12 The Safety and Efficiency of Addressing ARDS Using Stem Cell Therapies in Clinical Trials

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12.1 ARDS

Acute Respiratory Distress Syndrome (ARDS) is a complex and debilitating disease of the lungs, which continues to have a high mortality rate and huge disease burden on patients. Incidence is rising, possibly due to greater awareness leading to more diagnoses rather than a change in the underlying rate. It arises from multiple etiologies, though pathogenic infection, termed pneumonia, is the most prevalent and widely studied. The distinct pathophysiology and rapid evolution of ARDS makes it uniquely challenging with regard to therapeutics development and, to date, no medicines are licensed for specific therapy. Antibiotics, ventilation, and other organ support remain intervention standards.

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12.2 Definition and Diagnosis

In 1967 Ashbaugh and colleagues [1] recognized a specific clinical pattern characterized by an acute onset of elevated respiratory rate, hypoxemia resistant to high FiO_2, bilateral lung infiltrates on chest X-ray in the absence of cardiogenic edema and the presence of a heterogeneous number of risk factors that can lead to the same syndrome [2]. The first formal definition of ARDS was developed at the American-European Consensus Committee in 1994 [3].

1. Acute onset.
2. Presence of bilateral infiltrates at the chest X-ray.
3. Pulmonary wedge pressure ≤ 18 mmHg or no clinical evidence of high left atrial pressure (to rule out a cardiogenic cause of lung edema).
4. Hypoxemia, regardless of the applied levels of positive end expiratory pressure.

The levels of hypoxemia were used to stratify the severity of lung injury as ALI ($PaO_2/FiO_2 \leq 300$) or ARDS ($PaO_2/FiO_2 \leq 200$). An updated and improved version was proposed in 2012 during a task force meeting of experts in Berlin, from which the last ARDS definition takes its name [4].

1. Rapid onset of symptoms that cannot be attributed to any underlying cause.
2. Bilateral infiltration of leukocytes from surrounding tissue to the airspace, as identified by chest X-ray.

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3. Exclusion of hydrostatic causes of edema leading to respiratory failure.
4. Impaired blood oxygenation as assessed by arterial:alveolar oxygenation ratio (PaO_2/FiO_2), with relative levels denoting mild (≤300), moderate (≤200), or severe (≤100) ARDS.

Beyond initial diagnosis criteria, scoring systems have also been devised to assess degree of injury, including APACHE [5] and Murray [6] scales for adults and PRISM [7] and PIM [8] scales for pediatric patients. It was subsequently shown that the Berlin definition criteria of ARDS are adaptable also for the pediatric population [9, 10].

ARDS can arise from pneumonia, sepsis, and overaggressive ventilation strategies, while other less common causes include smoke inhalation, near-drowning, and poisoning [11, 12]. Only very recently has an accurate picture of in-hospital ARDS incidence been attained [13, 14], although prevalence as regards to the general population is still somewhat unclear.

12.3 ARDS Management

ARDS is an acute condition, generally arising within a week of an inciting event (e.g., pneumonia) essentially occuring and is resolved over a matter of days to weeks, and has a distinct acute hyperinflammatory phase [15–17]. The high and imminent mortality means issues such as eventual chronic fibrosis development may be secondary considerations compared to immediate restoration of lung function, specifically adequate blood oxygenation. Broad-spectrum antibiotics, given as early as possible where infection is known or suspected to be present, as is support of gas exchange, usually via assisted ventilation or in more severe cases, if available, extra-corporeal membrane oxygenation (ECMO) support [18]. The core treatment of ARDS is based on supportive measures that primarily aim at gaining time to allow the antibiotic treatment or the patient immunologic system to defeat the primary cause of ARDS. Mechanical ventilation strategy includes: the use of low tidal volume ventilation, inspiratory pressure, higher positive end expiratory pressure and using prone positioning, and administering neuromuscular blockers in higher severity ARDS [19–21]. Current guidelines supporting protective mechanical ventilation is aimed at preventing the risk of ventilator-induced lung injury [22] in patients with ARDS. The pathophysiologic reason behind this is based on the concept of the "baby lung" [23], which has reduced compliance caused by the severe decrease of lung aerated volumes, and is defined based on CT scans over the course of initial ARDS diagnosis (0–7 days) and the subsequent fibroproliferative response (15–20 days) [24, 25].

It might also be argued that despite the era of protective mechanical ventilation [26], large tidal volumes are still fundamental for mechanical ventilation daily management [13], and adjunctive measures such as proning [27] and neuromuscular blockers [28], while proven to have a positive effect on outcome, have not been fully implemented yet [13].

Furthermore, ARDS mortality remains high, despite considerable advances in terms of antibiotic stewardship and antibiotic treatment options [29–32] and fluid management [31, 33] to face up the two main risk factors leading to ARDS, pneumonia, and sepsis [13].

The multimodal nature of ARDS also necessitates a multimodal approach to treatment, which cannot be met with traditional small molecule or recombinant protein medicines. A wide variety of anti-inflammatory pharmacologicals, proteins, and antibodies have demonstrated promise in the laboratory but have failed in clinical trial (reviewed in [34]). Of relevance here is the fact that injury and repair responses are intimately linked phenomena at the cell signaling and transcriptional level, and blanket inhibition of inflammation may delay or even prevent essential regenerative processes that restore lung tissue to normal function [35–37]. An idealistic treatment of ARDS should target multiple mechanisms and biologic pathways instead of aiming at a single exclusive target. This hypothesis is supported by: (1) the heterogeneity of the mechanisms involved in the lung injury, (2) decades of negative randomized clinical trials

with pharmacologic and other therapies and (3) the new upcoming evidences about the role of biologically distinct pathways and response to treatment in specific subsets of ARDS patients – as recently observed by the identification of specific endotypes and phenotypes [38–40].

