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Mesenchymal stromal cells for acute respiratory distress syndrome (ARDS), sepsis and COVID 19 infection: optimising the therapeutic potential

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Summary

Mesenchymal stromal (stem) cell (MSC) therapies are emerging as a promising therapeutic intervention in patients with Acute Respiratory Distress Syndrome (ARDS) and sepsis due to their reparative, immunomodulatory, and antimicrobial properties.

Areas covered: This review provides an overview of Mesenchymal Stromal Cells (MSCs) and their mechanisms of effect in ARDS and sepsis. The preclinical and clinical evidence to support MSC therapy in ARDS and sepsis is discussed. The potential for MSC therapy in COVID-19 ARDS is discussed with insights from respiratory viral models and early clinical reports of MSC therapy in COVID-19. Strategies to optimise the therapeutic potential of MSCs in ARDS and sepsis are considered including preconditioning, altered gene expression, and alternative cell free MSC derived products, such as extracellular vesicles and conditioned medium.

Expert opinion: MSC products present considerable therapeutic promise for ARDS and sepsis. Preclinical investigations report significant benefit and early phase clinical studies have not highlighted safety concerns. Optimisation of MSC function in preclinical models of ARDS and sepsis has enhanced their beneficial effects. MSC derived products, as cell free alternatives, may provide further advantages in this field. These strategies present opportunity for the clinical development of MSCs and MSC derived products with enhanced therapeutic efficacy.

Keywords

Acute Respiratory Distress Syndrome, ARDS, Advanced Therapeutic Medicinal Product, ATMP, COVID-19, Mesenchymal stromal cell, MSC, Sepsis, Septic Shock

1.0 Introduction

Acute Respiratory Distress Syndrome (ARDS) and sepsis are associated with high mortality, morbidity and economic cost in critically ill patients. ARDS is defined clinically by the acute onset of hypoxaemic respiratory failure with bilateral opacities on chest imaging, not explained by the presence of heart failure or fluid overload [1]. The Third International Consensus Definition of Sepsis (Sepsis 3) describes sepsis as life-threatening organ dysfunction caused by a dysregulated host response to infection [2]. In septic shock, the most severe subset of sepsis, underlying circulatory, cellular and metabolic abnormalities are profound enough to substantially increase mortality. ARDS and sepsis are common in critically ill patients, accounting for 10% and >20% of ICU admissions respectively [3,4]. Mortality rates between 35% to 46% are reported for patients with ARDS [3]. Patients admitted to ICU with septic shock have an in-hospital mortality of 39% [5]. Long term morbidity in ARDS survivors includes persistent limitation of exercise capacity and psychological symptoms [6,7]. In sepsis survivors, cognitive decline, increased cardiovascular risk, and a reduction in quality of life at 5 years have been reported [8]. In the UK estimates of cost for ARDS and sepsis are £28,000 and £30,000 per patient per year at 1 year [9,10].

ARDS and sepsis frequently co-exist in critically ill patients and common pathogenic mechanisms are recognised. An initial insult drives a subsequent cascade of inflammatory pathways, activation and recruitment of immune cells, and the release of potent pro-inflammatory mediators. Endothelial and epithelial dysfunction occur in both syndromes as the result of inflammatory mediated tissue damage [11,12]. In ARDS, disruption of the alveolar epithelial – endothelial interface results in the efflux of an inflammatory exudate into the alveoli with loss of lung compliance and impaired gas exchange [12]. In sepsis, loss of integrity of the vascular endothelium with resultant increased vascular permeability causes widespread tissue oedema and multiorgan dysfunction [11]. ARDS and sepsis can occur in response to a variety of pulmonary and non-pulmonary infections and ARDS may be precipitated by non-infective insults such as trauma, surgery, and burns [3]. Most recently, COVID-19 has emerged as a precipitant of ARDS. International Severe Acute Respiratory and Emerging Infections Consortium (ISARIC) data suggests 17% of hospitalised patients require critical care and ARDS is reported in up to 85% of critical care patients [13,14].

Standard therapy for ARDS includes lung protective ventilation, conservative fluid management, and prone positioning [15]. Sepsis is managed by antibiotic therapy, source control, and organ supportive therapy;

haemodynamic resuscitation is a pillar of supportive therapy while immunomodulation (with corticosteroids and potentially vasopressin) has potential for some [16]. Numerous clinical trials of pharmacological interventions in ARDS and sepsis have been conducted with limited success [17]. In a recent Phase 3 randomised controlled trial, early dexamethasone administration in ARDS demonstrated benefit, significantly reducing mortality (21 % dexamethasone group, 36 % control group, $p = 0.0047$) [18]. However, this was a relatively small study (139 patients in dexamethasone group, 138 patients in control group) and follows a background of conflicting results from previous trials and meta-analysis of other corticosteroids [17]. In COVID-19, clinical trials have been rapidly mobilised and evidence is emerging of the benefit of an antiviral (Remdesivir) and interestingly also dexamethasone [19,20]. The benefit of dexamethasone is believed to be due to its potent immunosuppressive effects. Mesenchymal stromal cells (MSCs) are an emerging novel cellular therapy which also harness immunomodulatory, reparative, and antimicrobial properties which may be of benefit in targeting the complex pathogenesis of ARDS and sepsis [21]. In this review, we explore the current mechanistic understanding, preclinical, and clinical evidence for MSCs in ARDS and sepsis. MSCs as a potential therapy for COVID-19 ARDs is explored. Optimisation of MSC products for clinical translation is considered.

