FIFTY YEARS OF RESEARCH IN ARDS

Cell-based Therapy for Acute Respiratory Distress Syndrome

Biology and Potential Therapeutic Value

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Abstract

On the basis of several preclinical studies, cell-based therapy has emerged as a potential new therapeutic for acute respiratory distress syndrome (ARDS). Of the various cell-based therapy options, mesenchymal stem/stromal cells (MSCs) from bone marrow, adipose tissue, and umbilical cord have the most experimental data to support their potential efficacy for lung injury from both infectious and noninfectious causes. Mechanistically, MSCs exert their beneficial effects by release of paracrine factors, microvesicles, and transfer of mitochondria, all of which have antiinflammatory and pro-resolving effects on injured lung endothelium and alveolar epithelium, including enhancing the resolution of pulmonary edema by up-regulating sodium-dependent alveolar fluid clearance. MSCs also have antimicrobial effects mediated by release of antimicrobial factors and by up-regulating monocyte/macrophage phagocytosis. Phase 2a clinical trials to establish safety in ARDS are in progress, and two phase 1 trials did not report any serious adverse events. Several issues need further study, including: determining the optimal methods for large-scale production, reconstitution of cryopreserved cells for clinical use, defining cell potency assays, and determining the therapeutic potential of conditioned media derived from MSCs. Because ARDS is a heterogeneous syndrome, targeting MSCs to patients with ARDS with a more hyperinflammatory endotype may further enhance their potential for efficacy.

Keywords: mesenchymal stromal cells; pulmonary edema; acute lung injury

Acute respiratory distress syndrome (ARDS) constitutes a syndrome of acute hypoxemic respiratory failure that develops primarily from an increase in lung endothelial and epithelial permeability (1, 2). It accounts for more than 10% of intensive care unit (ICU) admissions worldwide, develops in up to 25% of patients receiving mechanical ventilation in the ICU, and has a mortality rate of approximately 30 to 40% (2, 3), although some of the mortality can be attributed to coexisting morbidities and not just to the respiratory failure itself. ARDS develops in response to multiple predisposing factors, including pneumonia, systemic sepsis, and major surgery or multiple trauma, with pulmonary and extrapulmonary sepsis accounting for 75% of all predisposing causes of ARDS (1). ARDS also confers a considerable long-term disability burden, with only 50% of survivors able to return to work at 1 year, whereas cognitive, psychological, and physical morbidity persist for up to 5 years (4).

The pathologic hallmark of ARDS is diffuse alveolar damage, with injury to both the lung endothelium and epithelium (2). Damage to the lung parenchyma results from multiple mechanisms, including direct injury by the inciting agent (e.g., bacteria and their products, viral invasion, acid injury after aspiration), by injury resulting from activation of the immune system, and by mechanical stretch–induced injury induced by mechanical ventilation (2). Immune system activation results in the release of several proinflammatory proteins and lipids and a predominantly neutrophilic influx into the alveolar space (2), leading to the generation of reactive oxygen species and other factors that can disrupt the alveolar–capillary barrier, resulting in protein-rich pulmonary edema and surfactant inactivation. Physiologically, ARDS is characterized by hypoxemia from ventilation–perfusion mismatch and intrapulmonary shunt and

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an increase in the pulmonary dead space, resulting in the need for positive pressure ventilation (2).

Lack of Therapies for ARDS

Despite 5 decades of research, there is no disease-modifying therapy for ARDS, and management remains supportive. Improved survival from ARDS over the past decade has been achieved with the use of lung-protective ventilation as well as prone positioning and the use of muscle relaxants in moderate to severe ARDS. After resolution of shock, fluid-conservative management increases the number of ventilator-free days (2). The complexity and diversity of the injury mechanisms, coupled with the failure of multiple clinical trials of pharmacologic therapies for ARDS (5), suggest that targeting an intervention to a single mediator or pathway may not be sufficient to achieve a therapeutic effect. In support of this perspective, the success of lung-protective ventilation was probably explained by multiple mechanisms of therapeutic benefit for reducing lung injury and enhancing repair with low tidal volume and airway pressure–limited ventilation (2). In addition, given the heterogeneity of ARDS, identification of ARDS subphenotypes or endotypes (6) may facilitate focusing of therapeutic interventions in ARDS subpopulations who are most likely to benefit. Alternatively, novel therapeutics that can target multiple mechanisms of injury, maintain or augment host defenses to pathogens, and facilitate the lung repair process are promising. In this regard, cell-based therapies with mesenchymal stem/stromal cells (MSCs) might fit this paradigm, with favorable results in preclinical models, and encouraging early-phase data suggesting safety and tolerability of MSC-based therapy in patients with ARDS. This Perspective focuses on these approaches as potential therapeutic agents for ARDS.

Stem Cells

Stem Cell Classification

There is a hierarchy of "stemness," from pluripotent cells, to multipotent cells, to progenitor cells, where the capacity to differentiate into different cell lineages is progressively reduced. Another important classification relates to their tissue source, whether they are derived from embryonic or adult tissues, and in the latter case, which specific tissues they originate from. The development of induced pluripotent cells, achieved by the reprogramming of mature adult cells (usually fibroblasts) via the overexpression of four primitive transcription factors, to produce cells with similar differentiation potential to embryonic stem cells, has blurred this distinction (7). Adult-derived stem cells, such as MSCs, have a much more limited differentiation potential (primarily *in vitro*), but they can modulate the immune response to tissue injury and facilitate repair. "Progenitor" cells, such as endothelial progenitor cells, which are circulating cells involved in the repair of endothelial cells and formation of new blood vessels, have a more limited differentiation capacity, being restricted to a single lineage. A prior publication in the *Journal* provided more detail on stem cell classifications and challenges in this field (8).