In light of these considerations, cell-based therapies with mesenchymal stem/stromal cells (MSCs) have been proposed as novel therapeutics in the treatment of ARDS, due to their broad immunomodulatory effect during inflammation, their enhancement of host defense through antimicrobial mechanisms, and their lung healing potential through the activation of repairing mechanisms [41].

12.4 Epidemiology

ARDS occurrence remains as high as 10.4% of all ICU admissions, rising up to 23.4% of mechanically ventilated patients. ARDS still appears to be an under-recognized syndrome, with up to 40% as recently reported in the Large observational study to Understand the Global impact of Severe Acute respiratory FailurE (LUNG SAFE) study [13]. Furthermore, ARDS mortality is still estimated as high as 40%.

12.5 Pathology

The pathophysiologic hallmark of ARDS is the well-known diffuse alveolar damage (DAD), which leads to the characteristic protein-rich nonhydrostatic pulmonary edema during ARDS [33, 42–44]. Considering that a match among clinical signs and pathology findings is far from being perfect, it is relevant that DAD is highly associated to the category of nonresolving ARDS [45] and that DAD predicts higher mortality in ARDS compared to patients with non-DAD ARDS [46]. Excess alveolar fluid blocks gas exchange at the alveolar epithelial surface, while surfactant inactivation by infiltrating albumin and other substances [47–49] damages fluid tension leading to alveolar collapse. Even for patients undergoing ventilation, eventually ventilation/perfusion (V/Q) mismatch occurs where oxygen delivered to the pulmonary space fails to reach the bloodstream and there is impairment of CO_2 clearance, leading to systemic hypoxemia and hypercapnia.

At the cellular and molecular level, the initial phase of ARDS arising from pulmonary infection involves resident pulmonary macrophage defenses being overcome, and the epithelial cell layer lining the alveolus begins to produce proinflammatory cytokines [50]. These signals initiate the recruitment of circulating leukocytes, including neutrophils, macrophages, and B-cells, to migrate up a chemokine concentration gradient to the lung tissue, where these cells release matrix metalloproteinases that enzymatically digest connective tissue facilitating entry to the airspace [51]. Recruited infiltrating cells produce a wide range of noxious substances including superoxide radicals [52–58], leukotrienes [59–64], and antimicrobial peptides [65–67] and further cytokine cocktails in an attempt to destroy the infectious agents. In ARDS, a section of these substances is uncontrolled and induce damage to the lung tissue itself leading to fluid buildup and surfactant loss. Finally as the alveoli fill with liquid and the integrity of the epithelial and endothelial barrier is compromised, gas exchange deteriorates and hypoxemia and hypercapnia result. In later phases of ARDS during systemic inflammatory response syndrome (SIRS), patients may suffer from functional immune-suppression and have heightened susceptibility to additional infection [68–70]. While ARDS presents an acute onset after the exposure to the causing factor, its evolving process starts with acute exudation and infiltrates of acute inflammatory cells into the alveoli within 1 week. During the second week a subacute deposition of collagen fibers are produced by fibroblasts. The syndrome typically resolves through a chronic stage characterized by alveolar macrophage infiltration into the alveoli and a fibrotic repairing process of the lung parenchyma [11].

12.6 MSCs for ARDS: A Promising Potential Therapy

MSCs have generated interest for a wide range of regenerative medicine applications and appear to

have broad immunomodulatory properties rendering them attractive for autoimmune and other inflammatory disorders. Initial safety has been amply demonstrated [71, 72], although some questions remain over hypersensitivity to repeat dosing in non-ARDS models which will only be answered over time [73, 74]. As further conditions are likely to have MSCs licenses as a medicinal therapeutic, this will be of added relevance where MSC therapy may be employed in the same patient for sequential separate disease instances.

MSCs accumulate in the lungs initially after IV administration [75–77] and can remain viable there for up to 24 h [78] after which this time MSCs disappear, suggesting any therapeutic effect has already been conferred to the host; however, it is not clear what happens to MSCSs after they have left the lungs. Interestingly, recent stem cell therapy research in other diseases has indicated the lung is crucial to licensing of MSC to ultimately allow their beneficial effects [79–81], suggesting an immunomodulatory effect that outlasts the MSC's presence in the body.

The MSC's responsiveness to the injury milieu [82–85] and diverse range of effects on multiple pathological and repair processes has made them, theoretically, an ideal candidate for ARDS interventional studies [86] (Fig. 12.1). Indeed, many of the leukocyte subpopulations involved in ARDS pathology have been shown to have direct interaction with MSCs [87–89], while direct antibacterial activity [65] is to be considered an added bonus. Also, the fact that most patients with ARDS require tracheal intubation to permit support of lung function opens up the possibility of direct delivery to the lung airspace of MSC or MSC derivatives [90], although since patients are also almost certain to have IV access obtained and there have been, to date, no demonstrated efficacy advantages observed in delivery of MSC intratracheally over IV [91] in ARDS models, this remains a point of urgent future investigation.

