the AETHER study ([NCT02013700](#)) where they used a single dose of bone marrow-derived MSCs at 20×10^6 , 100×10^6 , or 200×10^6 cells per infusion. This non-randomized and non-placebo controlled study included 9 patients, and while a few serious adverse events were reported, none was determined to be related to the MSC treatment. Similar to the other MSC trials, this study was not powered to detect efficacy, and no significant improvement in outcome was reported [32]. Taken together, clinical trials to date have shown safety and feasibility, but not benefit. Considering the general hypothesis that MSCs mainly act through rapid paracrine immunomodulatory mechanisms, advanced stages of IPF might not be the best suitable disease for MSC-based therapies.

Current Challenges and Hurdles in the Field that Needs to Be Overcome

MSC-based cell therapy for treatment of severe lung diseases has demonstrated promising results in experimental models; however, the lack of ability to translate these encouraging results into clinically relevant effects in patients has hampered the progression of the field. Despite an enormous interest in using MSCs for human clinical settings, the knowledge of MSC mechanisms of actions are limited and have predominantly been derived from pre-clinical studies [4, 33]. A growing literature suggests that the MSC phenotype and in vivo functions differ from the basic in vitro understanding. For

example, we and others have reported data suggesting that the *in vivo* mode of action changes depending on the inflammatory lung environment encountered [34•, 35•, 36••]. Moreover, MSCs exposed to the ARDS environment affects monocytes/macrophages by altering their phagocytic capacity and increasing anti-inflammatory M2 macrophage markers [35•]. In addition, Islam et al. recently demonstrated that the beneficial effect of MSCs was determined by the lung microenvironment. They further observed that high levels of both IL-6 and fibronectin in combination with low antioxidant capacity within the lung microenvironment resulted in poor outcome after MSC treatment in experimental lung injury [36••]. In addition, we recently demonstrated that MSCs stimulated with bronchoalveolar lavage fluid samples from patients with either ARDS or non-ARDS lung diseases exhibited both disease-specific and common MSC phenotypes [34•]. Taken together, the success of MSC-based clinical trials in lung diseases and other critical illnesses relies heavily on understanding the *in vivo* mode of action. Thus, a growing body of data suggests that the microenvironment within the diseased lung plays an important role in directing potential MSC therapeutic actions.

The generally accepted hypothesis has been that MSC therapeutic actions rely on the viability of the cells. However, this assumption has recently been challenged and recent studies suggest that dead or dying cells might improve the therapeutic effect by triggering host immune cells [37–40]. For example, administration of viable or heat-inactivated MSCs into an LPS induced lung injury mouse model evoked similar beneficial response [40]. More specifically, it is possible that systemically infused MSCs undergo apoptosis, which in turn activate the host immune system and lead to the immunomodulatory effects [37–40]. These are interesting data, however it is important to remember that these are xeno-transplantations (human into mouse), which may or may not contribute to these observations. The data on dead or dying MSCs in clinical trials are limited; nevertheless, a high portion of non-viable cells (85%) was reported in a recent multicenter double-blinded randomized trial of systemic bone marrow-derived MSCs in ARDS patients [12]. No efficacy was observed in this trial suggesting that non-viable MSCs may not have clinical benefit in ARDS. There are currently relative few publications investigating the effects of dead or dying MSCs, and this needs to be further studied.

Current Challenges and Hurdles for MSC Manufacturing for Clinical Applications

Despite an increasing number of early phase clinical trials using MSC for lung diseases, there is at present no established scientific consensus concerning the source of origin of MSCs, dosing strategies, therapeutic dose, and cell product

manufacturing (Table 2). MSC functionality changes depending on culture conditions including the isolation process, initial seeding density, number of passages, and culture surface. Despite this knowledge, a recent survey provided by the MSC committee of the International Society of Cell and Gene Therapy revealed that the current practice for manufacturing MSCs for clinical applications differs significantly in many of the above mentioned aspects amongst different US academic centers [41]. Although bone marrow was reported as the primary source of MSCs (93.3% of the facilities), MSCs are also isolated from adipose tissue, umbilical cord, umbilical cord blood, and placental tissue. Not surprisingly, MSCs isolated from different sources have different phenotypes and secretome profiles [42–46]. Furthermore, the survey revealed that multiple different isolation strategies were utilized. For example, 46.7% of centers used enzymatic digestion, 26.7% used an automated system, and 13.3% used Ficoll gradient centrifugation or filtration. In addition, 93.3% reported plastic adherence as the main enrichment method, with only 6.7% reporting use of FACS sorting, immuno-depletion, or immune-enrichment strategies. Utilizing standard plastic culture dishes is the most frequent way of cultivating MSCs, however three-dimensional cell culture systems may narrow the gap between pre-clinical and clinical research. For example, culture dishes coated with extracellular matrix molecules such as fibronectin and collagen have been demonstrated to support MSC growth [47, 48]. In addition, changing the composition of the matrix proteins resulting in altered elasticity or stiffness has been demonstrated to alter MSC phenotype [49]. However, the majority of the centers (80%) report using 2D culture methods with only three centers reporting exclusive use of 3D methods (bioreactor, bags, or hollow fibers) to expand MSCs. Also, seeding density affects MSC expansion and function. Fewer than 50% of centers report that they use low-density (30%, 50–500 cells/cm²) or intermediate density (16.7%, 500–2500 cells/cm²) meaning that the majority of facilities use a high plating density (>2500 cells/cm²). The same wide variation in current practice in producing MSCs for clinical use has also been found in a similar survey performed on MSC manufacturing facilities in Europe [50].

