Review Article of Critical Care Frontier and Horizon Corner

Cell therapy in acute respiratory distress syndrome

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Abstract: Acute respiratory distress syndrome (ARDS) is driven by a severe pro-inflammatory response resulting in lung damage, impaired gas exchange and severe respiratory failure. There is no specific treatment that effectively improves outcome in ARDS. However, in recent years, cell therapy has shown great promise in preclinical ARDS studies. A wide range of cells have been identified as potential candidates for use, among these are mesenchymal stromal cells (MSCs), which are adult multi-lineage cells that can modulate the immune response and enhance repair of damaged tissue. The therapeutic potential of MSC therapy for sepsis and ARDS has been demonstrated in multiple *in vivo* models. The therapeutic effect of these cells seems to be due to two different mechanisms; direct cellular interaction, and paracrine release of different soluble products such as extracellular vesicles (EVs)/exosomes. Different approaches have also been studied to enhance the therapeutic effect of these cells, such as the over-expression of anti-inflammatory or pro-reparative molecules. Several clinical trials (phase I and II) have already shown safety of MSCs in ARDS and other diseases. However, several translational issues still need to be addressed, such as the large-scale production of cells, and their potentiality and variability, before the therapeutic potential of stem cells therapies can be realized.

Keywords: Acute respiratory distress syndrome (ARDS); cell therapy; mesenchymal stromal cells (MSCs)

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Introduction

Acute respiratory distress syndrome (ARDS) is a multifactorial syndrome of severe lung injury causing hypoxemia, loss of lung compliance, pulmonary oedema, that can in some instances progress to multiple organ failure (1,2), and results in death in 30–45% of cases (3). ARDS occurs in 10% of all ICU patients and in 23% of all mechanically ventilated patients, with 5.5 ARDS cases per ICU bed each year globally (4). ARDS can develop in response to multiple predisposing factors including pneumonia, systemic infection, and major surgery or multiple traumas (5). This pathology is strongly associated with pulmonary sepsis and/or with a disordered immune response to a major insult (6). Severe lung inflammation is perpetrated by an invasion of neutrophils and macrophages into the alveolar space, which together with the production of pro-inflammatory cytokines, such as interleukin (IL)-6, IL-1β, IL-8 and tumour necrosis factor-alpha (TNF-α), results in damage to the endothelial and epithelial lung layers (7). This inflammatory environment enhances the production of reactive oxygen species, impairs lung barrier function and increases vascular permeability, and, where ARDS is prolonged or unresolved, it can lead to fibrosis (7) (*Figure 1*).