Cell-based Therapeutic Potential

Significant preclinical data support the therapeutic potential of several stem cell types for ARDS. Embryonic stem cells have been differentiated to produce functional airway epithelium (9), whereas MSCs derived from ESCs attenuate murine endotoxin and bleomycin–induced lung injury (10). Human induced pluripotent stem (iPS) cells can also generate functional respiratory cells (11), whereas in preclinical studies, iPS cells decrease cold ischemic injury in *ex vivo* perfused human lungs (12), and the iPS secretome (13) reduces rodent bleomycin-induced lung injury. Endothelial progenitor cells also show promise in several preclinical models, including endotoxin lung injury (14, 15). MSCs have been the most extensively studied in preclinical studies of acute lung injury, with more support for their potential as a treatment for ARDS.

MSCs

MSCs were originally discovered in the bone marrow, and criteria for identifying them focused on the presence and absence of cell-specific markers, adherence to plastic, and their *in vitro* differentiation potential (16). MSCs have a number of advantages. First, they express minimal immunogenicity because they have low expression of major histocompatibility antigens, which in turn permits allogeneic therapy without immunosuppression. Second, they can be stably expanded *in vitro* while maintaining an undifferentiated state, and it is feasible to generate sufficient quantities for clinical use and cryopreserve them before clinical use. Third, they exert multiple effects on the host immune response to injury, while maintaining or augmenting the host response to pathogens and facilitating tissue repair. Fourth, they are available for several tissue sources, including bone marrow, adipose tissue, umbilical cord, the placenta, and the pancreas (17, 18). MSCs also have an excellent safety record (search mesenchymal stem cell in clinicaltrials.gov); they are in clinical studies for a wide range of disease processes and are approved for clinical use for patients with steroid-resistant graft-versus-host disease in a number of countries, including Canada and Japan.

Mechanisms of Action of MSCs

Immune Modulation

MSCs can alter the behavior of both adaptive and innate immune cells and thereby reduce the deleterious effects of inflammation (Figure 1A). MSCs reduce proinflammatory cytokine responses to injury and enhance antiinflammatory cytokines, such as IL-10, in both infectious (19, 20) and sterile models of lung injury and repair (21–23). In a trauma model of unilateral lung contusion injury followed by hemorrhagic shock, MSCs decreased injury in part by increasing the number of regulatory T cells in the peripheral blood in rats (24). MSCs alter the polarization of alveolar macrophages to the M2-like (antiinflammatory or pro-repair) phenotype (25, 26).

Enhanced Bacterial Clearance

MSCs enhance the phagocytic ability of macrophages in bacterial pneumonia and peritoneal sepsis in mice and in an *ex vivo* perfused human lung preparation (26–28).

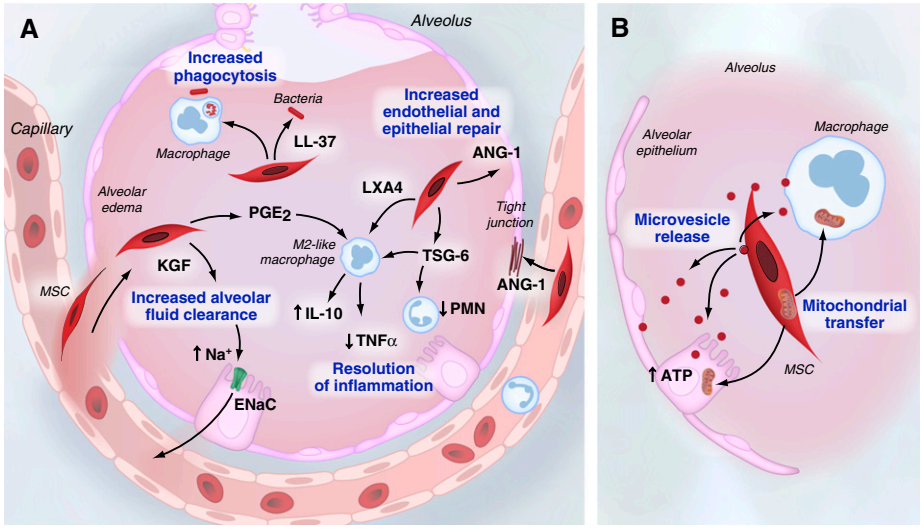

Figure 1. (*A*) The pathways by which mesenchymal stem/stromal cells (MSCs) can reduce lung injury are illustrated in this diagram of an injured alveolus filled with protein-rich alveolar edema fluid. Increased endothelial and epithelial repair can be mediated by release of angiopoietin-1 (ANG-1), tumor necrosis factor (TNF)-stimulated gene 6 protein (TSG-6), and lipoxin A_4 (LXA4), by reducing neutrophil influx, decreasing neutrophil activation, and direct effects on improving endothelial and epithelial barrier properties. Resolution of inflammation can be enhanced by the increased release of IL-10 and decreased release of proinflammatory molecules such as TNF-α, mechanisms that are mediated by LXA4 and prostaglandin E2 (PGE_2). Increased alveolar fluid clearance can be stimulated by the release of keratinocyte growth factor (KGF) by enhancing sodium-dependent alveolar fluid clearance through epithelial sodium channel (ENaC). Increased phagocytosis can be mediated by the transfer of microvesicles to macrophages from MSCs and also by release of the antimicrobial peptide β-cathelicidin (LL-37). (*B*) This illustration is intended to emphasize the capacity of MSCs to transfer mitochondria to injured alveolar epithelial cells, resulting in an increase in ATP, which can improve bioenergetics and increase alveolar epithelial function, improving surfactant release and potentially increasing alveolar fluid clearance. MSCs also release microvesicles (and exosomes not shown here) that can carry biologically active material to alveolar epithelium and macrophages (*see* text for further explanation). PMN = polymorphonuclear neutrophil.

MSCs increased *Escherichia coli* bacterial clearance and enhanced survival in rats (19).

Injury and Inflammation Resolution

Both rodent (21, 22) and human (23) bone marrow–derived MSCs (hMSCs) enhanced repair after ventilator-induced lung injury, enhancing resolution of inflammation and restoration of lung function and structure. MSCs also increased recovery from bleomycin-induced lung injury in mice (29).

