Chapter 14
Mesenchymal Stromal Cell-Based Therapies for Lung Disease

Daniel Chambers

Introduction

Although our current understanding of the identity of, and interactions between, lung stem/progenitor cells in the adult lung remains a work in progress, particularly in humans, great strides are being made. What is very clear is that understanding how the adult lung achieves homeostasis and repairs injury will be of fundamental importance if we are to completely understand the pathogenesis of lung diseases, particularly the increasingly common chronic, and mostly treatment refractory, degenerative lung diseases. As occurred for bone-marrow disorders three or four decades ago, it is highly likely that cellular therapies will play a role in the future management of these diseases [1]. This chapter will focus on mesenchymal stromal cell (MSC) therapy since this cell type, or a closely related cell, is likely to be the first of these new therapeutic modalities to enter the clinic and will highlight potential pulmonary disease targets, whilst also emphasising the limitations of the tools we currently use to isolate and characterise lung stromal cell populations. MSCs have a broad functional repertoire. Their immunosuppressive, antibacterial and antifibrotic activity; their ability to elaborate growth factors; and their proliferative potential and ability to differentiate into multiple cell lineages has generated great interest in their role in disease pathology, and as architects of organ repair and regeneration. Special emphasis will be given to idiopathic pulmonary fibrosis (IPF) as a potential target for regenerative medicine approaches utilising MSCs, since the incidence of this quintessential degenerative lung disease is rapidly increasing as populations age, and since evidence is accumulating that lung progenitor cell depletion or dysfunction may lie at the heart of its pathogenesis.

D. Chambers, MBBS, MRCP, FRACP, MD. (✉)
School of Medicine, The University of Queensland, Brisbane, QLD, Australia

Queensland Lung Transplant Service, The Prince Charles Hospital, Brisbane, QLD, Australia
e-mail: Daniel_Chambers@Health.qld.gov.au

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Therapeutic Potential of MSCs

MSCs are a specialised stromal cell type originally identified in the 1960s by heterotypic transplantation and liquid culture of rodent and guinea pig bone marrow and spleen cell suspensions [2, 3]. Originally characterised by their ability to form fibroblast-like, plastic adherent colonies from single cells when plated at clonogenic levels, and their multipotent (bone, cartilage, and adipose tissue) differentiation capacity when propagated in defined media, the minimal criteria endorsed by the International Society for Cellular Therapy (ISCT) for defining multipotent MSC now includes a $CD105^{+}CD90^{+}CD73^{+}CD45^{-}$ immunophenotypic signature profile [4]. MSCs or MSC-like cells have now been identified in many organs and tissues, including the lung [5]. Sabatini et al. first identified a population of cells consistent with the currently accepted ISCT definition of an MSC in human lung digests [6]. Similar cells have since been isolated from bronchoalveolar lavage of sex-mismatched lung transplant patients using plastic adherence [4]. Clonogenic cells derived from these patients retain the sex of the donor even many years after sex-mismatched lung transplantation, suggesting that they are very long-lived and retain an inherent capacity for self-renewal [4].

However, it is important to note that a similar phenotype is shared by many stromal cells, including the humble dermal fibroblast [7, 8], so many studies using the term 'MSC' have likely described the properties of a heterogeneous population of cells [9–11]. The 'stemness' (capacity for self-renewal assessed with serial transplantation) of putative MSCs differentiates them from other stromal cell types, but is rarely assessed in published studies [12]. Hence the term 'mesenchymal stromal cell' rather than 'mesenchymal stem cell' is favoured. It is possible, indeed even probable, that enrichment of heterogeneous MSC populations for stemness will improve therapeutic efficacy [10], Consequently, the development of assays to quantitate potency is an active area for research [13].

Notwithstanding this lack of precision with respect to definition, MSCs perform a remarkable, perhaps even surprising, array of functions and hence carry considerable therapeutic potential. This broad functionality may of course be related to cellular heterogeneity rather than a sweeping repertoire of cell-specific skills. However the currently available literature almost exclusively uses the ISCT definition of an MSC, with its inherent limitations, so this possibility remains largely unexplored. Despite this caveat, a large body of literature, some of which now includes well-controlled randomised trials, suggests that MSCs are highly likely to provide new therapeutic options for a broad range of diseases, and particularly to provide regenerative options for degenerative disease [14].