In addition to the challenges discussed above, efforts to map out strategies to foresee which patient groups are most likely to respond to MSC-based treatments need to be made. Many of the experimental lung injury models used in pre-clinical studies have been optimized to detect maximum effects, and might therefore not reflect the truly *in vivo* situation (reviewed in [51]). A compelling amount of pre-clinical data suggest that MSCs can act by secretion of a spectrum of paracrine factors, and tracking studies of systemically administered MSCs have demonstrated that, following initial lodging of the majority of cells in the pulmonary vascular bed, the majority are cleared within a few days [1, 27•, 52]. It is therefore unlikely that MSC-based treatments can remodel

Table 2 Completed and active clinical trials listed on ClinicalTrials.gov investigating MSCs for treatment of lung diseases including cell source, dose, and route or administration

NCT number	Conditions	Cell source	No. of cells	Route	Dosing	Comments	PMID
NCT00683722	COPD	BM-MSC (Prochymal)	Allogeneic 100.0×10 ⁶ cells/infusion	IV	1 dose/month (total 4 months)	MSCs were produced under good manufacturing practice conditions (Therapeutic Goods Administration)	23,172,272
NCT01175655	BO	BM-MSC	2.0×10 ⁶ cells/kg	IV	2 doses/week (for 2 weeks)	Female donors age 17–30 years	28,186,707
NCT01297205	BPD	UCB-MSC	1.0×10 ⁷ or 2.0×10 ⁷ cells/kg	IT	2 doses (1 week apart)	In combination with LVRS	24,508,444
NCT01306513	Emphysema	BM-MSC	Autologous 1.0–2.0×10 ⁶ cells/kg	IV	Single dose		26,819,296
NCT01385644	IPF	Placenta-MSC	1.0×10 ⁶ or 2.0×10 ⁶ cells/kg	IV	Single dose		25,039,426
NCT01775774	ARDS	BM-MSC	Allogeneic 1.0×10 ⁶ , 5.0×10 ⁶ , or 10.0×10 ⁶ cells/kg	IV	Single dose		25,529,339
NCT01828957	BPD	UCB-MSC (Pneumostem)	1.0×10 ⁷ cells/kg	IT	Single dose		
NCT01872624	Emphysema	BM-MSC	1.0×10 ⁸ cells (in 30 ml saline)	IB		In combination with EBV insertion	28,186,686
NCT01919827	IPF	BM-MSC	Autologous	EB			
NCT01977131	Silicosis	BM-MSC	Autologous 2.0×10 ⁶ cells/kg	IV	1 dose/week (for 3 weeks)	transfected by a vector containing human HGF cDNA	26,400,297
NCT02013700	IPF	BM-MSC	Allogeneic 2.0×10 ⁶ , 1.0×10 ⁸ , or 1.0×10 ⁸ cells/infusion	IV	Single dose	Male donors age 24 and 25 years	27,890,713
NCT02023788	BPD	UCB-MSC (Pneumostem)	1.0×10 ⁵ or 2.0×10 ⁷ cells/kg	IT	Single dose	5-year long-term follow-up study	
NCT02097641	ARDS	BM-MSC	Allogeneic 10.0×10 ⁶ cells/kg	IV	Single dose	One female and two male donors age 18–45 years	30,455,077
NCT02277145	Post-radiotherapy pulmonary fibrosis	UC-MSC	1.0×10 ⁶ cells/kg	FB		After fully lavage of the localized lesions	
NCT02381366	BPD	UCB-MSC	1.0×10 ⁷ or 2.0×10 ⁷ cells/kg	IT	2 doses at interval of 7 days (every 3 months for 1 year)		30,992,220
NCT02594839	ILD	BM-MSC	2.0×10 ⁸ cells/infusion	IV	2 doses		
NCT02625246	Bronchiectasis	BM-MSC	2.0×10 ⁷ or 10.0×10 ⁷ cells	IV			
NCT02680668	Pneumocystosis	UC-MSC	1.0×10 ⁶ cells/kg	IV			
NCT02804945	ARDS	MSC	max. dose of 3.0×10 ⁶ cells/kg	IV	Single dose	Injected after whole-lung lavage	
NCT01795950	PAH	Placenta-ASC	Allogeneic 0.5×10 ⁶ , 1.0×10 ⁶ , or 2.0×10 ⁶ cells/kg	IV	Single dose		
NCT01632475	BPD	UCB-MSC (Pneumostem)	1.0×10 ⁷ or 2.0×10 ⁷ cells/kg	IT	Single dose	2-year long term follow-up study	28,341,525
NCT02161744	COPD	Adipose-MSCs	Autologous	IV	Single dose		
NCT02192736	Asthma	MTF factors UC-MSC	Allogeneic	IN	1 dose/week (for 4 weeks)	Trophic factors from UC-derived MSCs	