Restoration of Capillary Barrier Function

The MSC secretome or conditioned media from MSCs prevented an increase in alveolar epithelial permeability and injury by restoring vectorial sodium and fluid transport in injured rat alveolar epithelial cells (30), repaired cytokine-injured human alveolar epithelial type 2 cell monolayers by release of angiopoietin-1 (31), and enhanced alveolar fluid clearance in an *ex vivo* endotoxin-injured human lung model, in part by release of keratinocyte growth factor (KGF) (27).

How MSCs Produce Their Effects

Paracrine Mediators

Multiple MSC effects are mediated by paracrine factors. The MSC-conditioned media protected rats from bleomycin injury by alleviating inflammatory markers and apoptosis in the lung, reducing collagen deposits and fibrosis (32). MSCs accelerated the resolution of endotoxin-induced lung injury in mice partly by the release of the pro-resolving lipid mediator lipoxin A_4, a molecule derived from arachidonic acid (33). The effects of MSCs on resolution of ventilator-induced lung injury may be dependent on KGF secretion (22). The secretion of antimicrobial peptides, such as LL-37, contributes to the antimicrobial effects of MSCs (28). These pathways are summarized in Figure 1A.

MSC-derived Microvesicles

MSC-derived microvesicles (MSC-MVs) and exosomes can transfer cellular contents, including mitochondria, mRNA, and microRNA, to injured cells (34). MVs derived from hMSCs reduced lung injury, reduced bacterial load, and improved survival in murine *E. coli* pneumonia (34) (Figure 1B). MSC-MVs decreased LPS-induced lung injury in mice in part via a KGF-dependent mechanism (35). MSC-MVs promoted alveolar fluid clearance and improved airway compliance in *E. coli*–injured *ex vivo* human lungs, effects blocked with anti-CD44 treatment (36).

MSC-Dependent Effects

Some effects of MSCs appear to require the cells to be present. Early recovery from ventilator-induced lung injury was enhanced by MSC therapy to a greater extent than with the MSC secretome (37). There is also evidence that exosomes from MSCs can prevent neonatal lung injury in mice (38).

Mitochondrial Transfer

MSCs can also transfer mitochondria to injured alveolar epithelium (Figure 1B), an effect that reduced endotoxin-induced lung injury and increased survival in mice, perhaps by a connexin-43–dependent mechanism that enhanced intracellular ATP levels in the injured epithelial cells (39). More recent work has shown that mitochondrial transfer occurs from MSCs to macrophages (40), in part via tunneling nanotubes (41), and this transfer can enhance macrophage phagocytosis.

Recent Translational and Clinical Studies

Preclinical studies have focused on validating mechanistic work in clinically relevant models of ARDS with hMSCs, in an effort to clearly test the clinical translation potential and guide early-phase clinical trial design.

Bacterial and Viral Infection

More than 75% of ARDS develops from pulmonary or systemic sepsis (3). MSCs can exert direct antibacterial effects via secretion of antimicrobial peptides (28) and modulate the immune system to enhance their effectiveness in countering bacterial

infection. Bone marrow–derived hMSCs reduced lung injury, decreased bacterial load, and improved survival in rats with *E. coli*–induced pneumonia (19). Also, cryopreserved, clinical-grade bone marrow–derived hMSCs reduced bacterial-induced injury to the alveolar epithelium and enhanced *E. coli* clearance in an *ex vivo* perfused human lung preparation (27). The effects of MSCs in viral-induced lung injury appear to depend on the specific viral strain. MSCs significantly attenuated H9N2 avian influenza virus–induced acute lung injury and inflammation in mice (42), and they were also effective in H5N1 lung injury in mice (43). In contrast, MSCs failed to protect mice from lung injury caused by influenza A pneumonia (a mouse-adapted H1N1, PR8) (44, 45).

Large Animal Model

Bone marrow–derived hMSCs decreased endotoxin-induced acute lung injury in a sheep model (46). There was no organ toxicity (cardiovascular, liver, pancreas, and kidney) with hMSC treatment (46). To test safety and efficacy, cryopreserved, clinical-grade bone marrow–derived hMSCs were studied in a ventilated sheep model of *Pseudomonas aeruginosa* pneumonia with severe lung injury and sepsis for 24 hours. Compared with plasmalyte controls, there were no adverse effects on hemodynamics or other safety parameters, and the MSC-treated sheep had improved oxygenation (at both 5 and 10 million cells/kg) and reduced pulmonary edema (only at 10 million cells/kg) (47).

Ex Vivo Human Lung

Bone marrow–derived hMSCs or the conditioned media of MSCs can restore alveolar fluid clearance in an *ex vivo* preparation of perfused human lungs, in part by up-regulating sodium-dependent resolution of alveolar edema (48). Subsequent studies in the *ex vivo* perfused human lung showed that microvesicles from MSCs could enhance alveolar fluid clearance, an effect that was blocked by anti-CD44 antibody treatment, indicating that the CD44 receptor plays a role in the internalization of the microvesicles in the lung epithelial cells (36). These findings are supported by an experimental study in which endobronchial-delivered hMSCs improved lung compliance in lungs transplanted from non–heart-beating donors in pigs (49).

Insights from Clinical Studies

MSCs have been reported to be safe in clinical trials for several diseases (50) and are in clinical use for steroid-resistant graft-versus-host disease. For patients with ARDS from pneumonia, sepsis, or aspiration, there were no safety issues in a phase 1b dose-escalation study that tested three doses of human bone marrow–derived MSCs (1, 5, and 10 million MSCs/kg ideal body weight) given intravenously over 1 hour in nine patients with moderate to severe ARDS defined as a Pa_{O_2}/FI_{O_2} less than 200 mm Hg ventilated with at least 8 cm H_2O positive end-expiratory pressure (NCT 01775774) (51). This research group is now conducting a randomized, blinded phase 2a safety trial in 60 patients (40 treated with MSCs at 10^6 MSCs/kg ideal body weight and 20 with plasmalyte placebo; NCT 02097641). The primary endpoint for this 60-patient trial is safety; it is not powered for efficacy endpoints. The Food and Drug Administration mandated clear evidence of safety in a phase 2a ARDS trial before the conduct of a larger phase 2b trial that could be powered for respiratory endpoints. Zheng and colleagues demonstrated that a low dose of 1 million adipose-derived hMSCs/kg was well tolerated in a phase 1b study (52). Recently published trials of hMSCs for ARDS and studies in progress are summarized in Table 1.