Relative immune privilege: Aside from this broad functionality, MSCs have the added advantage of being easy to propagate and relatively immune-privileged. Since MSCs can escape lysis by cytotoxic T-cells and natural killer cells, they may be transplantable between HLA-mismatched individuals without the need for immunosuppression. This relative immunoprivilege has been confirmed in multiple studies using xenogeneic and major histocompatibility mismatched models. For

instance, when human bone-marrow-derived MSCs were transplanted into lambs they were able to engraft and persist for up to 13 months [15]. While human MSCs do not express HLA class II antigen, they do weakly express HLA class I, however in co-culture experiments human MSCs fail to induce proliferation of allogeneic lymphocytes [16]. More recently, the degree of immunological privilege accorded MSCs has been questioned. Studies in small animal cardiac models suggest that the immunogenicity of MSCs increases with the differentiation state of the cells—so that multipotent cells remain immune-privileged, but major histocompatibility complex expression is upregulated in their terminally differentiated progeny (e.g., myocytes and endothelial cells) leading to destruction by complement- and/or cell-mediated lysis [17, 18], re-enforcing the objective to enrich MSC populations for stemness when devising optimal therapies.

On the other hand, even multipotent cells have been found to be more immunogenic than previously anticipated. In a primate model, multiple administrations of high-dose allogeneic MSC resulted in the production of alloantibodies in two of six animals [19]. MSCs have also been reported to induce memory T-cell responses in a murine model [20] and furthermore, MSCs express the activating NK cell-receptor ligands NKG2D and UL16 [21] limiting their ability to avoid lysis by NK cells [22]. The practical implication is that preclinical work in major histocompatibility complex-matched and/or immunosuppressed animals needs to be cautiously interpreted in the planning of human Phase I studies which are likely to involve HLA-mismatching, particularly if treatment with relatively well differentiated cells is proposed.

Immunosuppression and tolerance: One of the most consistently observed properties of MSCs has been their immunosuppressive function. They are able to abrogate T-cell responses by production of paracrine factors such as PGE_2 [23, 24], and the induction of a regulatory phenotype in $CD4^+$ lymphocytes [25–27]. This provides a strong rationale for preclinical and clinical studies utilising MSC in transplantation, where tolerance remains the Holy Grail. The possibility that MSC-treatment may foster operational tolerance as part of an immunosuppression-minimisation protocol holds great promise [27] with the potential for substantially improved post-transplant outcomes, and has already been confirmed in a randomised controlled trial in renal transplantation [28]. In the lung, MSCs show promise as an adjunct therapy in patients undergoing lung transplantation with encouraging efficacy data from animal models [29–31], and safety data from a Phase 1 study of human transplant patients with chronic lung rejection [32].

Lung homeostasis and epithelial repair: Complex organisms possess a remarkable capacity for extensive and sustained tissue renewal throughout a lifetime. This regenerative capacity is maintained by reservoirs of self-renewing somatic tissue stem cells which are responsible for organ homeostasis and repair following injury [33]. Analysis of the organisation and regulation of the archetypal hematopoietic stem cell (HSC) hierarchy has revealed that bone-marrow MSCs are a critical element of the HSC niche responsible for maintaining stem cell potential, facilitating hematopoiesis [34], as well as playing a key role in the mobilization of HSC into

the circulation, and their homing and lodgement in the marrow following transplantation [12].

Since the stroma provides critical cues to support respiratory epithelial progenitors during lung development, homeostasis and repair, it follows that an analogous niche may also exist in adult lung. The critical importance of stromal inputs to lung regeneration and repair has been apparent for many years, but has been brought into sharper focus by the development of assays for identifying and characterising candidate lung epithelial stem cells which have provided powerful tools for analysis of the niche interactions between stem and stromal cells [35–37]. While the cell types providing these cues and the cues themselves remain enigmatic in humans [11], the stem cell function of type 2 pneumocytes during alveolar repair was recently proven in mice and is dependent on cross-talk from a population of stromal cells including alveolar fibroblasts and lipofibroblasts within a niche [38]. Furthermore, ablation of MSCs in the murine lung has been associated with experimental bleomycin-induced fibrosis [39]. MSCs were more recently shown to increase the proliferative potential of a key epithelial progenitor cell—the bronchoalveolar stem cell [40] and, remarkably, to restore bioenergetics in lung epithelium and induce repair programmes through donation of mitochondria [41, 42]. Fully understanding the components and relationships within the niche, or, as is much more likely, niches, is likely to be crucial to understanding the pathogenesis of degenerative lung disease. It is also for this reason that the exogenous delivery of stromal cells is an attractive idea for the treatment of degenerative lung disease.