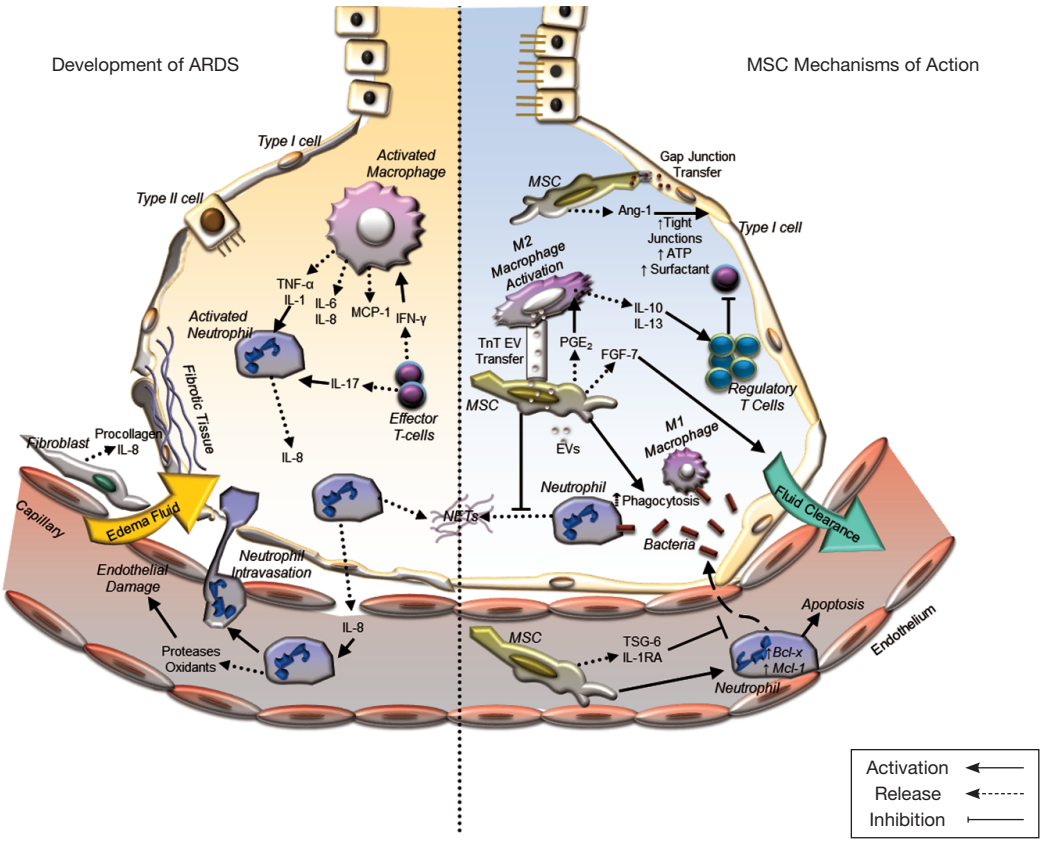

Figure 1 Schematic diagram of the therapeutic effects of MSCs in ARDS. In the acute phase of ARDS the injury manifests as damaged epithelial and endothelial layers allowing the influx of protein-rich edema fluid and inflammatory neutrophils. The release of proteases, oxidants, IL-8, and other injurious factors from inflammatory white cells and damaged tissues leads to a progression of injury and worsening of the condition. The activation of effector T-cells results in the release of IFN-γ and other cytokines such as IL-17 which induces the activation of macrophages and neutrophils, in turn inducing the production of further inflammatory factors producing a greater neutrophil infiltration. The presence of these pro-inflammatory cytokines, and the release by neutrophils of oxidants, proteases, and generation of NETs, damage the alveolar epithelium and capillary endothelium, producing fibrosis and edema. The mechanisms of action of MSCs are depicted on the right-hand side, with cells distributed IV or IT. MSCs inhibit neutrophil intravasation, release PGE2 which induces production of anti-inflammatory cytokines from macrophages, activates the proliferation of regulatory T-cells which inhibit effector T-cells proliferation and activation. MSCs also inhibit the pro-inflammatory activity of neutrophils, mainly inhibiting the formation of NETs, and increase their phagocytic activity, favoring clearance of bacteria. MSCs enhance the differentiation of macrophages towards the M2 phenotype, which produce anti-inflammatory cytokines and favor tissue repair. In M1 macrophages, MSCs increase phagocytic capacity enhancing bacterial clearance. Taken together, MSCs generate an anti-inflammatory environment that enhances the resolution of the pathology, the recovery of function, and tissue repair in the alveolar space. These MSC effects are mediated through cytokine release, cell - cell contact, and the release of EVs. MSC, mesenchymal stromal cell; ARDS, acute respiratory distress syndrome; IL, interleukin; IFN, interferon; NETs, neutrophil extracellular traps; IT, intratracheal; IV, intravenous; PGE2, prostaglandin E2; TnT, tunneling nanotubules; EVs, extracellular vesicles; TNF, tumor necrosis factor; TSG-6, TNF-stimulated gene 6 protein.

Current therapies and their shortfalls

There are no direct therapies for ARDS currently, while management strategies such as protective mechanical ventilation and fluid-restrictive strategies minimize iatrogenic harm while providing organ support. Patients with ARDS by definition are extremely hypoxic and require mechanical ventilation, which can exacerbate the acute lung injury (ALI). This is commonly caused by the initial

volutrauma that intensifies the inflammatory response and is known clinically as ventilator-induced lung injury (VILI) (8). In ARDS which has developed from a pulmonary or systemic infection, early broad-spectrum antibiotics and source control where possible, are the treatment of choice. Antibiotic therapy presents a low success rate in the treatment of ARDS due to the ongoing inflammatory response which continues to cause injury even after eradication of the pathogen. The emergence of antibiotic-resistant strains and the timing of antibiotic administration can also contribute to this low success (9). Pharmacologic treatments, including glucocorticoids, surfactants, inhaled nitric oxide, antioxidants, protease inhibitors, and a variety of other anti-inflammatory treatments have been tested in clinical trials (10). Unfortunately, these pharmacologic treatments have proven to be completely ineffective (11). In contrast, protective lung ventilation strategies (low tidal volume or limited driving pressure strategy) are currently the accepted gold standard for improving mortality rates in ARDS.

Rationale for cell-based therapies for ARDS

There is a pressing need for a safe and effective treatment for ARDS and attention has turned to the use of cell therapy. The first successful use of cell therapy involved the use of bone marrow (BM) aspirates in a transplant procedure for leukaemia patients (12,13). Originally thought to be the definitive solution for the replacement of damaged tissues by differentiating to replace the damaged cells, further investigations have highlighted that in fact this is not a major mechanism of adult stromal/stem cell action. However, other investigations have highlighted an array of capabilities that some of these cells possess. Their immune-modulating effects, anti-bacterial action, lack of rejection molecules as well as relative ease of isolation and characterisation make these cells an ideal therapeutic for ARDS. In fact, several different cell types have been examined for therapeutic potential.