Advancing the Therapeutic Potential of MSCs for ARDS

Better MSC Characterization

There is no single specific cell surface marker that identifies MSCs. Furthermore, there is no clear relationship between cell surface markers or functional characteristics and their therapeutic properties in various preclinical models. A recent publication from the International Society for Cellular Therapy addressed several of these issues and recommended approaches that could improve characterization of MSCs with quantitative RNA analysis of selected gene products, flow cytometry of functionally relevant surface markers, and protein-based assays of the paracrine products also known as the secretome (18).

Alternative MSC Sources

Although most of the preclinical and translational work has focused on bone marrow–derived MSCs, recent interest has focused on adipose- and umbilical cord–derived MSCs as potentially more plentiful MSC sources. MSCs from these different tissues exhibit differential toll-like receptor expression and immunosuppressive potential (53), which may cause differential immune-modulating effects that may be important in ARDS. Adipose tissue–derived MSCs have demonstrated efficacy in preclinical models of ischemia–reperfusion lung injury (54), radiation injury (55), and bacterial (*P. aeruginosa*) infection (56). Umbilical cord MSCs have also demonstrated efficacy in relevant preclinical models, and placenta-derived MSCs demonstrated efficacy both *in vitro* and in an endotoxin-injured perfused human lung preparation (57). Most recently, cryopreserved, xenogeneic material–free umbilical cord–derived MSCs showed comparable efficacy to bone marrow–derived MSCs (58). Both umbilical cord– and adipose-derived MSCs are being tested in early-phase clinical trials (NCT 02444455 and NCT 01902082, respectively) for ARDS.

Determining Cell Potency

A key translational step is the validation of an assay that constitutes an index of cell potency. Although there are several potential candidate potency assays, including components of the MSC secretome, none have been clearly validated yet for this purpose. Cell viability assays after thawing of cryopreserved cells before administration provide data regarding the viability but not the potency of the cells. The Food and Drug Administration normally requires cell potency assays for phase 3 trials, but more work needs to be done to develop cell potency assays in phase 2 trials. Our recommendation is that the cell lysates of MSC be tested for potency by measuring several of the best-established paracrine factors that mediate some of the benefit in acute lung injury, including KGF, angiopietin-1, tumor necrosis factor-α–stimulated gene 6 (TSG-6), and IL-1ra. There have been some experimental approaches designed to enhance the potency of MSCs, including serum deprivation, exposure to hypoxia, and transfection, although none of these strategies has been well validated in preclinical models yet (59).

Batch Heterogeneity and Cryopreservation

A challenge in translating hMSCs to clinical use in ARDS is the issue of batch-to-batch

Table 1. Selected Clinical Trials of Mesenchymal Stem/Stromal Cells for Acute Respiratory Distress Syndrome

Study	Study Type	MSC Type and Dose	Study Status/Key Findings
START (51)	Phase 1b dose escalation clinical trial in moderate to severe ARDS	Intravenous infusion of 1, 5, or 10 million hBM MSCs/kg	Study complete; all hMSC doses well tolerated. No adverse effects detected
Adipose-derived Mesenchymal Stem Cells in Acute Respiratory Distress Syndrome (NCT 01902082) (52)	Phase 1b clinical trial in patients with ARDS	Intravenous infusion of human adipose MSCs; dose 1 million/kg	Low dose of 1 million adipose-derived hMSCs/kg was well tolerated
START (NCT 02097641)	Phase 2a safety clinical trial in moderate to severe ARDS	Intravenous infusion, hBM-derived MSCs; 10 million cells/kg	Patient recruitment in progress
UCMSC-ALI (NCT 02444455)	Phase 1–2 clinical trial	Intravenous infusion, human umbilical cord–derived MSCs; 5×10^5/kg once a day, three doses	Patient recruitment in progress
A Phase 1/2 Study to Assess MultiStem Therapy in Acute Respiratory Distress Syndrome (NCT 02611609)	Phase 1–2 clinical trial	Intravenous infusion, "low" and "high" dose hBM-derived MSCs	Patient recruitment in progress
REALIST (NCT 03042143)	Phase 1–2 clinical trial	Intravenous infusion; 1, 5, or 10 million human umbilical cord–derived CD362-positive MSCs	Not yet recruiting patients
Mesenchymal Stem Cells (MSCs) for Treatment of Acute Respiratory Distress Syndrome (ARDS) in Stem Cell Transplant Patients (NCT 02804945)	Phase 2 clinical trial	Intravenous infusion; 1×10^7 cells/kg; hBM-derived MSCs	Not yet recruiting patients

Definition of abbreviations: ARDS = acute respiratory distress syndrome; hBM = human bone marrow; hMSC = human mesenchymal stem/stromal cell; MSC = mesenchymal stem/stromal cell; REALIST = Repair of Acute Respiratory Distress Syndrome by Stromal Cell Administration; START = Human Mesenchymal Stem Cells for Acute Respiratory Distress Syndrome; UCMSC-ALI = Human Umbilical-Cord–derived Mesenchymal Stem Cell Therapy in Acute Lung Injury.

MSC heterogeneity. Although cell donors are extensively screened to rule out systemic illnesses and undergo screening for a panel of infectious diseases (including HIV-1, HIV-2, hepatitis B, hepatitis C, human T-lymphotropic virus I/II, cytomegalovirus, and syphilis), other donor-related variables, such as age, may be important. MSCs from aging (murine) donors demonstrate reduced therapeutic efficacy (60). Umbilical cord MSCs may have less batch variability, as the donor conditions are more uniform, but this remains to be determined. Differences in expression of effector molecules may also be important. Lee and colleagues demonstrated that the expression of TSG-6 in MSCs paralleled efficacy *in vivo* (61). The impact of other donor-related variables, such as genetic variants, is unknown but potentially important.