But could MSCs not just orchestrate, but actually participate in epithelial repair by respecification and transdifferentiation of MSC to generate epithelial cell lineages? As is the case for MSC-like cells derived from other organs, MSC-like cells derived from human lung have been shown to differentiate into non-mesodermal cell lineages, including epithelium [43]. Others have also shown that MSCs derived from lung tissue differentiate into cells expressing club cell secretory protein and aquaporin-5, markers of small airway and alveolar epithelial cells respectively when cultured in suitable media [44]. However it is now broadly accepted that any such trans-differentiation occurs rarely, if at all, in vivo, and is not extensive or robust enough to contribute meaningfully to epithelial repair [45].

Despite this caveat, there remains intense interest in delivering MSCs to the lung to treat inflammatory diseases such as chronic obstructive pulmonary disease (COPD) and asthma or post-transplant rejection; and to manage acute lung injury (ALI) and chronic degenerative diseases such as IPF.

Delivering Cell Therapy to the Lung

There are two potential routes of pulmonary delivery of a candidate cell product–endobronchial and intravenous. A third possible route of delivery, during ex vivo perfusion of whole organs, is feasible and may prove important in lung

bioengineering [46] but will not be further discussed here. Whilst the endobronchial route has been used [47] and is readily accessible via bronchoscopy, delivery of large numbers of cells to the distal lung is problematic and unpredictable. In contrast the intravenous route is highly attractive because of the so-called 'first-pass effect', whereby cells delivered intravenously are required to transit the lung so that there is extensive and homogeneous, although admittedly temporary, retention of MSCs as they pass through the pulmonary circulation [48]. Hence, only a small proportion of infused cells pass through into the systemic circulation [49]. This effect is particularly pertinent to MSC-based cell therapy due to the large physical size of MSCs.

While the first-pass effect has impeded the development of regenerative therapy approaches such as MSC therapy for non-pulmonary target organs including the heart [50], the ability to deliver cellular therapy to the lung via a simple intravenous approach is a major advantage and affords the opportunity for large-scale retention of reparative cells. Even more attractively, the apparent preferential retention of infused cells at sites of lung injury [51], provides a distinct advantage for designers of lung cell therapies.

A downside of intravenous delivery of cell therapy is the risk that embolization of MSC will lead to adverse hemodynamic events, but this problem has been largely discounted in early phase human studies [52, 53]. The need for infused cells to bind to and then transit the endothelial layer, in order to reach the site of injury and provide any conceivable therapeutic effect is also a potential impediment to the delivery of an effective cellular therapy. However, since evidence points increasingly to a perivascular location of lung MSCs [10], and hence the epithelial stem cell niches, this problem may not be insurmountable. Despite these advances, large evidence gaps remain, not the least of which are the elucidation of the mechanisms of MSC homing and engraftment to targeted tissue microenvironments. In summary, whilst it must be kept in mind that mere transit to the lung microvasculature does not equal functional engraftment; nevertheless the ease of pulmonary delivery of cellular therapy via the intravenous route is attractive. Future studies will need to focus on the chemokine signals which encourage MSC homing and on the ligand/receptor interactions at the endothelial surface which encourage migration, margination, extravasation and engraftment.

MSC source: At present most MSCs used in human trials are derived from unrelated bone-marrow donors, although placenta, umbilical cord and adipose tissue are other, potentially more convenient sources. The literature presently sheds little light on the similarities and differences between these cell types, with all meeting the (admittedly broad) current ISCT-endorsed definition of an MSC. Further complicating the field, MSCs prepared for clinical use, regardless of source, are heterogeneous in therapeutic efficacy despite attempts to standardise ex vivo expansion protocols. Determining the most accessible and potent source of MSC for therapeutic product development will of course be highly dependent on the target disease, and in the case of degenerative disease, will further depend upon accurately defining the extent of and mode of delivery of stromal cell support to the

pulmonary epithelium and enriching heterogeneous cell populations for the relevant activity.