Stem cell candidates for ARDS

Embryonic stem cells (ESCs)

ESCs are pluripotent cells derived from the inner blastocyst cell mass of the developing embryo (14).These cells can differentiate into all other progenitor cell types (15,16) and their capacity for self-renewal makes them a viable treatment option for tissue regeneration. Studies using human ESCs have shown efficacy in a number of disease models including diabetes mellitus (17), Parkinson's disease (18), ischemic stroke (19) and some have progressed to clinical trial (20,21). A study by Wang *et al.*, observed that ESC-derived alveolar-epithelial type II cells (AECII) attenuated bleomycin-induced lung injury in mice and thus showed potential promise as a therapeutic (22). There are ethical concerns with the use of this cell type however, and in many countries their use is limited or banned. Another major limiting factor is the safety concerns of ESC-based therapy. The pluripotency of ESCs is a double-edged sword; the same plasticity that permits ESCs to generate hundreds of different cell types also makes them difficult to control after *in vivo* transplantation, with one of the definitions of ESCs being that after implantation they form teratomas containing cells from all three primary germ layers (23).

Induced pluripotent stem cells (iPSCs)

iPSCs are originally somatic cells of animal or human origin that undergo an induced differentiation treatment, resulting in the overexpression of Oct3/4, Sox2, Klf-4 and c-Myc transcription factors that licence pluripotency (24). iPSCs solve the ethical concerns of ESCs, retaining plasticity and also allowing for autologous transplants. However, iPSCs still present the risk of teratoma formation, for example c-Myc activity has been linked to tumorigenesis (25) while mutagenesis may occur due to the use of lentivirus and adenovirus during the reprogramming process (26). Recent studies have focused on identifying new molecular strategies that can increase cell reprogramming efficiency and that avoid the use of viral transduction (27). A recent study showed that iPSCs significantly alleviated histological damage and cell leakage in a murine model of endotoxin-induced lung injury (28). There are several phase I clinical trials using iPSCs in the treatment of Leukemia (NCT02564484), chronic granulomatous disease (NCT02926963) and retinoblastoma (NCT02193724) for example. iPSCs represent a promising strategy for the therapeutic use of a pluripotent cell type, however much research remains to be conducted to ascertain the safety and enhanced benefits (if any) of these cells over multipotent stem cells.

Mesenchymal stromal/stem cells

MSCs are multipotent adult progenitor cells that can be

isolated from numerous sources, including BM, umbilical cord (UC) and adipose tissue (AD), and can be differentiated into mesenchymal lineage cells (29). MSCs are considered to be hypoimmunogenic because they exhibit low levels of MHC-I expression, and no expression of either MHC class II markers or costimulatory molecules, which allows them to avoid immunosurveillance (30) and thus allows allogenic and autologous transplantation (31,32). MSCs have already shown therapeutic efficacy in preclinical models and exhibited safety clinically in a number of phase I trials. Their therapeutic potential, low immunogenicity, ease of harvest and isolation, and low production costs compared with other stem cells have made them the focus of research and consequently, the rest of this review.

While MSCs are traditionally isolated from BM, they can also been found in many other adult tissues such as lung, liver, cord blood, placenta, dental pulp and AD (33), providing alternative, more readily available and cheaper sources of MSCs. These cells have some common morphological and immunophenotypic properties and studies have shown that MSCs derived from UC and AD tissue among others have demonstrated therapeutic efficacy in pre-clinical models of ARDS (34-36). It was recently demonstrated that UC-MSCs could protect against LPS-induced lung injury in a mouse model, with examination of the MSC secretome and identification of factors responsible for the immune regulation leading to a beneficial outcome (37). A study using human AD-MSCs in a mouse model of bleomycin-induced pneumonia has also shown these cells to play a role in immune regulation whereby they reduce the production of pro-inflammatory cytokines and also reduce the proliferation and differentiation of Th2-type CD4+ T-cells, the major T-cell population involved in inflammation (38). The most recent and relevant research studies using MSCs from different tissues are shown in *Table 1*.

Progenitor cell candidates for ARDS

Progenitor cells are tissue-specific cells with a limited differentiation capacity, distinguishing them from stem cells. They are found in most tissues and can differentiate and replace injured/damaged cells within organ systems. These cells reside within specific tissue/organ niches; accordingly preclinical studies suggest their therapeutic potential for disease conditions relating to their source tissue, having properties most associated with the repair and regeneration of that tissue (47).