NCT clinical trial registry number in [ClinicalTrials.gov](https://clinicaltrials.gov) database, BPD bronchopulmonary dysplasia, IPF idiopathic pulmonary fibrosis, ARDS acute respiratory distress syndrome, PAH pulmonary arterial hypertension, COPD chronic obstructive pulmonary disease, BO bronchiolitis obliterans, ILD interstitial lung disease, max maximal, BM bone marrow-derived, UC umbilical cord-derived, UCB umbilical cord blood-derived, ASC mesenchymal-like adherent stromal cells, MTF allogeneic mesenchymal trophic factors, No number, IV intravenous, IT intratracheal, IB intrabronchial, EB endobronchial, IN intranasally, FB fibroepithelial bronchiolitis, LVRS lung volume reduction surgery, EBV one-way endobronchial valve, HGF hepatocyte growth factor

chronically injured and destroyed tissue such as found in advanced lung fibrosis or emphysema by differentiating into other cell types. Rather, it is more likely that MSC-based cell therapies will be more beneficial in diseases involving acute inflammation and infection such as ARDS and sepsis/septic shock. Moreover, ARDS is a multifaceted and heterogeneous disorder and there may be very meaningful differences in the host environment that will affect the therapeutic activity of MSCs. As such, we need to find strategies for selecting patients most likely to respond to the treatment. Another way to improve the clinical outcome would be to design MSC potency assays. However, without knowing the *in vivo* mode of action makes it difficult to determine what this assay or assays should be [51, 53]. In addition, effort should also be made to optimize and unify outcome parameters measured in clinical trials for lung diseases in order to design successful clinical trials and to obtain maximum biologic and mechanistic information.

Stem Cell Tourism

In parallel with the progression of the field of cell-based therapies, a growing problem with commercial stem cell therapies has developed both in the USA and globally [54, 55]. In modern life with daily access to internet and social media platforms, desperate patients, families, and caregivers can easily be misled into participating in very expensive and unproven stem cell treatments, which are not covered by insurance. Importantly, many stem cell clinics fail to prove safety and do not fulfill recognized biological and medical standards, exposing patients to unnecessary risks. These unethical clinics not only harm patients and their families, they also have the potential to hamper the progression of scientifically rigorous potential stem cell therapies. Therefore, the FDA, the International Society for Cell and Gene Therapies (ISCT), the International Society for Stem Cell Research (ISSCR) and an increasing number of respiratory disease foundations have taken stances against these stem cell clinics [56–62]. In an attempt to increase the knowledge about these clinics to patients, families, and caregivers, the American Thoracic Society (ATS) Respiratory Cell and Molecular Biology Assembly Stem Cell Working Group posted online statements and several other related publications [58–62]. In addition, earlier this year Google announced that they are not any longer accepting advertising for “unproven or experimental medical techniques such as most stem cell therapy, cellular (non-stem) therapy, and gene therapy” [63].

Conclusions

MSC-based therapies for severe lung diseases such as ARDS, COPD, IPF, and BPD have demonstrated promising results in

experimental lung models; however, this has not translated into significant improved clinical outcome in patients to date. Importantly, current clinical trials have all demonstrated that MSC-based therapies are safe for lung disease patients; however, no significant efficacy or improved lung function has currently been demonstrated except for one promising investigation in patients with ARDS [14^{***}]. There are several challenges ahead for this field including revealing the *in vivo* mechanism of action, selecting the patient group most likely to respond, development of relevant potency assays, and the accelerating problem with the unethical stem cell clinics. Despite these challenges, there has been significant progression in the field and hopefully ongoing clinical trials will bring positive and encouraging clinical data with improved outcomes and hope for the future.

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Compliance with Ethical Standards

Conflict of Interest The authors declare no conflicts of interest.

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- Of major importance

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