Whole cells, *microvesicles or conditioned medium*? Whilst it is now clear that MSCs are unable, at least in any significant number, to transdifferentiate to aid in epithelial repair, it remains to be determined whether the support signals orchestrating epithelial repair by MSCs are soluble and delivered in a paracrine manner or cell contact dependent. However, recent exciting studies in the lung provide evidence that cell–cell contact and the transfer of cytoplasmic contents, including mitochondria, from the donor MSC to epithelial progenitors may be critical in providing the cues regulating stem cell proliferation, differentiation and repair [41].

Mitochondria were donated to eukaryotes approximately 1.5 billion years ago by an ancient prokaryote, facilitating aerobic respiration and the subsequent evolution of the eukaryotic cell, and later, complex multicellular organisms. Mitochondrial dysfunction has recently been identified as a key trigger for cellular senescence and apoptosis, and may act as a break on tissue stem cell proliferation in organs with a generally slow turnover of stem cell pools, such as the lung [54]. The potential for MSCs (and other cell types) to have retained the ancient ability to transmit and donate mitochondria and hence restore mitochondrial function to tissue stem cells to effectively slow tissue ageing is of considerable interest. While the literature in this field remains scant in the lung, there is emerging evidence that delivery of cell components (microvesicles or exosomes) carrying organelles and microRNAs, but not the cells themselves, can provide substantial therapeutic benefit [55]. This exciting possibility opens the way to the development of novel therapeutic approaches whereby MSCs may become the ‘postmen’ of the regenerative medicine world, delivering their designer therapeutic packages to specific target cells.

But what are the likely disease targets? Whilst the answer to this question must of course be founded on a sound understanding of MSC biology, it would be a mistake to design early human studies without considering what the path to successful translation might look like.

Ensuring That the Therapeutic Potential of MSCs Is Not Lost in Translation

The term ‘translational research’ is used, somewhat confusingly, to refer to two distinct phases of therapeutic product development and application. To add to the confusion, very often, the phase being referred to is not specified. The first translational ‘step’, or as is sometimes the case, ‘leap’ is that taken when moving from preclinical studies to first-in-human trials or from the ‘bench’ to the ‘bedside’. The decision to move to a first-in-human study is difficult, but can be considered within the framework outlined in Fig. 14.1. The case for conducting a first-in-human study is strongest when there is an important unmet clinical need for a new therapy; when the therapy has been successfully tested in appropriate preclinical studies which

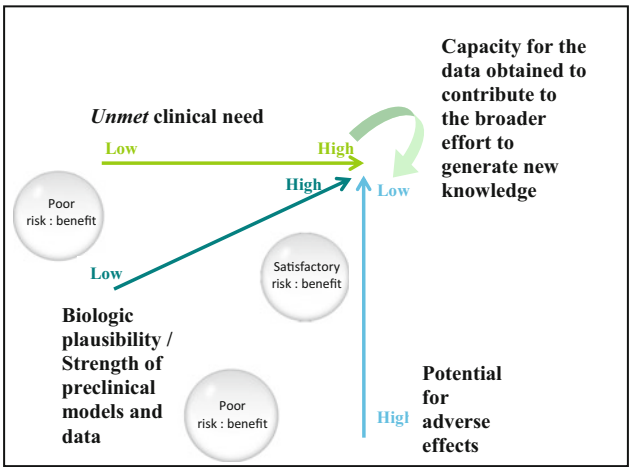

Fig. 14.1 Turning the translational 'leap' into a step. The decision to move to a first-in-human study is difficult, but can be considered within the framework outlined in the figure. The case for conducting a first-in-human study is strongest when there is a sound biologic rationale for a new treatment; when the therapy has been successfully tested in appropriate preclinical studies which reliably reflect the human disease; when the potential for adverse events is low; when the probability that the data generated during early phase human studies will shed new light on the pathogenesis of a serious human disease is high; and when there is an important unmet clinical need. It is important to make the distinction between 'unmet' and 'met' clinical need. In the latter case, while there may be a large burden of disease, effective therapeutic strategies are available and the burden of remaining disease largely relates to ineffective implementation of these strategies (the second translational step). Naturally it is common for not all of these pieces of the puzzle to fall neatly into place, but careful consideration of the weight of evidence along each axis will improve the risk: benefit of a first-in-human study

reliably reflect the human disease; and when the potential for adverse events is low. Of course the proposed new therapy also needs to make biologic sense. The probability that the data generated during an early phase human study will shed important light on a serious human disease further enhances the attractiveness of a first-in-human study. Naturally it is common for not all of these pieces of the puzzle to fall neatly into place. The second translational step refers to the adoption and dissemination of a practise or product which is already known from well-designed clinical trials to be beneficial to human health. The implementation of evidence-based treatment regimens and management algorithms for asthma is a good example of this second step.