Pulmonary epithelial progenitor cells (EpPCs)

EpPCs have the potential to be used as a therapy in pulmonary diseases both as a direct treatment and also as a potential target *in vivo* due to their involvement and disruption in certain syndromes (48). A Wnt-responsive alveolar epithelial progenitor cell population expressing AECII surface markers has been recently demonstrated to enhance lung alveoli regeneration in a mouse model of influenza (49).

AEC-IIs, the pulmonary surfactant-producing cells of the lung (48), are a sub-population of EpPCs and their therapeutic potential stems from their ability to rapidly differentiate to AEC-Is, which regulate and control the fluid homeostasis in the alveolar wall and express diverse ion and water channels, and tight junction proteins (50). Intratracheal administration of AEC-IIs aided lung repair through AEC-I transformation and regulated the immune response by synthesizing surfactant and other anti-inflammatory proteins and lipids such as prostaglandin E_2 (PGE_2) and surfactant protein A (SPA) in a rodent LPS injury model (46). However, the isolation of these cells can be difficult, and the number of cells obtained low.

Endothelial progenitor cells (EnPCs)

EnPCs are circulating cells and their role, isolation and identification has not been fully elucidated. EnPCs express the surface marker CD34 (hematopoietic marker) and seem to have a pivotal role in the repair of the endothelium, adhering to it and other areas under hypoxia or ischemia, releasing growth factors that induce angiogenesis (51,52). One study showed that autologous transplantation of EnPCs improved endothelial function and ameliorated pulmonary oedema following oleic acid-induced ALI in rabbits (53), while another study observed that higher counts of circulating EnPCs correlated to higher survival rates in patients with ALI (54,55). These cells have also shown potential therapeutic use for vascular diseases such as pulmonary arterial hypertension (PAH) as demonstrated during a clinical study by Zhu and colleagues (56). A more recent study has examined the effects of EnPCs transfected to express endothelial nitric oxide synthase in patients with PAH (57). This phase I trial demonstrated the therapeutic potential and safety of this treatment, however further investigations are required to understand the exact mechanism of action of these cells.

Table 1 MSC efficacy in recent pre-clinical studies

Injury model	Cell therapy	Effect/mechanisms	Reference
Unmodified MSC therapy			
IT administration of LPS in Mice	Mouse BM-MSC—retro-orbital injection	• Improved survival • Reduced white cell influx to the lung • Reduced COX-2, NF-κB expression • Reduced NETS formation	Pedrazza *et al.*, 2017 (39)
IT administration of LPS in increasing doses to mice	IT administration of human MSC from Wharton's jelly	• Improved survival • Increased production of PGE_2 and IL-10	Cóndor *et al.*, 2016 (40)
IT administration of LPS in mice	IV Administration of human menstrual MSCs	• Decreased lung oedema • Decreased BAL IL-1β • Enhanced lung repair	Xiang *et al.*, 2017 (41)
100% O_2 48 hours + CLP in rats	IV Administration of human UC-MSCs 1 and 24 h post CLP	• Improved survival in earlier administration group	Lee *et al.*, 2017 (42)
Modified MSC therapy			
IT administration of LPS to mice	IV Administration of Nrf-2 over-expressing human amniotic MSCs	• Reduced inflammation • Enhanced anti-apoptotic effect • Enhanced transformation to ATII cells • Inhibited fibrosis	Zhang *et al.*, 2018 (43)
IT administration of LPS in mice	MSCs over-expressing IL-10	• Increased numbers of B- and T-cells producing IL-10 • Decreased TNF-α and total BAL protein	Wang *et al.*, 2018 (44)
IT administration of LPS in mice	Combination therapy using human UC-MSCs + S1P and FTY720	• Improved survival • Decreased vascular permeability and inflammation	Zhang *et al.*, 2017 (45)
IT administration of LPS in mice	IT administration of ATII cells	• Increased lung function recovery • Increased survival • Decreased pulmonary inflammation	Guillamat-Prats *et al.*, 2017 (46)

IT, intratracheal; LPS, lipopolysaccharide; COX, cyclooxygenase, NF-κB, nuclear factor kappa B; PGE, prostaglandin; IL, interleukin; IV, intravenous; MSC, mesenchymal stromal/stem cell; UC, umbilical cord; ATII, alveolar type II; CLP, cecal ligation puncture; TNF, tumor necrosis factor; BAL, bronchoalveolar lavage; NET, neutrophil extracellular trap; S1P, sphingosine 1 phosphate.