Inviting as MSC therapy may appear, the timecourse and whole body nature of ARDS demand a specific set of considerations around preparation, storage, and administration to be resolved before deployment of MSCs to the clinic. Translation of experimental findings from animal models to the patient are also problematic, with uncertainty regarding dose scaling and testing of the human MSCs destined for patients in nonhuman models, where DNA/RNA sequence and protein/ligand binding incompatibilities warrant extra attention.

12.7 Ex Vivo Human Lung Models

In a first report in 2009, Lee et al. explored the potential role of allogenic human MSCs in the treatment of ARDS induced by *E. coli* endotoxin in an ex vivo perfused human lung [92]. The authors administered allogeneic human MSCs or MSCs-derived conditioned medium at 1 h after the injury induction. Fluid balance was normalized by the decrease of the extravascular lung water, restoring the alveolar fluid clearance (AFC) and by improvement of lung endothelial barrier permeability. The alveolar epithelial fluid transport was in part coordinated by the keratinocyte growth factor (KGF), secreted by the MSCs, which restored the correct function of the amiloride-dependent sodium transport.

Some years later, it was observed that clinical-grade MSCs, administered via the lung perfusate or directly into the right middle lobe, could decrease neutrophil influx and inflammation, effectively cleared bacteria, confirming the contributing role of the KGF, and restored the clearance of the alveolar fluid, with a relevant improvement of the lung histology [93]. In 2014, it was observed in a follow-on study that intravenous administration of clinical-grade allogenic human MSCs could increase the AFC at 4 h. The role of KGF in the AFC was confirmed by the study of a neutralizing antibody of KGF that could decrease the AFC activity [94]. Recently, the same group explored the effects of microvesicles (MVs) released by human mesenchymal stem cells in their established ex vivo human lung perfusion model of bacterial pneumonia. The investigators reported positive results highlighting the beneficial effects of MSC MVs in increasing lung antibody forming cells, in decreasing the lung permeability, and improving the bacterial clearance, particularly when MSCs were pre-