The burden of lung disease in the twenty-first century—where is the unmet clinical need going to be? Lung disease remains the third most common cause of death globally, behind ischemic heart disease and cerebrovascular disease [56], with most of these respiratory deaths being related to pneumonia. In developing countries tuberculosis remains a very serious problem and smoking-related lung disease (overwhelmingly lung cancer and COPD) causes significant morbidity and

mortality worldwide. However a major change is underway in developed nations, with declining cigarette smoking rates, effective antibiotic therapy and population ageing meaning that non-communicable, chronic degenerative diseases will become, beside lung cancer, the major cause of respiratory death in the coming decades [56].

Currently, the most common chronic respiratory diseases in developed nations remain asthma and COPD. In Australia the prevalence of asthma is approximately 10 % and COPD 3 %. Highly effective and safe therapies are already available for asthma so that mortality is now rare, with the major challenges being in achieving a timely and accurate diagnosis, ensuring adherence to treatment to minimise morbidity and mortality, and in identifying the underlying causes to reduce prevalence. COPD on the other hand remains difficult to treat as the structural nature of the disease means that pharmacologic agents are only partially effective, but COPD is completely preventable if cigarette smoking is avoided. Hence COPD-related mortality is declining in developed nations. In contrast, lung cancer remains a leading cause of death, even with declining smoking rates, since a minority of cases are unrelated to cigarette smoking.

If we look ahead to the latter half of the twenty-first century then, the prevalence of COPD and lung cancer will be decreasing but these diseases will remain significant sources of morbidity and mortality. In contrast the prevalence of degenerative and age-related lung diseases will be increasing rapidly without effective treatments to meet this increasing burden. Indeed this change is already underway, with the incidence of IPF, the most common of these diseases, increasing to somewhere (depending on case definition) between 30 and 93/100,000 [57–59] and the annual cumulative prevalence rising rapidly from 202 cases per 100,000 people in 2001 to 494 in 2011 [59]. IPF is a lethal disease with the median survival from diagnosis being 3.5–4 years, even in the modern era [57–59].

Acute lung injury (ALI) is a clinical syndrome of diverse aetiology characterised by widespread pulmonary infiltration and rapidly developing respiratory failure. It is also prominent among lung diseases for its high mortality, lack of effective treatments, and increasing incidence with population ageing. This incidence is now 60–80/100,000 [60], and the mortality rate is 40 % and static [60]. It is likely that ALI will become an increasing problem in developed nations in the coming years. Several pieces of evidence point to a role for MSC treatment in ALI [41, 61–64], and early phase human studies are now underway.

Although therapeutic options for both IPF and ALI remain limited, there has been significant recent progress for patients with IPF, for the first time, confirmation of efficacy of two small molecules in large randomised controlled trials [65, 66]. However, due to a lack of truly effective preventative or therapeutic measures and a rising incidence, in developed nations this century the clinical need will be most pressingly unmet for lung cancer, ALI and IPF. The therapeutic potential of MSCs comes into clear focus when considered from this perspective. It is unlikely that regenerative strategies will play a role in the management of cancer, however they may well go some way to expanding the therapeutic options available for patients with ALI and IPF.

IPF—a degenerative disease in need of a regenerative solution. It is against this backdrop that focus is shifting toward understanding and developing effective therapeutic strategies to manage degenerative lung disease. These non-communicable, non-malignant lung diseases are characterised by failed or ineffective organ repair after injury, with the clinical phenotype (for instance ALI vs. IPF) in large part depending on the nature, severity and acuity of the lung injury as well as host factors such as the effectiveness of repair. In fact IPF is characterised by sudden deteriorations, called exacerbations, which are a form of ALI [67]. In order to appreciate the potential place of future regenerative strategies for human lung disease, it is instructive to take a step back and review recent advances in our understanding of IPF pathogenesis.