Variations in production and cryopreservation methods may impart variability in the function of MSCs when tested in preclinical models or in patients for specific clinical disorders. One approach has been to thaw the cryopreserved MSCs and then suspend them in plasmalyte before administration; with this approach, the MSCs are given with the dimethyl sulfoxide (DMSO), an agent frequently used in cell cryopreservation, and without removing cell debris, a standard approach in most industry-supported trials that have used MSCs. However, concerns exist regarding the potential for DMSO to exert toxic effects on stored cells, reducing efficacy after transplantation (62). Unpublished data from the studies by Asmussen and colleagues demonstrated that hMSCs were less effective in their ovine bacterial pneumonia model (47) when given directly after thawing, compared with hMSCs that had been washed to remove DMSO before administration (S. Asmussen, personal communication). In the phase 1 and 2 ARDS clinical trials from the University of California in San Francisco, DMSO and cell debris are removed by centrifugation after the cryopreserved MSCs have been thawed and the cells suspended in plasmalyte before intravenous administration. This latter approach retained efficacy of the MSCs in both small and large animal preclinical studies of lung injury (26, 27, 51).

hMSC Dosage Regimens

Although preclinical data exist demonstrating efficacy for intravenous and intrapulmonary MSC administration, there are no clear data demonstrating the superiority of either route (21, 22). The therapeutic potential of additional hMSC doses also remains to be determined. Consequently, current clinical studies are focused on single-dose hMSC administration via the less-invasive intravenous route, with the START (Stem Cells for Acute Respiratory Distress

Syndrome) trial demonstrating safety for this approach (51).

Other Adverse Effects

The SafeCell systematic review of eight randomized controlled clinical trials of MSC therapy did not find any evidence for MSC-induced complications, including acute infusional toxicity, organ system complications, infection, death, or malignancy (63). The START trial demonstrated that doses of hMSCs up to 10 million/kg were well tolerated in patients with ARDS (51). Nevertheless, the potential remains for MSCs to exert longer-term adverse effects in patients with ARDS, such as pulmonary fibrosis, underlining the need for long-term follow-up studies in these patients.

Target ARDS Population

ARDS is a clinical syndrome rather than a disease. The clinical criteria are sensitive but lack specificity. Therefore, although the criteria are a good screening tool for ARDS, a significant proportion of patients who fulfill the clinical criteria may not actually have primarily acute lung injury. In the ARDS network trials, 29% of patients had an elevated pulmonary arterial wedge pressure greater than 18 mm Hg at the time of entry into the trial (64). In addition, recent studies that used latent class analysis of ARDS network clinical trials with measurement of plasma biomarkers and clinical variables have indicated that about 35% of patients with ARDS have a hyperinflammatory endotype, which correlated with a higher mortality and differential responses to therapy with positive end-expiratory pressure and a conservative fluid strategy (6, 65). Conceivably, patients with ARDS with a hyperinflammatory endotype might be better candidates for therapy with MSCs. As an experimental example of this hypothesis, MSCs were not effective in a mouse model of H1N1 influenza lung injury (44) but were effective in the more inflammatory H5N1 influenza mouse model of lung injury (43). Inclusion of all patients in a clinical trial to determine efficacy of MSCs may dilute any potential signal for efficacy, reducing the effective power of a clinical study and degrading statistical power and effect size estimates of clinical studies in ARDS, reducing the power of these studies to detect important differences (66).

Conclusions

Cell-based therapies offer promise for the treatment of ARDS. MSC-based therapies can be administered as an allogeneic therapy, have strong preclinical data demonstrating efficacy, and can be scaled up for clinical use. More studies are needed to reduce variability in cell batches and to identify potency assays that measure products of MSCs that are important in mediating the therapeutic effects in ARDS. MSCs are in early-phase clinical trials for safety, and the next steps should be to conduct phase 2b trials that are powered for respiratory endpoints, perhaps in patients with a more proinflammatory endotype. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