Effects of MSCs on the immune system in ARDS

Modulation of the inflammatory response

Cytokine networks between immune and non-immune cells of the alveolar-capillary membrane are necessary for cellular communication during pulmonary inflammation. The subsequent events of these cellular/humoral interactions are pivotal to the initiation and propagation of the inflammatory response leading to pulmonary injury (58). Several studies demonstrate a reduction of the pro-inflammatory cytokines (IL-1α, -1β, -6, -12, -17, TNF-α, TNF-γ and IFN-γ) and an increase in the concentration of anti-inflammatory cytokines and molecules (IL-1 receptor antagonist, IL-10, cyclooxygenase-2 and PGE_2) in the lung environment after MSC treatment (41,59-65). Miao *et al.*, demonstrated that MSCs can regulate the NLRP3 inflammasome which regulates the activation of caspase-1 and a subsequent inflammatory response to infectious microbes and molecules in Kupffer cells via secretion of PGE_2, leading to increased Kupffer cell production of IL-10. This ameliorated the inflammatory response and ensuing organ dysfunction (66).

Effects on neutrophil response

Upon infection, a series of chemical signals are released, which induce the activation and recruitment of neutrophils

to the site of injury (67). Neutrophils kill microorganisms that cause the infection via: phagocytosis, the release of antibacterial peptides, and by creating neutrophil extracellular traps (NETs) (67). If the infection or injury is not resolved, over-stimulation of neutrophils causes the overproduction of inflammatory cytokines at the site of injury (68), thus leading to more damage than resolution (69).

Neutrophils can also migrate from inflamed tissues to other tissues and organ systems causing widespread host injury and organ dysfunction (69). NETs are structures released from neutrophils comprising a core of chromatin DNA and histones, surrounded by specific antimicrobial proteins (lactoferrin, cathepsin G, defensins, LL-37, and bacterial permeability increasing protein), proteases (neutrophil elastase, proteinase-3, and gelatinase), and reactive oxygen species-generating enzymes (myeloperoxidase) (67). However, excessive increases in the release of NETs can also cause damage to lung tissue. Pedrazza *et al.* demonstrated that MSC treatment enhanced survival in a LPS injury model by reducing NETs, formation (39,40). Numerous pre-clinical ARDS and sepsis studies have shown that MSCs reduce the infiltration of neutrophils to the damaged tissue (64,70) while also enhancing neutrophil-mediated phagocytosis and thus bacterial clearance (71). Németh *et al.* showed that PGE_2 released by MSCs increases the production of IL-10, reducing neutrophil trans-endothelial migration, protecting the organ function and reducing pathogen load (64).

Effects on macrophages

Macrophages are present in almost all tissues, where they coordinate developmental, metabolic, and immunologic functions and thus contribute to the maintenance of homeostasis (72). Upon activation, macrophages develop into two broad phenotypes: M1 or pro-inflammatory macrophages are involved in initiating and sustaining inflammation in response to injury or infection and are required for bacterial killing and clearance, and M2 macrophages, involved in the clearance of dead/injured host cells and tissue repair and immune resolution (73,74). Thus, they play a pivotal role in most aspects of pathologies occurring in the lung. Research has focused on the ability of MSCs to modulate macrophage function by inducing their differentiation to different phenotypes (75,76). Studies suggest that MSCs favour the differentiation of macrophages to the M2 phenotype thus improving the resolution of inflammation and enhancing repair while MSC promotion of the M1 phenotype leads to enhanced phagocytic activity (59,77-79).

Effects on the T-cell response

Regulatory T-cells are a subpopulation of T-cells that modulate the immune system, maintaining self-antigen tolerance and preventing autoimmune disease (80). MSCs promote regulatory T-cell expansion, which causes the suppression of the proliferation of effector T-cells and dampens the immune response, potentially providing a mechanism by which MSCs may enhance ARDS resolution (80). Furthermore, MSCs can modify T-cells, dendritic cells, and natural killer cells, decreasing pro-inflammatory cytokine release and enhancing anti-inflammatory molecule release (81). These effects may be direct or may occur indirectly via effects on dendritic cells and/or other antigen presenting cells (82).

Therapeutic efficacy of MSCs in pre-clinical models of ARDS

As previously described, MSCs offer therapeutic promise for ARDS for several reasons including their immunomodulating ability, reprogramming the immune system to reduce host tissue damage while preserving the immune response to microorganisms and also their capacity to enhance tissue repair after lung injury (83). A study demonstrated that in a septic cecal ligation and puncture (CLP) murine model, MSC therapy modulates transcription of up to 13% of the genome, with immune response–related effects including; down-regulation of toll-like receptor and nuclear factor-κB (NF-κB) activation, a decrease in IL-6 signalling pathways, up-regulation of nuclear factor of activated T-cell (NFAT)-related genes, and genes involved in antigen presentation and cell-to-cell interactions which regulates endothelial integrity, increased phagocytosis and bacterial killing, decreased complement activation, and coagulation regulation including platelet activation (58,84). Furthermore, the long-term effects of MSCs are mitigated by the fact that they disappear from the tissue within days of administration.