Although the moniker 'idiopathic' remains appropriate, compelling insight into IPF pathogenesis comes from the relatively rare germline mutations in the human TERT and TERC genes [68]. Together, TERT and TERC form the specialised enzyme telomerase. In stem/progenitor cells the telomerase complex functions to synthesise telomeric DNA and so protect the chromosome ends during cell division. TERT provides reverse transcriptase activity to the complex, and uses TERC, the RNA component of telomerase, as a template. Hence together the TERT and TERC genes maintain telomere length and prevent cellular senescence during recurrent cycles of cellular replication. Telomerase activity is thus a commonly used measure of 'stemness', and overexpression of TERT prevents replicative senescence in MSCs and other cells [69]. The most common phenotype in humans with a germline loss-of-function TERT/TERC mutation is a form of pulmonary fibrosis indistinguishable from IPF [70]. Short telomere length, independent of TERT/TERC mutations, is also a strong risk factor for IPF itself, with a clear dose–response relationship between telomere length and survival [71]. These pieces of evidence, along with the epidemiology of IPF (it is overwhelmingly a disease of ageing [58]) indicate that cellular processes which rely on the maintenance of telomere length are fundamental to IPF pathogenesis. A provocative but tantalising conclusion is that IPF results from dysfunction and/or depletion of lung stem/progenitor cell pools over a lifetime. Candidate cells include epithelial progenitors [72], the stromal progenitors which orchestrate epithelial repair, and potentially others, although recent evidence points to depletion of lung resident MSCs in both animal [39] and human [10] lung fibrosis. Repletion of these pools through the delivery of exogenous MSCs or epithelial progenitors [14], or pharmacologic, cell or exosome treatment to improve native adult lung progenitor cell function thus holds promise for the management of IPF [52, 73], ALI [62] and other diseases.

Given this background, it is clear that understanding lung homeostasis and regeneration, and in turn defining the cells which complete these functions, is highly likely to provide new regenerative treatment options. However, currently the ultimate and, at this point, only, regenerative strategy for lung disease is whole organ transplantation, which has now been a viable and evidence-based treatment for selected patients with IPF and other end-stage lung diseases for over three decades. However, lung transplantation necessarily involves the allogeneic replacement of all lung cells, even those with normal function, via a highly invasive

operative approach and utilising a very precious and scarce, but also highly immunogenic, resource. More targeted, less invasive and potentially even non- or 'hypo'-allogeneic approaches to lung regenerative medicine should be possible.

Given the fundamental defects in epithelial repair which underlie these conditions, delivering a cell product like an MSC with the aim of improving repair makes biologic sense, especially since depletion of the MSC pool has now been demonstrated in human IPF [10]. Furthermore, and notwithstanding the limitation of these models, multiple preclinical studies provide robust support for the therapeutic efficacy of MSCs (reviewed by Sinclair et al. [11]) in animal models of lung fibrosis resulting from exposure to bleomycin. Table 14.1 demonstrates the diversity in these studies with respect to cellular source, the use of immunodeficient or immunocompetent animals, and the timing and route of MSC delivery. Most of these studies have utilised allogeneic MSCs which have been isolated from bone marrow using plastic adherence and delivered intravenously or endobronchially. If we return to Fig. 14.1, there appears to be strong evidence for efficacy of MSC treatment in preclinical models of these diseases, but the ability of these models to accurately reflect the corresponding human disease is questionable. This is particularly so for the bleomycin model of pulmonary fibrosis where over the last few decades many compounds have been apparently effective, only to prove ineffective in human trials.

Although there appears to be a consistent effect of MSCs if delivered soon after the administration of bleomycin, the therapeutic effect diminishes considerably if treatment is delayed until 7 days after administration [51, 74, 75]. This effect highlights a well-known deficiency of the bleomycin model, and is particularly important to recognise since the timing of MSC delivery appears to determine the fate of the engrafting cell, with later delivery favouring the differentiation of MSCs into cells which are pro-fibrotic [51]. The latter possibility remains an ongoing concern for investigators contemplating human IPF trials and will be a key safety outcome. Thus the potential efficacy of MSC-based cell therapy in IPF and ALI remains controversial [80, 81], with the potential for profibrosis being the main drawback. Since MSCs can be driven down a myofibroblastic differentiation pathway given the correct context, these paradoxical findings again highlight the importance of the fundamental work being carried out to discover the secrets of the lung stem cell niche(s), and potentially provide a rationale for delivering therapy earlier in the disease course when epithelial disrepair is at its height, but when extensive fibrosis has not yet ensued. In summary, there is biologic plausibility around MSC treatment for IPF and ALI, there is a large body of preclinical data (admittedly in models which have a questionable relationship to the human disease), there is substantial evidence for safety from human studies for other indications, and there is a large unmet clinical need. From Fig. 14.1, the pieces of the puzzle are in place to proceed to human studies of MSC treatment for IPF and ALI [73], with perhaps a combination of these two conditions, the acute and often lethal, exacerbation of IPF being a prime target.