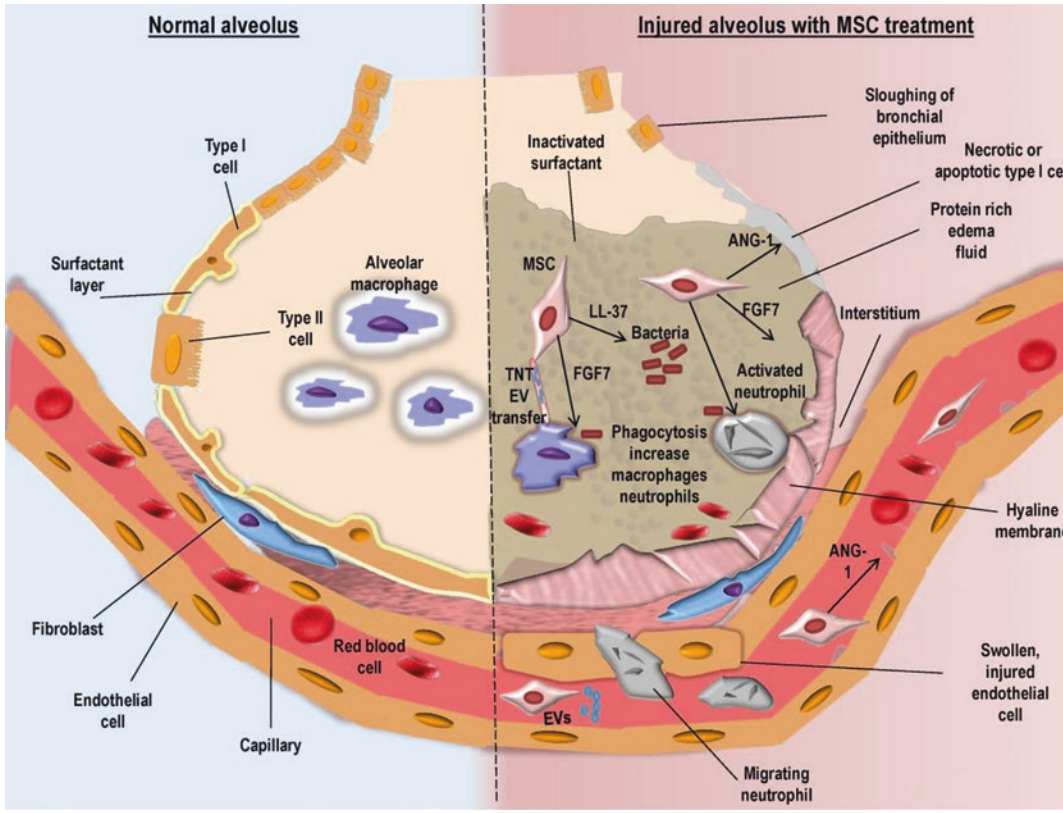

Fig. 12.1 Possible mechanisms of action of the MSC in the ARDS alveolus and surrounding vasculature. Left side: healthy alveolus. Right side: during injury, protein-rich edema fluid and inflammatory cells permeate the alveolus. MSCs have been demonstrated to alleviate the pathophysiological symptoms of ARDS through the secretion of paracrine factors, cell-to-cell contact and mitochondrial transfer (TNT and EV release). Indirect and direct contact of MSCs has been shown to reduce the permeability of alveolar epithelium and increase fluid clearance. Through the secretion of ANG-1, endothelial and epithelial repair is increased. Reduction of neutrophil migration also improves endothelial and epithelial barriers. Release of KGF promotes an increase in alveolar fluid clearance. Bacteria clearance is achieved through the direct release of the antimicrobial peptide LL-37 or through increased phagocytosis by neutrophils and macrophages mediated by the release of cytokines including FGF-7 or by transfer of micro vesicles through TNTs. Mitochondrial transfer to epithelial cells also increases surfactant release. A few mechanisms of action of MSCS in ARDS have been displayed in this diagram. *EVs* extracellular vesicles, *TNT* Tunnelling Nanotubules, *ANG-1* angiopoietin-1, *PGE2* prostaglandin E2, *KGF* keratinocyte growth factor, *LL-37* peptide β-cathelicidin

treated before isolation of MVs with a Toll-like receptor 3 agonist, polyinosinic:polycytidylic acid (Poly(I:C)) [95].

12.8 Mechanistic Considerations for Clinical Therapy

Allogenic MSCs have the ability to avoid detection of the immune system and it is assumed that this evasion is due to the low expression of the major histocompatibility complexes (MHC) I and II, while MSCs also do not express CD80 and CD86 which are identified T-cell stimulators [96]. Therefore autologous MSC administration is considered a viable therapeutic option as the likelihood of an immune response is extremely low.

The routes of administration will influence the MSCs ability to differentiate, their immunogenic

effect, and ultimately their survival [97]. Some studies have contradicted the MSC's proposed ability to evade immunological detection. MHC II protein expression analysis on MSCs has been shown to be higher than originally documented [98–100]. In vivo studies have also shown that allogenic MSCs are not immune privileged and have the potential to cause an immune response, while other research has contradicted findings and stated MSCs are immune privileged [101–104].

MSC efficacy has been demonstrated in multiple preclinical models of ARDS [90, 93, 94, 105–111], while MSC products including conditioned medium and extracellular vesicles have also shown promise [91, 94, 107, 112–117]. These are interesting in that they avoid safety and cryostorage issues associated with whole cell delivery and may be more compatible with direct delivery to the airspace by nebulizer. MSCs have been shown to reduce inflammation and improve bacterial clearance [107] through direct antimicrobial peptide release such as LL-37 and indirectly through the modulation of phagocytic activity in BAL monocytes [108] as well as alveolar resident monocytes specifically [93].

More recently, MSCs have been documented to protect from injury via direct interaction through tunneling nanotubules (TNT) or formation of connexin 43 gap junctions [118–125]. It has been demonstrated that mitochondrial transfer from MSCs to alveolar epithelial cells improved survival after endotoxin injury [118], while in a rat model of COPD, iPS-derived MSC mitochondrial transfer to bronchial epithelial cells was also observed [126]. MSCs have been shown to transfer mitochondria to macrophages in vitro and in vivo, improving macrophage function and enhancing phagocytosis [127]. In a mouse model of *E. coli*-induced pneumonia it was reported that therapeutic effect was dependent on transfer of MSC mitochondria to alveolar macrophages through TNT, enhancing antimicrobial activity and phagocytosis [128]. While it is unclear whether the mechanism of action in vivo is due to an enhancement of a normal mitochondrial function or restoration of dysfunctional mitochondria, there is some evidence for damaged mitochondrion, e.g., downregulated NDUFB8 (complex I) and ATP synthase (complex V), in ARDS meaning the latter is a distinct possibility [129, 130].

12.9 Production Considerations for Clinical Cell Therapies

For ARDS, autologous stem cell therapy is not an option as there is insufficient time to isolate and expand patient MSCs while the rapid onset nature of ARDS demands a cryopreserved MSC product. Cryoprotective agents are used to protect the cellular components from crystal formation and osmotic shock and membrane damage during the slow freezing process, preserving the fine structures of cells [131]. For clinical applications, MSCs are typically frozen to at least −150 °C at a controlled rate of 1–5 °C per minute in 5% or 10% dimethyl sulfoxide in an electrolyte solution and added protein, typically human serum albumin [132]. Despite extensive optimization, the process can cause damage and affect cell viability [133–135] and inadequate insight into how MSCs function after systemic infusion remains an issue [134, 136–139].

Freeze-thawed MSCs, in comparison to cells harvested from continuous cultures, have diminished immunomodulatory properties as well as a reduced responsiveness to proinflammatory cytokines [140]. The immunomodulatory effects of MSCs is affected by cryopreservation, launching a heat shock protein response [141]. In vivo experiments have shown that cryopreserved are less well tolerated. In a clinical application where predominant indications included graft versus host disease (GvHD) and tissue injury in hemorrhagic cystitis, therapeutic properties of freeze-thawed and freshly harvested MSCs were compared. A 100% response rate was observed in patients treated with fresh cells at a low passage compared to patients treated with cryopreserved freeze-thawed cells at a higher passage, with cryopreserved MSCs eliminated faster by compliment after exposure to recipient blood [140]. The thawing process can damage cell surface proteins and this abnormality attracts the binding of complement initiating clearance by phagocy-

tosis [142–144]. After complement exposure, there is an 80% decrease in cell viability in cryopreserved cells compared to a 50% decrease in fresh MSCs [141, 145]. However activation of complement may not be negative as recognition of opsonized MSCs are hypothesized to induce an M2 phenotype, producing anti-inflammatory mediators [140]. Macrophages can display various phenotypes, with the two being described as M1 and M2. M1 phenotypes are generated by the classical pathway [146, 147] and produce abundant inflammatory cytokines such as tumor necrosis factor (TNF)-α and interleukin (IL)-12, and reactive oxygen species. M2 phenotypes are generally activated by the alternative pathway and express a variety of lectins, protein, and scavenger receptors [146–148].