Means by which MSCs exert effects

Cell-to-cell contact mediated effects

MSCs can migrate to the damaged lung, and without the need to engraft in the tissue, perform their antimicrobial

and tissue repair functions, residing in the tissue for a limited time (85). Liu *et al.* demonstrated that in ALI, MSCs migrate to the lung, reducing inflammation through direct cell-cell contact (86). Specifically, MSCs showed enhanced therapeutic efficacy after pulmonary lung injury (LPS) versus extra-pulmonary lung injury (LPS/zymosan) due to greater cell recruitment to the lung (86). Islam *et al.* demonstrated that MSCs in the lung transfer cellular products, including mitochondria via gap junctions to epithelial cells, elevating the ATP levels and improving their function and survival (87). Recently, Jackson *et al.* demonstrated that MSCs conducted a transfer of mitochondria to macrophages in EVs, via cell-cell contact through tunnelling nanotubules (TnTs), which induced the transformation of these macrophages to a highly phagocytic phenotype and ameliorated *E. coli*-induced lung injury *in vivo* (88).

Soluble MSC secretome

Numerous studies have shown that MSCs exert part of their therapeutic efficacy via paracrine mechanisms through the release of an array of soluble molecules known as the 'MSC secretome'. Curley *et al.* demonstrated that MSC conditioned medium (MSC-CM) containing the MSC secretome attenuated injury and enhanced repair in a VILI rat model partly by a keratinocyte growth factor (KGF)-dependent mechanism (63). However, Hayes *et al.* demonstrated in the same animal model, that MSCs produced a better early phase recovery in blood oxygenation and respiratory compliance, and reduction in lung edema when compared to the MSC-CM (89). In another study, MSC-CM caused the down-regulation of inflammatory NF-κB signalling in the lung, which reduced the expression of Bcl-x and Mcl-1 in neutrophils and induced apoptosis of these cells in an endotoxin-induced ALI mouse model (90). In an LPS-induced ALI mouse study it was further demonstrated that MSC-CM produces an improvement in the physiology and histology of the lung (91). Furthermore, this study showed that MSC-CM can induce the differentiation of monocytes to an M2 macrophage phenotype, enhancing the anti-inflammatory and pro-healing environment in part due to the production of insulin-like growth factor (IGF-1) from MSCs (91).

MSC-derived EVs and exosomes

As mentioned previously, MSCs release EVs which incorporate cellular components including mitochondria (87), and gene products such as mRNA and microRNAs (miRNA) (92). Zhu *et al.* demonstrated that these EVs reduced extravascular lung water and protein levels, decreased pulmonary edema, reduced the alveolar influx of neutrophils, and decreased alveolar macrophage inflammatory protein-2 concentrations after endotoxin-induced ALI in mice (93). Monsel *et al.* observed that human MSC-derived EVs enhanced survival in a mouse model of *E. coli* pneumonia, increased ATP levels of epithelial cells, reduced bacterial load, and decreased protein and inflammatory cytokine concentrations—effects that were mediated in part by KGF secretion (92). In recent ARDS research, MSCs were shown to promote an anti-inflammatory and highly phagocytic macrophage phenotype through EV-mediated mitochondrial transfer, reducing lung damage (94,95). Song *et al.* showed that MSCs stimulated with IL-1β, produce exosomes with high concentration of miR-146a, an anti-inflammatory micro-RNA. This exosomal miR-146a was transferred to macrophages and resulted in M2 polarization. When these exosomes were administered to septic CLP mouse models they lead to an increased survival and were internalised by macrophages *in vivo* (96). These properties suggest a potential use of stem cell derived EVs for therapy in lung diseases and gives insight to the mechanism of action of MSCs *in vivo*.

Strategies to enhance MSC therapeutic potential for ARDS

Different methods have been employed to improve the therapeutic effect of MSCs. MSCs treated with poly (I:C), a toll-like receptor-3 ligand, inhibited micro-RNA-143 which increased MSC expression of cyclooxygenase-2, leading to increased PGE_2 production and enhanced MSC effects on macrophage function in an *in vivo* CLP sepsis model (97). Human MSCs overexpressing soluble IL-1 receptor-like-1, the IL-33 antagonist, attenuated endotoxin-induced ALI (65). Recently, Han *et al.* demonstrated that MSCs transduced with the E-prostanoid 2 receptor enhanced their migration to the injured lung, decreased lung inflammation and reduced endothelial permeability (98). Cai *et al.* showed that overexpression of orphan receptor tyrosine kinase (ROR2) facilitates MSCs to repair lung injury, enhancing their retention in the lung, and reducing inflammation and pathological impairment (99). MSCs overexpressing the anti-inflammatory cytokine IL-10 resulted in enhanced migration and engraftment,

increased wound healing and improved survival rates in a murine model of endotoxin-induced ALI (44).