Aside from lung fibrosis (Table 14.1) and ALI [61, 62], animal studies have provided support for therapeutic efficacy in other lung diseases where the need is

Table 14.1 Preclinical studies of MSCs or MSC-like cells in pulmonary fibrosis

Author	Intervention	Model	Outcome	Engraftment?
Ortiz et al. [74]	Allogeneic 5×10^5 BM-MSC @ 0, 7 days via jugular vein	Mouse bleomycin	↓ Hydroxyproline—not significant with day 7 infusion	Yes, increased in fibrotic areas
Cui et al. [75]	BM-MSC @ 1, 7 days via tail vein	Rat bleomycin	↓ Hydroxyproline and lung fibrotic score—more pronounced with day 1 infusion	Yes
Zhao et al. [76]	5×10^6 BM-MSC @ 12 h via tail vein	Rat bleomycin	↓ Hydroxyproline and pro-fibrotic cytokines	Yes
Moodley et al. [77]	Xenogeneic umbilical cord-derived MSC 1×10^6 @ 1 day	Mouse bleomycin	↓ Hydroxyproline, collagen and pro-fibrotic cytokines	Yes, only in fibrotic areas
Bitencourt et al. [78]	Autologous MSC engraftment encouraged by hyaluronidase	Mouse bleomycin	↓ Collagen content and fibrotic score	Yes
Choi et al. [79]	Xenogeneic BM-MSC 2×10^5 IV or microvesicles @ 12 and 14 weeks	Mouse silica	↓ Collagen content and fibrotic score, more pronounced with MSC	Yes + ATII differentiation
Yan et al. [51]	Isogeneic BM-MSC 2×10^5 IV @ 0, 60, 120 days	Mouse radiation	↑ Fibrosis with late delivery	Yes
Jun et al. [39]	Isogeneic Lung MSC (Hoechst) 2.5×10^5 IV day 0	Mouse bleomycin	Bleomycin causes lung MSC depletion with repletion attenuating fibrosis	No—?rescue of lung resident MSC

currently unmet. This evidence base is well summarised in Sinclair et al. [11] and so will not be repeated here, but is significant for the increasingly common (as survival following extreme preterm birth continues to improve) bronchopulmonary dysplasia [40] and also for pulmonary hypertension [82–84]. With respect to bronchopulmonary dysplasia it is noteworthy that these babies are often left with obstructive lung disease which manifests in adulthood as emphysema [85]. Amongst the preclinical studies in pulmonary hypertension, of particular note are the studies which demonstrated that genetic engineering of MSCs, for instance to overexpress heme oxygenase [83] or prostacyclin [84], conferred enhanced efficacy, perhaps providing a glimpse of the future of the field.

Human Studies of MSC Therapy in Lung Disease

The multifaceted activity of MSC has translated into a large body of clinical trial activity outside the lung, most notably in the treatment of steroid refractory graft versus host disease following allogeneic bone-marrow transplant, but also in other

immune-mediated diseases like Crohn's disease, multiple sclerosis, lupus and in the renal transplant setting [28]. The tissue repair capability of MSCs is being investigated in clinical trials for cardiac repair, bone disorders (osteogenesis imperfecta), bone fracture and following meniscectomy. As a result, many thousands of human subjects have received intravenous MSC therapy with very few adverse effects [86], providing key safety data for moving to clinical trials in lung disease. While relatively few human trials are underway for patients with lung disease, and while even fewer have been published, the diseases which have been targeted reflect MSC biology, the strength of the preclinical data, and the seriousness and lack of availability of alternate treatments for the target disease.