While various research have demonstrated that umbilical cord (UC) [149–152] and adipose tissue (AT)-derived [153] MSCs have a faster population doubling time than bone marrow (BM) sourced, attaining human-sized doses of the order of 10^9 cells is still a daunting task. Additionally, there is currently no data published on whether there is an upper limit on MSC population doubling that still retains therapeutic efficacy. Kern et al. compared the senescence ratio of AT-MSC to BM-MSC and found that BM-MSC had a growth threshold of passage 7, whereas AT-MSC had a threshold of 8 [154], but efficacy itself may be lost long before senescence arises. In addition, MSCs isolated from patients with advanced age [155, 156], diabetes [157], rheumatoid arthritis [158], or indeed ARDS itself [159] have decreased activity, including lower regenerative and differential potential and therefore autologous MSC therapy in patients with significant chronic comorbidities may not be a promising approach in any case. Downregulation of inflammatory marker receptors may render MSCs isolated from such patients less responsive to the injury microenvironment and hence of lower overall therapeutic value [146, 147, 159].

Beyond the conventional MSC therapeutics, human embryonic stem cells (ESC) and induced pluripotent stem cells (iPSC) as new cell types have also been investigated in immunoregulation and have shown encouraging results [160–163]. iPSCs are immunomodulatory in a mouse model of allergic inflammation [164], while their systemic administration inhibited serum levels of IgE and TH2 cytokines (IL-4, IL-5 or IL-13) with better survival and engraftment rate after transplantation compared to adult tissue derived MSCs [163, 165]. There are also, variations in age related to DNA methylation levels, which is correlated to differential abilities, with ESC-derived iPSC having a higher proliferation and regenerative capacity [166, 167]. However, caution is still required as genetic abnormalities remain an area of concern in iPSCs [168]. Clinical studies using iPSCs found some cells in the study contained genetic abnormalities, and were consequently not used. Cells taken from elderly patients to be reprogrammed for administration can come with increased risks of genetic abnormalities [168], demonstrating the need for screening of cells before infusion if autologous cells are ever to be used in elderly patients.

12.10 Clinical Trials—Demonstrating Safety in ARDS

Clinical trials utilizing MSCs for ARDS patients are in phase 1-2 studies and focused on safety, tolerability and feasibility concerns. We are far from being able to claim MSC therapy is a viable option for ARDS. However, during the last decade, promising preclinical evidence supports the hypothesis of a potential benefit in treating ARDS patients with MSCs [169]. Despite the many studies into the potential clinical benefit of MSCs in ARDS that have been proposed over the last years and posted on clinicaltrials.gov, most of them are still currently ongoing or they lack a status update. Furthermore, few studies have disclosed the initial safety results (Table 12.1). The first clinical trial where MSCs were used to treat ARDS was reported in 2014 (NCT01902082), where Zheng and coworkers administered 1×10^6 AT-MSCs/kg of body weight or saline in a 1:1 fashion in 12 patients with moderate and severe ARDS. The investigators reported that the administration of allogeneic MSCs was feasible and

Table 12.1 List of clinical trials reported on clinicaltrials.gov about mesenchymal stem/stromal cells in ARDS—current status and key findings

Study	NCT	Reference/url	Design	MSC type	Dose	Population	Status	Findings
Mesenchymal stem cells (MSCs) for treatment of acute respiratory distress syndrome (ARD) in patients with malignancies	02804945	https://clinicaltrials.gov/ct2/show/results/NCT02804945	Phase 2—open label	Allogeneic human Mesenchymal stem cells (hMSCs)	3×10^6 cells/kg one time on day 1 over about 1–2 h	ARDS $PaO_2/FiO_2 \leq 200$	Recruiting	In progress
Adipose-derived Mesenchymal stem cells in acute respiratory distress syndrome	01902082	https://clinicaltrials.gov/ct2/show/NCT01902082	Phase 1—"Triple blinded" (participant, care provider, investigator)	Allogeneic adipose-derived hMSCs	1×10^6 cells/kg body weight within 48 h of enrollment	ARDS $PaO_2/FiO_2 < 200$	Unknown	Published: no infusion toxicities or serious adverse events related to MSCs administration
Human umbilical cord Mesenchymal stem cells (MSCs) therapy in ARDS (ARDS)	03608592	https://clinicaltrials.gov/ct2/show/NCT03608592	Early phase 1	Umbilical cord derived mesenchymal stem cells (UCMSCs)	60×10^6 cells suspended in 100 ml normal saline after randomization in 30–60 min	ARDS $PaO_2/FiO_2 < 200$	Not yet recruiting	In progress
Human Mesenchymal stem cells for acute respiratory distress syndrome	01775774	https://clinicaltrials.gov/ct2/show/NCT01775774	Phase 1—open label	Allogeneic Bone marrow-derived hMSCs	3 cohorts with 3 subjects/cohort who receive doses of 1, 5 and 10×10^6 cells/kg predicted body weight (PBW)	ARDS $PaO_2/FiO_2 < 200$	Completed	Published: No prespecified infusion-associated events or treatment-related adverse events
Human mesenchymal stem cells for acute respiratory distress syndrome (START)	02097641	https://clinicaltrials.gov/ct2/show/NCT02097641	Phase 2—Triple masking (participant, care provider, investigator)	Allogeneic Bone marrow-derived hMSCs	A single dose of 10×10^6 cells/kg PBW over approximately 60–80 min	ARDS $PaO_2/FiO_2 < 200$	Completed	Published: No predefined MSC-related