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References

1. Ranieri VM, Rubenfeld GD, Thompson BT, Ferguson ND, Caldwell E, Fan E, Camporota L, Slutsky AS; ARDS Definition Task Force. Acute respiratory distress syndrome: the Berlin Definition. *JAMA* 2012;307: 2526–2533.
2. Matthay MA, Ware LB, Zimmerman GA. The acute respiratory distress syndrome. *J Clin Invest* 2012;122:2731–2740.
3. Bellani G, Laffey JG, Pham T, Fan E, Brochard L, Esteban A, Gattinoni L, van Haren F, Larsson A, McAuley DF, *et al*.; LUNG SAFE Investigators; ESICM Trials Group. Epidemiology, patterns of care, and mortality for patients with acute respiratory distress syndrome in intensive care units in 50 countries. *JAMA* 2016;315:788–800.
4. Herridge MS, Tansey CM, Matté A, Tomlinson G, Diaz-Granados N, Cooper A, Guest CB, Mazer CD, Mehta S, Stewart TE, *et al*.; Canadian Critical Care Trials Group. Functional disability 5 years after acute respiratory distress syndrome. *N Engl J Med* 2011;364: 1293–1304.
5. Tonelli AR, Zein J, Adams J, Ioannidis JP. Effects of interventions on survival in acute respiratory distress syndrome: an umbrella review of 159 published randomized trials and 29 meta-analyses. *Intensive Care Med* 2014;40:769–787.
6. Calfee CS, Delucchi K, Parsons PE, Thompson BT, Ware LB, Matthay MA; NHLBI ARDS Network. Subphenotypes in acute respiratory distress syndrome: latent class analysis of data from two randomised controlled trials. *Lancet Respir Med* 2014;2:611–620.
7. Takahashi K, Yamanaka S. A decade of transcription factor-mediated reprogramming to pluripotency. *Nat Rev Mol Cell Biol* 2016;17:183–193.
8. Kotton DN. Next-generation regeneration: the hope and hype of lung stem cell research. *Am J Respir Crit Care Med* 2012;185:1255–1260.
9. McIntyre BA, Alev C, Mechael R, Salci KR, Lee JB, Fiebig-Comyn A, Guezguez B, Wu Y, Sheng G, Bhatia M. Expansive generation of functional airway epithelium from human embryonic stem cells. *Stem Cells Transl Med* 2014;3:7–17.
10. Hao Q, Zhu YG, Monsel A, Gennai S, Lee T, Xu F, Lee JW. Study of bone marrow and embryonic stem cell-derived human mesenchymal stem cells for treatment of *Escherichia coli* endotoxin-induced acute lung injury in mice. *Stem Cells Transl Med* 2015;4:832–840.
11. Huang SX, Islam MN, O'Neill J, Hu Z, Yang YG, Chen YW, Mumau M, Green MD, Vunjak-Novakovic G, Bhattacharya J, *et al*. Efficient generation of lung and airway epithelial cells from human pluripotent stem cells. *Nat Biotechnol* 2014;32: 84–91.
12. La Francesca S, Ting AE, Sakamoto J, Rhudy J, Bonenfant NR, Borg ZD, Cruz FF, Goodwin M, Lehman NA, Taggart JM, *et al*. Multipotent adult progenitor cells decrease cold ischemic injury in ex vivo perfused human lungs: an initial pilot and feasibility study. *Transplant Res* 2014;3:19.
13. Gazdhar A, Grad I, Tamò L, Gugger M, Feki A, Geiser T. The secretome of induced pluripotent stem cells reduces lung fibrosis in part by hepatocyte growth factor. *Stem Cell Res Ther* 2014;5:123.
14. Rafat N, Dacho C, Kowanetz G, Betzen C, Tönshoff B, Yard B, Beck G. Bone marrow-derived progenitor cells attenuate inflammation in lipopolysaccharide-induced acute respiratory distress syndrome. *BMC Res Notes* 2014;7:613.
15. Yin X, Liang Z, Yun Y, Pei L. Intravenous transplantation of BMP2-transduced endothelial progenitor cells attenuates lipopolysaccharide-induced acute lung injury in rats. *Cell Physiol Biochem* 2015;35:2149–2158.
16. Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D, Deans R, Keating A, Prockop Dj, Horwitz E. Minimal criteria for defining multipotent mesenchymal stromal cells: the International Society for Cellular Therapy position statement. *Cytotherapy* 2006;8:315–317.
17. Kode JA, Mukherjee S, Joglekar MV, Hardikar AA. Mesenchymal stem cells: immunobiology and role in immunomodulation and tissue regeneration. *Cytotherapy* 2009;11:377–391.