Chronic obstructive pulmonary disease: Weiss et al. conducted what remains the largest trial in humans with lung disease using allogeneic bone-marrow-derived MSCs [53]. They randomised 62 patients with COPD to receive 4 monthly intravenous infusions of either MSCs (100×10^6 cells) or vehicle control in a double-blind manner. Patients were followed for 2 years. There were no infusional toxicities and no treatment-related deaths or serious adverse events. Although MSC treatment was not associated with any improvement in the efficacy measures (lung function, walk distance, or dyspnea score), this study does provide excellent safety data in humans with moderate-to-severe lung disease [53]. Another study targeting emphysema is currently listed as recruiting (NCT01849159, www.clinicaltrials.gov, accessed 20th Feb 2015).

Obliterative bronchiolitis: Recruitment to a phase I trial of MSC therapy for post-transplant obliterative bronchiolitis has recently been completed (http://clinicaltrials.gov/ct2/show/NCT01175655). In this study ten patients with moderate or severe chronic lung allograft dysfunction received allogeneic bone-marrow-derived MSCs (2×10^6 cells/kg twice weekly for 2 weeks) and will be followed for 1 year. MSC therapy was feasible and appeared well tolerated in the short-term, with long-term results awaited [32]. A similar Phase 1 study has recently commenced recruitment (NCT02181712) in the United States. A Phase 2 study is now being planned in Australia.

IPF: The short-term safety of MSC treatment in IPF was recently confirmed in a Phase 1 study of intravenous, allogeneic, placenta-derived MSC in moderate-severely affected patients [52]. In this study, eight patients were treated with 1–2×10^6 MSCs/kg and were followed for 6 months. There was no evidence of acute hemodynamic or gas exchange compromise and no evidence of worsening fibrosis [52]. In the only other published human study, 14 patients with IPF received 0.5×10^6/kg autologous adipose-derived stromal cells endobronchially. No adverse effects of this treatment were seen. Two other clinical trials are ongoing in IPF. A US trial with very similar design to the study by Chambers et al. but involving intravenous delivery of allogeneic bone-marrow-derived MSCs, has almost completed recruitment (NCT02013700, M. Glassberg personal communication) while the other trial (NCT01919827) involves the non-randomised endobronchial delivery of allogeneic adipose-derived MSCs.

ALI: No studies have been published for MSC treatment of ALI, but there are currently two randomised clinical trials and one non-randomised trial underway.

The first (NCT01775774) is a dose-escalation (1×10^6, 5×10^6, and 10×10^6 cells/kg) study delivering allogeneic bone-marrow-derived MSCs to three cohorts of patients ($n = 3$/cohort). The other randomised, double-blind, placebo-controlled trial (NCT01902082) delivers allogeneic adipose-derived MSCs. The inclusion criteria are similar for these two studies. In the third non-randomised study, patients with viral infection-induced and extra-corporeal membrane oxygenation-dependent ALI will receive open-label allogeneic bone-marrow-derived MSCs (NCT02215811). In all three studies MSCs are delivered intravenously.

Other disease targets: The only other clinical trials of MSC therapy for lung disease listed as active at www.clinicaltrials.gov are in bronchopulmonary dysplasia, where a Phase 1 study has recently reported promising results [87]; in pulmonary hypertension (NCT01795950) where a Phase 1 study involves three dosing cohorts of $0.5–2 \times 10^6$ placenta-derived MSCs/kg delivered intravenously; and in radiation-induced lung fibrosis (NCT02277145).

Conclusions

Together, this preclinical and clinical trial activity showcases the diversity and potential of MSCs in regenerative medicine. MSCs have been shown to be much more than a cellular population with immune-suppressive activity and multipotent capacity. MSCs can also promote tissue repair by regulating the functionality of tissue stem cell pools and rescuing aged, senescent or damaged tissue. While a number of lung diseases, most notably asthma, are now able to be relatively safely and effectively treated due to significant improvements in available pharmacologics, substantial therapeutic gaps remain, particularly for degenerative and fibrotic lung diseases. It is pleasing to think that a deeper understanding of the role of tissue-resident stem cells in the maintenance of lung health, and hence the development of pharmacologic and/or cell therapies aimed at restoring that health, may one day fill these gaps. However in order for this promise to be achieved safely, and in order to avoid a repetition of the problematic headlong introduction of gene therapies in large-scale clinical trials [88], a deeper understanding of basic MSC biology is needed, alongside the careful conduct of early phase human trials. The elucidation of the interrelationships between epithelial and MSC hierarchies; the role of MSCs in the construction of lung stem cell niches; and the dynamics of the cellular interactions within these niches are key to achieving this objective.

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