Mesenchymal stem cells for multiple organ Failure after cardiac surgery	03552848	https://clinicaltrials.gov/ct2/show/NCT03552848	Pilot—open label	Mesenchymal stem (stromal) cells (MSC)	1×10^6 cells/kg of body weight intravenously once every 4 days for four times	Failure >2 organs and: (1). In case of ventilation $PaO_2/FiO_2 < 100$,100% oxygen demand (2). Sequential organ failure assessment (SOFA) ≥ 10, multiple organ dysfunction (MOD) ≥ 10	Not yet recruiting	In progress
Mesenchymal stem cell in patients with acute severe respiratory failure (STELLAR)	02112500	https://clinicaltrials.gov/ct2/show/NCT02112500	Phase 2—open label	Bone marrow derived mesenchymal stem cells	Not reported	ARDS $PaO_2/FiO_2 < 200$ Not clear if AHRF or ARDS?	Unknown	
Repair of acute respiratory distress syndrome by stromal cell administration (REALIST)	03042143	https://clinicaltrials.gov/ct2/show/NCT03042143	Phase 1 dose escalation pilot study /phase 2—Quadruple masking (participant, care provider, investigator, outcomes assessor)	Orbcel-C (human umbilical cord derived CD362 enriched MSCs)	Dose not specified - maximum tolerated dose from the phase 1—Single infusion	ARDS $PaO_2/FiO_2 \leq 27$ kPa	Recruiting	In progress
Human umbilical-cord-derived mesenchymal stem cell therapy in acute lung injury (UCMSC-ALI)	02444455	https://clinicaltrials.gov/ct2/show/NCT02444455	Phase 1/2—open label	Umbilical-cord-derived mesenchymal stem cell (UC-MSC)	5×10^5/kg once a day, a total of three times—maximum tolerated dosage without side effects	ARDS oxygenation index:200 < $PaO_2/FiO_2 \leq 300$ mmHg	Unknown	
Using human menstrual blood cells to treat acute lung injury caused by H7N9 Bird Flu virus Infection	02095444	https://clinicaltrials.gov/ct2/show/NCT02095444	Phase 1/2—open label	Menstrual blood stem cells	$1–10 \times 10^7$ cells/kg, two times a week, 2 weeks for infusion	H7N9 infection and critical lung tissue injury	Unknown	

(continued)

Table 12.1 (continued)

Study	NCT	Reference/url	Design	MSC type	Dose	Population	Status	Findings
A phase 1/2 study to assess multi stem therapy in acute respiratory distress syndrome (MUST-ARDS)	02611609	https://clinicaltrials.gov/ct2/show/NCT02611609	Phase 1/2—Quadruple masking (participant, care provider, investigator, outcomes assessor)	MultiStem	Low versus high multistem dose	Moderate to severe ARDS	Recruiting	Active, not recruiting
Human umbilical-cord-derived mesenchymal stem cell therapy in paraquat poisoning induced lung injury (UCMSC-PQLI)	02444858	https://clinicaltrials.gov/ct2/show/NCT02444858	Phase 1/2—single masking (participant)	Umbilical-cord-derived mesenchymal stem cell (UC-MSC)	5×10^5 cells/kg, once a day, a total of three times	Paraquat-induced lung injury	Unknown	
Treatment of severe acute respiratory distress syndrome with Allogeneic Bone marrow-derived mesenchymal stromal cells	02215811	https://clinicaltrials.gov/ct2/show/NCT02215811	Phase 1—open label	Allogenic bone marrow-derived mesenchymal stromal cells (BM-MSC)	Not reported	ECMO patients with viral-induced ARDS	Unknown	
A pilot study using placenta derived Decidual stromal cells for toxicity and inflammation with special focus to the allogeneic hematopoietic cell transplantation setting	02175303	https://clinicaltrials.gov/ct2/show/NCT02175303	Phase 1/2—open label	Decidual stromal cell therapy	1×10^6 cells/kg, at one or more occasions at weekly intervals dependent on clinical response	Unclear criteria including: — acute lung injury — Decidual stromal cells–stem cell transplantation —inflammation	Unknown	

safe with no infusion toxicities or serious adverse events related to MSCs in the treatment group and with no differences in terms of adverse events and biomarkers of lung injury between the MSCs and the placebo group [170].

In 2015, Wilson J.G. and coworkers tested the safety of BM-MSCs in a multicenter phase 1b dose-escalation study in patients with moderate-severe ARDS, with PaO_2/FiO_2 less than 200 mm Hg, a positive end-expiratory pressure (PEEP) ≥ 8 cmH_2O, and bilateral infiltrates at the frontal chest X-ray (NCT01775774). Nine patients were enrolled and three groups of three received three doses of MSCs intravenously (1, 5, or 10 × 10^6 MSC/kg ideal body weight). The investigators reported no significant difference in biomarkers of inflammation (IL-6, IL-8), lung epithelial (receptor for advanced glycation end products—RAGE) and endothelial injury (Ang-PT2) among the groups. MSC administration was safe and the authors reported neither infusion-associated events nor serious adverse events. The viability of the MSCs infused ranged from 50–63% [171].