Another strategy being investigated is the overexpression of proteins that may have a key function in tissue repair. Wang *et al.* found that human placental MSCs overexpressing platelet-derived growth factor (PDGF) receptor (PDGFR)-β exhibited greater proliferation rates, expressed higher levels of pro-angiogenic factors such as Ang1, VEGF, bFGF and PDGF, and thus enhanced wound repair (100). Overexpression of ANG-1 in MSCs was more effective than naïve MSCs in reducing endotoxin-induced alveolar inflammation and lung permeability (101). Min *et al.* demonstrated that MSCs overexpressing angiotensin-converting enzyme 2 (ACE_2) caused an enhanced reduction in inflammation, and reduced lung edema, collagen deposition and fibrosis after bleomycin-induced lung injury in mice (36). The overexpression of other genes, such as fibroblast growth factor 2 (FGF-2) or KGF have been demonstrated to enhance MSC efficacy in attenuating endotoxin-induced lung injury (102,103).

Other studies have also aimed to improve MSC functionality or longevity; Liu Y *et al.* showed that inhibition of miRNA-24a enhances survival of BM-MSCs under oxidative stress (104), however the functionality of these MSCs after rescue was not ascertained. Zhang *et al.*, demonstrated that nuclear factor (erythroid-derived 2)-like 2 (Nrf2) transfection of human amniotic mesenchymal stem cells enhances the efficacy of these stem cells to reduce lung damage in an LPS ALI model by decreasing epithelial apoptosis and inflammatory cytokine production (43). Further to this, combination therapies have been investigated to complement and enhance the MSC effect *in vivo*. The use of a sphingosine 1 phosphate (S1P) analogue FTY720, previously shown to be effective in murine lung injury models (105), administered with UC-MSCs has been demonstrated to yield a better outcome than either treatment alone in terms of mortality and lung injury indices (45). While Chen *et al.* showed the enhanced effective protection against ARDS with peritoneal sepsis by combined administration of AD-MSCs and pre-activated disaggregated platelets (106). Other substances which may prove beneficial in combination therapy with MSCs include nebulized heparin which has been shown to inhibit coagulation and inflammatory pathways and modulate alveolar macrophages in ALI (107), or using targeting molecules such as glycogen synthase kinase 3 beta inhibitor (GSK-3β) which improves indices of lung injury and promotes the differentiation of MSCs to AT-II cells in *in vivo* pre-clinical ALI (108)

Progress and challenges

Clinical studies in ARD

Based on their therapeutic promise in pre-clinical studies, MSCs have entered early phase clinical testing in patients with ARDS. Zheng *et al.* administered allogeneic MSCs to 12 ARDS patients in an intravenous dose of 1 million cells per kilogram, or placebo in a 1:1 ratio (NCT01902082). A study by Wilson *et al.* included nine patients that received 1, 5, or 10 million cells per kilogram of a single intravenous dose of allogeneic human BM-MSCs, using a three-by-three dose escalation design (NCT01775774). Both studies have shown that MSCs appear to be well tolerated by ARDS patients (*Table 2*). Zheng *et al.* also measured IL-6, IL-8, and surfactant protein D levels and found that surfactant protein D concentrations were lower after MSC delivery at day 5 but that MSC administration had no effects on IL-6 or IL-8 levels. There were no significant differences in total length of hospital stay, ICU–free days, and ventilator–free days between treatment arms (*Table 2*) (109). Another study reported beneficial results of MSCs when administered to two patients in a compassionate use setting (110).

Barriers to clinical translation

Despite these important advances, several issues must still be investigated before MSCs can be considered as a potential "off the shelf" treatment. Robust assays of MSC batch potency for ARDS are lacking. Factors such as the stage of ARDS, type of MSCs, viability and purity of MSCs, and donor variability, are all poorly understood. The timing of MSC therapy is also relevant, with pre-clinical studies to date generally focused on early MSC delivery. A concern regarding delivery is that during intravenous administration due to the risk of MSC clumping into micro emboli, an obstruction of the pulmonary circulation could occur. The longer-term effects of MSC administration should also be considered with a concern that MSCs could potentially enhance tumorigenesis either by direct malignant transformation or indirectly by facilitating growth of tumor cells, although studies suggest that this is quite unlikely. Frozen cells are often used in studies, as this is necessary for cell transport to clinical sites. In contrast, the majority of preclinical studies use freshly harvested cells. The optimization of cryopreservation strategies for MSCs that maintain cell viability, potency, and efficacy is an important translational challenge.