18. Galipeau J, Krampera M, Barrett J, Dazzi F, Deans RJ, DeBruijn J, Dominici M, Fibbe WE, Gee AP, Gimble JM, *et al*. International Society for Cellular Therapy perspective on immune functional assays for mesenchymal stromal cells as potency release criterion for advanced phase clinical trials. *Cytotherapy* 2016;18:151–159.
19. Devaney J, Horie S, Masterson C, Elliman S, Barry F, O'Brien T, Curley GF, O'Toole D, Laffey JG. Human mesenchymal stromal cells decrease the severity of acute lung injury induced by E. coli in the rat. *Thorax* 2015;70:625–635.
20. Nemeth K, Keane-Myers A, Brown JM, Metcalfe DD, Gorham JD, Bundoc VG, Hodges MG, Jelinek I, Madala S, Karpati S, *et al*. Bone marrow stromal cells use TGF-beta to suppress allergic responses in a mouse model of ragweed-induced asthma. *Proc Natl Acad Sci USA* 2010;107:5652–5657.
21. Curley GF, Ansari B, Hayes M, Devaney J, Masterson C, Ryan A, Barry F, O'Brien T, Toole DO, Laffey JG. Effects of intratracheal mesenchymal stromal cell therapy during recovery and resolution after ventilator-induced lung injury. *Anesthesiology* 2013;118:924–932.
22. Curley GF, Hayes M, Ansari B, Shaw G, Ryan A, Barry F, O'Brien T, O'Toole D, Laffey JG. Mesenchymal stem cells enhance recovery and repair following ventilator-induced lung injury in the rat. *Thorax* 2012;67:496–501.
23. Hayes M, Masterson C, Devaney J, Barry F, Elliman S, O'Brien T, O'Toole D, Curley GF, Laffey JG. Therapeutic efficacy of human mesenchymal stromal cells in the repair of established ventilator-induced lung injury in the rat. *Anesthesiology* 2015;122:363–373.
24. Gore AV, Bible LE, Song K, Livingston DH, Mohr AM, Sifri ZC. Mesenchymal stem cells increase T-regulatory cells and improve healing following trauma and hemorrhagic shock. *J Trauma Acute Care Surg* 2015;79:48–52. [Discussion, p. 52.]
25. Curley GF, McAuley DF. Stem cells for respiratory failure. *Curr Opin Crit Care* 2015;21:42–49.
26. Krasnodembskaya A, Samarani G, Song Y, Zhuo H, Su X, Lee JW, Gupta N, Petrini M, Matthay MA. Human mesenchymal stem cells reduce mortality and bacteremia in gram-negative sepsis in mice in part by enhancing the phagocytic activity of blood monocytes. *Am J Physiol Lung Cell Mol Physiol* 2012;302:L1003–L1013.
27. Lee JW, Krasnodembskaya A, McKenna DH, Song Y, Abbott J, Matthay MA. Therapeutic effects of human mesenchymal stem cells in ex vivo human lungs injured with live bacteria. *Am J Respir Crit Care Med* 2013;187:751–760.
28. Krasnodembskaya A, Song Y, Fang X, Gupta N, Serikov V, Lee JW, Matthay MA. Antibacterial effect of human mesenchymal stem cells is mediated in part from secretion of the antimicrobial peptide LL-37. *Stem Cells* 2010;28:2229–2238.
29. Ortiz LA, Dutreil M, Fattman C, Pandey AC, Torres G, Go K, Phinney DG. Interleukin 1 receptor antagonist mediates the antiinflammatory and antifibrotic effect of mesenchymal stem cells during lung injury. *Proc Natl Acad Sci USA* 2007;104:11002–11007.
30. Goolaerts A, Pellan-Randrianarison N, Larghero J, Vanneaux V, Uzunhan Y, Gille T, Dard N, Planès C, Matthay MA, Clerici C. Conditioned media from mesenchymal stromal cells restore sodium transport and preserve epithelial permeability in an in vitro model of acute alveolar injury. *Am J Physiol Lung Cell Mol Physiol* 2014;306:L975–L985.
31. Fang X, Neyrinck AP, Matthay MA, Lee JW. Allogeneic human mesenchymal stem cells restore epithelial protein permeability in cultured human alveolar type II cells by secretion of angiopoietin-1. *J Biol Chem* 2010;285:26211–26222.
32. Shen Q, Chen B, Xiao Z, Zhao L, Xu X, Wan X, Jin M, Dai J, Dai H. Paracrine factors from mesenchymal stem cells attenuate epithelial injury and lung fibrosis. *Mol Med Rep* 2015;11:2831–2837.
33. Fang X, Abbott J, Cheng L, Colby JK, Lee JW, Levy BD, Matthay MA. Human mesenchymal stem (stromal) cells promote the resolution of acute lung injury in part through lipoxin A4. *J Immunol* 2015;195:875–881.
34. Monsel A, Zhu YG, Gennai S, Hao Q, Hu S, Rouby JJ, Rosenzwajg M, Matthay MA, Lee JW. Therapeutic effects of human mesenchymal stem cell-derived microvesicles in severe pneumonia in mice. *Am J Respir Crit Care Med* 2015;192:324–336.
35. Zhu YG, Feng XM, Abbott J, Fang XH, Hao Q, Monsel A, Qu JM, Matthay MA, Lee JW. Human mesenchymal stem cell microvesicles for treatment of *Escherichia coli* endotoxin-induced acute lung injury in mice. *Stem Cells* 2014;32:116–125.
36. Gennai S, Monsel A, Hao Q, Park J, Matthay MA, Lee JW. Microvesicles derived from human mesenchymal stem cells restore alveolar fluid clearance in human lungs rejected for transplantation. *Am J Transplant* 2015;15:2404–2412.
37. Hayes M, Curley GF, Masterson C, Devaney J, O'Toole D, Laffey JG. Mesenchymal stromal cells are more effective than the MSC secretome in diminishing injury and enhancing recovery following ventilator-induced lung injury. *Intensive Care Med Exp* 2015;3:29.
38. Mitsialis SA, Kourembanas S. Stem cell-based therapies for the newborn lung and brain: possibilities and challenges. *Semin Perinatol* 2016;40:138–151.
39. Islam MN, Das SR, Emin MT, Wei M, Sun L, Westphalen K, Rowlands DJ, Quadri SK, Bhattacharya S, Bhattacharya J. Mitochondrial transfer from bone-marrow-derived stromal cells to pulmonary alveoli protects against acute lung injury. *Nat Med* 2012;18:759–765.
40. Phinney DG, Di Giuseppe M, Njah J, Sala E, Shiva S, St Croix CM, Stolz DB, Watkins SC, Di YP, Leikauf GD, *et al*. Mesenchymal stem cells use extracellular vesicles to outsource mitophagy and shuttle microRNAs. *Nat Commun* 2015;6:8472.
41. Jackson MV, Morrison TJ, Doherty DF, McAuley DF, Matthay MA, Kissenpfennig A, O'Kane CM, Krasnodembskaya AD. Mitochondrial transfer via tunneling nanotubes is an important mechanism by which mesenchymal stem cells enhance macrophage phagocytosis in the in vitro and in vivo models of ARDS. *Stem Cells* 2016;34:2210–2223.
42. Li Y, Xu J, Shi W, Chen C, Shao Y, Zhu L, Lu W, Han X. Mesenchymal stromal cell treatment prevents H9N2 avian influenza virus-induced acute lung injury in mice. *Stem Cell Res Ther* 2016;7:159.
43. Chan MC, Kuok DI, Leung CY, Hui KP, Valkenburg SA, Lau EH, Nicholls JM, Fang X, Guan Y, Lee JW, *et al*. Human mesenchymal stromal cells reduce influenza A H5N1-associated acute lung injury in vitro and in vivo. *Proc Natl Acad Sci USA* 2016;113:3621–3626.
44. Gotts JE, Abbott J, Matthay MA. Influenza causes prolonged disruption of the alveolar-capillary barrier in mice unresponsive to mesenchymal stem cell therapy. *Am J Physiol Lung Cell Mol Physiol* 2014;307:L395–L406.
45. Darwish I, Banner D, Mubareka S, Kim H, Besla R, Kelvin DJ, Kain KC, Liles WC. Mesenchymal stromal (stem) cell therapy fails to improve outcomes in experimental severe influenza. *Plos One* 2013;8:e71761.
46. Rojas M, Cárdenes N, Kocyildirim E, Tedrow JR, Cáceres E, Deans R, Ting A, Bermúdez C. Human adult bone marrow-derived stem cells decrease severity of lipopolysaccharide-induced acute respiratory distress syndrome in sheep. *Stem Cell Res Ther* 2014;5:42.
47. Asmussen S, Ito H, Traber DL, Lee JW, Cox RA, Hawkins HK, McAuley DF, McKenna DH, Traber LD, Zhuo H, *et al*. Human mesenchymal stem cells reduce the severity of acute lung injury in a sheep model of bacterial pneumonia. *Thorax* 2014;69:819–825.
48. McAuley DF, Curley GF, Hamid UI, Laffey JG, Abbott J, McKenna DH, Fang X, Matthay MA, Lee JW. Clinical grade allogeneic human mesenchymal stem cells restore alveolar fluid clearance in human lungs rejected for transplantation. *Am J Physiol Lung Cell Mol Physiol* 2014;306:L809–L815.
49. Wittwer T, Rahmanian P, Choi YH, Zeriouh M, Karavidic S, Neef K, Christmann A, Piatkowski T, Schnapper A, Ochs M, *et al*. Mesenchymal stem cell pretreatment of non-heart-beating-donors in experimental lung transplantation. *J Cardiothorac Surg* 2014;9:151.
50. Walter J, Ware LB, Matthay MA. Mesenchymal stem cells: mechanisms of potential therapeutic benefit in ARDS and sepsis. *Lancet Respir Med* 2014;2:1016–1026.
51. Wilson JG, Liu KD, Zhuo H, Caballero L, McMillan M, Fang X, Cosgrove K, Vojnik R, Calfee CS, Lee JW, *et al*. Mesenchymal stem (stromal) cells for treatment of ARDS: a phase 1 clinical trial. *Lancet Respir Med* 2015;3:24–32.
52. Zheng G, Huang L, Tong H, Shu Q, Hu Y, Ge M, Deng K, Zhang L, Zou B, Cheng B, *et al*. Treatment of acute respiratory distress syndrome with allogeneic adipose-derived mesenchymal stem cells: a randomized, placebo-controlled pilot study. *Respir Res* 2014;15:39.
53. Raicevic G, Najar M, Stamatopoulos B, De Bruyn C, Meuleman N, Bron D, Toungouz M, Lagneaux L. The source of human mesenchymal stromal cells influences their TLR profile as well as their functional properties. *Cell Immunol* 2011;270:207–216.