The same investigators recently reported the findings of a double-blind multicenter randomized phase 2a clinical trial testing the safety of BM-MSCs versus placebo in ventilated patients with moderate-severe ARDS, with PaO_2/FiO_2 < 27 kPa and PEEP ≥8 cmH_2O. Patients randomly received in a 2:1 fashion 1 × 10^6 BM-MSCs/kg ideal body weight or placebo. The primary objective of this investigation was the safety of MSCs in an intention to treat analysis (NCT02097641). No patient in the MSCs treatment group experienced any adverse respiratory or hemodynamic events. The treatment group had higher APACHE III score, minute ventilation, and PEEP compared to placebo. No statistically significant 28-day mortality difference was observed among treatment and placebo group, even after adjustment with the APACHE III score, while a trend in a lower number of ICU-free days to day 28 was reported in the treatment group compared to placebo. Of importance, there was higher absolute 28 day and 60 day mortality in the MSC group, although it is unclear at this point if there is any clinical significance to this result or if it was related to variability in MSC viability on administration or some other quality issue.

Furthermore, a higher severity of illness at baseline—quantified by the SOFA and APACHE III scores—was present in the MSC group compared to the placebo group and mortality in the MSC group and in the placebo group was lower and higher than anticipated, respectively.

However, this RCT was not powered for efficacy, as per the Food and Drug Administration mandate to clearly demonstrate safety before targeting lung oxygenation or compliance as in a phase 2b trial. The viability of the MSCs infused ranged from 36–85% [71].

The range in MSC viability was unanticipated and only discovered after study completion. The authors reported a significantly higher MSCs viability after centrifugation when MSCs were thawed compared to when the cells were washed to remove dimethyl sulfoxide during preparation. Based on these findings the investigators conducted a post-hoc analysis and observed that plasma angiopoietin-2 levels in the intermediate and highest tertiles of MSCs viability were significantly lower in the MSCs treatment group at 6 h after administration compared to placebo, and albeit nonsignificantly, the oxygenation improved at day 2.

These results suggest that the administration of MSCs with a high viability is required to target an improvement in efficacy. Recent experimental data on the comparison of different cell products reports that fresh BM-MSCs are 14% more viable compared to cryopreserved ones [172]. Furthermore, delivery of MSC immediately upon thawing instead of thawing and washing the MSCs could enhance MSC viability, as observed by Matthay MA et al [71]. This is an unusual finding, as washing of MSC in physiological buffer has not been considered traditionally to have any impact on viability, and warrants further investigation.

Simonson and colleagues reported data on the clinical outcomes of two patients with severe ARDS who received allogenic BM-MSCs. MSC administration was safe and no adverse events were reported during infusion. The investigators reported a decrease of plasma and BAL proinflammatory cytokines, chemokines, miRNAs,

and biomarkers of epithelial apoptosis and alveolar-capillary fluid leakage. One patient developed pneumonia 5 days after cell administration, which resolved after antibiotic therapy and the patient was subsequently extubated 4 weeks after MSCs administration. The second patient was extubated 12 days later [92].

Very recently, Athersys disclosed in a press release the positive results for the MUST-ARDS study about MultiStem® Cell Therapy in patients with moderate-severe ARDS (NCT02611609). After an initial dose confirmation phase ($n = 6$), Athersys confirmed the tolerability and the safety profile of the MultiStem® treatment ($n = 20$) with no adverse events during administration, and lower levels of inflammatory biomarkers compared to the control group ($n = 10$). Furthermore, despite the study was not powered for efficacy outcomes, MultiStem® cell therapy was associated with better short term prognosis, as shown by a lower mortality rate (25% versus 40%), higher ventilator-free (12.9 versus 9.2) and ICU-free days (10.3 versus 8.1) compared to control. Further findings will be unveiled at end of the collection of the 1-year follow-up data, as aimed according to the study design. (http://www.athersys.com/news-releases/news-release-details/athersys-announces-positive-results-its-exploratory-clinical).

All the studies currently ongoing in the field of MSCs and ARDS are safety studies (Phase 1, 2, 1/2) (Table 12.1). At the moment there are still additional issues that need to be overcome: (1) improvement of MSCs bioavailability by the optimization of the cell preparation and storage [71]; (2) the modulation of the microenvironment [173]; (3) the characterization of the specific phenotypes/endotypes of ARDS potentially more suitable to respond to cell therapy [39, 40]. This might enhance the likelihood of success in subsequent efficacy (Phase 3) studies.

12.11 Future Directions

12.11.1 Patient Stratification

ARDS is classified by the Berlin definition into different severity categories, according to the degree of hypoxemia, and each associated with increasing mortality rates. However, other evidence suggests that: (1) either the etiology (i.e. pulmonary versus extrapulmonary ARDS) [174–177], or (2) the macroscopic ARDS presentation at radiological imaging [178] or (3) the levels of different inflammatory biomarkers contributing to different biological patterns of ARDS might play a key role in stratifying the outcome of this syndrome [179]. Pulmonary ARDS was associated with longer total ventilation time and longer ICU stay compared to extrapulmonary ARDS [180]. ARDS patients with a higher epithelial injury, as observed by higher levels of soluble form of the receptor for advanced glycation end product (sRAGE), showed a specific nonfocal CT lung pattern, which was associated with higher mortality compared to the focal pattern [178].