Table 2 Clinical studies of MSC safety and efficacy

Study title	Clinical trial identification/ study phase	Cell type used	Dose/frequency/ route	Country	Expected end date/publication reference
Treatment of severe acute respiratory distress syndrome with allogenic bone marrow derived MSCs	NCT02215811, Phase I	Allogenic BM-MSCs	Not stated	Sweden	December 2015
Human umbilical cord derived mesenchymal stem cell therapy in acute lung injury (UCMSC-ALI)	NCT02444455, Phase I/II	UC-MSC	5×10^6/kg ×3 doses, IV	China	December 2017
A phase 1/2 study to assess MultiStem therapy in ARDS (MUST-ARDS)	NCT02611609, Phase I/II	MultiStem BM-MSC	Not stated	USA/UK	November 2018
Mesenchymal stem cells (MSCs) for treatment of ARDS (ARDS) in stem cell transplant patients	NCT02804945, Phase II	Allogenic BM-MSC	3×10^6 cells/kg, single dose, IV	USA	February 2020
Repair of ARDS by stromal cell administration (REALIST)	NCT03042143, Phase I/II	Cyndacel-C BM-MSC	Dose escalation: 1×10^6, 5×10^6, or 10×10^6 cells/kg, single dose, IV	UK	September 2020
Human mesenchymal stem cells for ARDS (START-2)	NCT02097641, Phase IIa	Allogenic BM-MSC	1×10^7 cells/kg IV	USA	February 2018

UC, umbilical cord; BM, bone marrow; ARDS, acute respiratory distress syndrome; MSC, mesenchymal stromal/stem cell; IV, intravenous.

Commercial production of MSC for the clinical setting

The number of patients receiving MSC based therapies is growing and this increasing demand must be managed legitimately to avoid complications in production and use. The challenges facing the successful marketing of cell therapies has been discussed in several articles (111) which highlight critical hurdles including the scalability, manufacturing and distribution concerns, navigating regulation, cost management, and indeed dealing with the complexity of the cells themselves. In addition to this, focus must be placed on patient safety with regulations needed to avoid exploitation of patients who partake in what is termed 'stem cell tourism' and regarding the unlicensed use of stem cell-based therapies (112).

For cell therapy to be viable in patients with critical illnesses, cells must be available within hours and in sufficient numbers from a reproducible production process and be available at relatively low cost. Therefore, there is a need for a strict production process which is highly regulated and licenced by an international authority, however it has been stated that due to complexities of disease states, cell types, and administration procedures, a strict repeatable, reproducible process in cell production may be unfeasible (113). A recent study was conducted to better understand the relationships between commercial and regulatory environments regarding cell-based therapies and products in Canada (114) concluding that there is a 'reverse governance process' whereby the regulatory authority relies on the scientific input of researchers and developers to develop the framework. Currently, commercially produced MSCs are manufactured to the companies own (often patented) protocols and are thus the preferred method of MSC acquisition for research rather than 'in house' production due to a reduction in variability and an availability of large quantities of cells. However, there are then concerns regarding commercial interests from company entities, the discrepancies in protocols between products, and the ability for research groups to fund and manage clinical trials without vested interests coming into play. The efficacy of MSCs in ARDS has yet to be proven in a large scale clinical trial, requiring the availability of huge quantities of clinical-grade, validated MSCs which would necessitate the involvement of a commercial process. Indeed, there are many issues that need to be addressed to aid in the progress of the development and routine use of cell therapies.

Conclusions

Many challenges remain before MSC therapy becomes the "go to" treatment for ARDS. Further effort is required to optimise their isolation, preparation, and administration in addition to having a better understanding of their mechanism of action. The need for a more abundant source of MSCs is apparent, with UC- and AD-derived MSCs potentially filling this need. Preclinical studies have demonstrated the abundant therapeutic potential of MSCs in ARDS, specifically their capacity to modify the inflammatory response and promote repair. Although MSC treatment seems encouraging in patients in different phase I and II clinical trials, there are still significant hurdles to overcome before these cells can be a truly viable therapy in the clinical setting. The therapeutic efficacy of MSCs can be enhanced by various methods including pre-activation and gene therapy but a complete understanding of their mechanism of action will clarify specific targets to create the most effective phenotype. In understanding the mechanism of action of MSCs, markers of cell potency can be identified, making selection and batch testing more reliable and repeatable, thus ultimately paving the way to an enhanced MSC therapy for the ARDS patient. In an ideal clinical scenario MSCs will constitute a readily available, off the shelf product, with reliable, reproducible effects, that can be tailored to the condition and patient being treated, at an affordable price. In some aspects we are not far from this reality, however some critical hurdles must be overcome to fulfil the criteria of an 'ideal medicine'.

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Footnote

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