54. Sun CK, Leu S, Hsu SY, Zhen YY, Chang LT, Tsai CY, Chen YL, Chen YT, Tsai TH, Lee FY, *et al*. Mixed serum-deprived and normal adipose-derived mesenchymal stem cells against acute lung ischemia-reperfusion injury in rats. *Am J Transl Res* 2015;7:209–231.
55. Jiang X, Jiang X, Qu C, Chang P, Zhang C, Qu Y, Liu Y. Intravenous delivery of adipose-derived mesenchymal stromal cells attenuates acute radiation-induced lung injury in rats. *Cytotherapy* 2015;17: 560–570.
56. Mao Y-X, Xu J-F, Seeley EJ, Tang X-D, Xu L-L, Zhu Y-G, Song Y-L, Qu J-M. Adipose tissue-derived mesenchymal stem cells attenuate pulmonary infection caused by pseudomonas aeruginosa via inhibiting overproduction of prostaglandin E2. *Stem Cells* 2015;33: 2331–2342.
57. Nazarov I, Lee JW, Soupene E, Etemad S, Knapik D, Green W, Bashkirova E, Fang X, Matthay MA, Kuypers FA, *et al.* Multipotent stromal stem cells from human placenta demonstrate high therapeutic potential. *Stem Cells Transl Med* 2012;1:359–372.
58. Curley GF, Jerkic M, Dixon S, Hogan G, Masterson C, O'Toole D, Devaney J, Laffey JG. Cryopreserved, xeno-free human umbilical cord mesenchymal stromal cells reduce lung injury severity and bacterial burden in rodent *Escherichia coli*-induced acute respiratory distress syndrome. *Crit Care Med* 2017;45:e202–e212.
59. Sung PH, Chang CL, Tsai TH, Chang LT, Leu S, Chen YL, Yang CC, Chua S, Yeh KH, Chai HT, *et al*. Apoptotic adipose-derived mesenchymal stem cell therapy protects against lung and kidney injury in sepsis syndrome caused by cecal ligation puncture in rats. *Stem Cell Res Ther* 2013;4:155.
60. Bustos ML, Huleihel L, Kapetanaki MG, Lino-Cardenas CL, Mroz L, Ellis BM, McVerry BJ, Richards TJ, Kaminski N, Cerdenes N, *et al*. Aging mesenchymal stem cells fail to protect because of impaired migration and antiinflammatory response. *Am J Respir Crit Care Med* 2014;189:787–798.
61. Lee RH, Yu JM, Foskett AM, Peltier G, Reneau JC, Bazhanov N, Oh JY, Prockop DJ. TSG-6 as a biomarker to predict efficacy of human mesenchymal stem/progenitor cells (hMSCs) in modulating sterile inflammation in vivo. *Proc Natl Acad Sci USA* 2014;111: 16766–16771.
62. Ruiz-Delgado GJ, Mancías-Guerra C, Tamez-Gómez EL, Rodríguez-Romo LN, López-Otero A, Hernández-Arizpe A, Gómez-Almaguer D, Ruiz-Argüelles GJ. Dimethyl sulfoxide-induced toxicity in cord blood stem cell transplantation: report of three cases and review of the literature. *Acta Haematol* 2009;122:1–5.
63. Lalu MM, McIntyre L, Pugliese C, Fergusson D, Winston BW, Marshall JC, Granton J, Stewart DJ; Canadian Critical Care Trials Group. Safety of cell therapy with mesenchymal stromal cells (SafeCell): a systematic review and meta-analysis of clinical trials. *Plos One* 2012; 7:e47559.
64. Wiedemann HP, Wheeler AP, Bernard GR, Thompson BT, Hayden D, deBoisblanc B, Connors AF Jr, Hite RD, Harabin AL; National Heart, Lung, and Blood Institute Acute Respiratory Distress Syndrome (ARDS) Clinical Trials Network. Comparison of two fluid-management strategies in acute lung injury. *N Engl J Med* 2006;354: 2564–2575.
65. Famous KR, Delucchi K, Ware LB, Kangelaris KN, Liu KD, Thompson BT, Calfee CS; ARDS Network. Acute respiratory distress syndrome subphenotypes respond differently to randomized fluid management strategy. *Am J Respir Crit Care Med* 2017;195:331–338.
66. Sjoding MW, Cooke CR, Iwashyna TJ, Hofer TP; Potential Effect on Clinical Study Results. Acute respiratory distress syndrome measurement error. *Ann Am Thorac Soc* 2016;13:1123–1128.