The ARDS Network proposed a novel classification of ARDS with two distinct subphenotypes, which included different clinical and laboratory characteristics [39]. Interestingly, in a secondary analysis of the ARMA [26, 181, 182] and the ALVEOLI trials [183], the investigators could identify a specific pattern of ARDS that the investigators named hyperinflammatory subphenotype, phenotype 2. Phenotype 2 showed higher plasma concentrations of inflammatory biomarkers greater prevalence of vasopressor use and lower serum bicarbonate concentrations than phenotype 1. The hyperinflammatory subphenotype could differentiate a subgroup of patients with a higher mortality rate.

In light of the heterogeneity of ARDS, attempts have been made to optimize treatment regimens [173], and stratification parameters are emerging among recipients of MSC therapy which may be of relevance to ARDS patients [184].

12.11.2 Large-Scale Cell Manufacture

As detailed earlier, generation of human-sized doses of GMP quality MSCs, for the numbers of patients needed for large-scale clinical trials (and subsequent clinical therapy), is not a trivial undertaking. Preclinical studies typically use between 1×10^6 and 10×10^6 MSCs per kg of

bodyweight or ideal bodyweight of the patient, and clinical trials have been designed with these doses in mind. In light of the observed relatively low cell viability in ongoing trials, production of the order of 10^9 MSC may be required to reach the upper doses when allowance for dead cells is calculated. Coupled with the lack of information with regard to passage or population doubling at which efficacy is lost, this will likely necessitate pooled donor batches to enter the MSC isolation and production process. Considerable work is being concentrated on this area both academically and industrially to optimize and automate, including the utilization of xeno-free media that allays fears of contamination with viruses or other as yet unknown contaminating factors.

The preponderance of preclinical work with MSCs has involved freshly harvested MSCs and this will remain an impractical and unlikely therapeutic for the clinic. Despite the development of rapid shipping solutions from manufacturing facility to clinical site, and research into supportive media to extend the MSCs' effective lifespan in suspension prior to administration, it is probable that a cryopreserved MSC will become the choice in the long term. Cryopreservation at the clinical site or expedited transport from manufacturing facilities will be required, but it remains to be determined what further equipment such as centrifuges and viability assessment assays will be needed to prepare the MSC dose and allow quality control prior to administration to the patient.

12.11.3 Lack of Clearly Defined Mechanism of Action

Despite a myriad of possible effector mechanisms by which the MSC may alleviate ARDS severity, including secreted antimicrobials, cytokines, extracellular vesicles, and other factors, and the observed influence MSCs have on leukocytes, it has remained difficult to ascertain which of these mechanisms are of importance to the MSCs' efficacy. Indeed several studies that have sought to replicate the various mechanisms proposed through administration of MSC-produced factors have failed or not reproduced the efficacy of the MSC itself, indicating critical gaps in our knowledge of MSC action in ARDS and suggesting interpretation of unsuccessful or even successful follow on clinical trial will be difficult. If the mechanism remains unknown, then interactions with other drugs or comorbidities will always be unpredictable.

12.11.4 Lack of MSC Potency Assay

Related to both production and mechanism, a critical limiting factor in successful deployment of MSC therapy to the clinic is the lack of defined assays to accurately predict MSC potency in the ARDS patient. Many cell manufacturers and research groups have proposed small, easily quantified molecules such as aldehyde dehydrogenase (ALDH) [185] or indoleamine 2,3-dioxygenase (IDO) [186], which correlated well with in vitro tests such as T-cell expansion inhibition or in vivo tests in ARDS animal models. However, as the MSC's mechanism of action in ARDS remain unclear, these factors can only be considered correlative and not conclusive proof of likely efficacy in the human patient.

12.11.5 Beyond the MSC?

Determining the mechanism(s) of action of the MSC specifically, however, will lead to us a question: do we need the cell at all? A suite of effectors produced by MSC cultures, or indeed by similar cell types engineered to replicate or improve upon the MSC secretome while being more open to manipulation and expansion, could replace cell therapy entirely. Also, as alluded to already, these factors will be likely easier to analyze, store, and deliver than the MSC they are derived from. Presuming cell-contact dependent mechanisms such as TNTs are the sole means underlying the MSC's efficacy in ARDS, we may ultimately see an MSC product cocktail available in stable, off-the-shelf format that can be delivered IV or intratracheally by nebulizer that will reproduce the efficacy initially demonstrated with the IV-delivered cryopreserved whole cell.

12.12 Conclusions

ARDS has been a stubbornly challenging syndrome to address clinically for decades. Despite gradual improvement in supportive care for the patient, specific therapies have proven elusive. The MSC is an exciting prospect, as it is a real paradigm shift from traditional approaches, due to its ability to respond to the level and nature of injury, having both direct and immunomodulatory properties, and a multimodal mechanism of action that targets multiple pathologies seen in the ARDS patient. Issues around dosing, MSC production, and potency reproducibility remain but are being addressed. We look forward to the conclusion of the many current and planned clinical trials to determine the true therapeutic potential of MSCs for those suffering from this devastating disease.

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