2.0 Mesenchymal Stromal Cells (MSCs)

The International Society of Cellular Therapy (ISCT) define Mesenchymal Stromal Cells (MSCs) according to the following criteria: 1) plastic adherent; 2) surface antigen expression profile (Positive (>95%) for CD105, CD73 and CD90; Negative (< 2%) CD45, CD34, CD14/CD11b, CD79a/CD19, HLA-DR); 3) multipotent differentiation potential with *in vitro* potential to differentiate into osteoblasts, adipocytes and chondroblasts [22]. In the United Kingdom and European Union, MSCs, as therapeutic products, are regarded as advanced therapy medicinal products (ATMP, Directives 2001/83/EC and 1294/2007/EC) and must adhere to relevant ATMP regulatory requirements.

Previous descriptions of MSCs as a 'stem cell' reflected their ability to differentiate into multiple cell lines *in vitro*, however, *in vivo* engraftment and differentiation capacity is now known to be limited [23-27]. MSCs can be isolated from multiple sources including bone marrow, adipose tissue, perinatal tissues (including placenta, umbilical cord blood, and umbilical cord tissue known as Wharton's Jelly), dermal tissue, dental tissue and peripheral blood [28,29]. Allogeneic donation of MSCs, even from HLA-unmatched donors, is possible due to lack of expression of MHC Class 2 antigens on MSC surfaces and evasion of T-cell lymphocyte recognition [30]. This is advantageous in critically ill patients as the potential for autologous administration is limited by the time required for culture and expansion of an autologous cell product and the additional risk of an invasive procedure to source the starting material.

Cell-free products derived from MSCs may provide alternatives to MSCs that exploit their therapeutic properties. Extracellular vesicles (including microvesicles (MVs), nanovesicles (NVs) and exosomes) are anuclear, membrane bound vesicles released by many cell types, including MSCs [31]. They are important mediators in intracellular communication, transporting substances such as proteins, mitochondria, DNA, and RNA. MSC conditioned medium (CM) contains the MSC secretome and may exploit paracrine mediated therapeutic properties of MSCs [32].

3.0 Mechanism of action in ARDS and Sepsis

Figure 1 illustrates the immunomodulatory, reparative, and antimicrobial effects of MSCs in ARDS and sepsis.

3.1 Immunomodulatory effects

In ARDS and sepsis, MSCs shift the balance of the inflammatory environment with downregulation of pro-inflammatory cytokines and upregulation of anti-inflammatory cytokines [24,25,33-52]. MSCs directly secrete soluble factors which exert modulatory effects on immune cells, these include IL-6 [37,53], IL-10 [54] transforming growth factor β (TGF-β) [53], indoleamine 2,3-dioxygenase (IDO) [53], intercellular adhesion molecule (ICAM) [55], TNF-stimulated gene protein-6 (TSG-6) [52,53], prostaglandin E2 (PGE_2)[25,37], among others. While the relative contribution of each of these factors to the beneficial actions of MSCs in ARDS and sepsis remains to be elucidated, some have been investigated in *in vivo* models. Zhang *et al*, 2014, demonstrated in a murine lipopolysaccharide (LPS) model, knockdown of TSG-6 in MSCs by siRNA abrogated the anti-inflammatory effects of MSCs [52]. In a murine caecal ligation and puncture (CLP) sepsis model, MSCs released PGE_2 which acted on macrophage prostaglandin receptors increasing IL-10 production [25]. In this model, pre-treatment with IL-10 antibodies abrogated the beneficial effects of MSCs. This illustrates the important role of IL-10 in mediating the anti-inflammatory effects of MSCs, though in *in vivo* models of ARDS and sepsis only modest, and often non-significant, increases in serum or alveolar IL-10 levels have been seen [33-37]. In contrast the cytokine IL-6 has been reported to be significantly reduced by MSC administration in several models of ARDS and sepsis [37-45]. IL-6 is often considered to be a pro-inflammatory cytokine, therefore it is of interest that in a rodent LPS model, administration of IL-6 knockdown MSCs abrogated many of the therapeutic effects of MSCs [53], supporting evidence that IL-6 exhibits both pro- and anti-inflammatory properties [56]. Other important cytokines have also been shown to be modulated in models of ARDS and sepsis, including IL-1α [46], IL-1β [33,38,46-48], IL-1 receptor antagonist [49], TNF-α [24,38,40,45,46,50,51], IFN-γ [41] and chemokines RANTES (CCL5) [40,47,52], MCP-1 (CCL2) [47,52] and IL-8 (CXCL8) [40,47].

The immunomodulatory effects of MSCs regulate innate and adaptive immune cellular responses, including neutrophils [44,51,57,58], monocytes/macrophages [35,59,60], dendritic cells [61] and T cell populations [50,62]. Nemeth *et al*, 2009, demonstrated in a murine CLP model of sepsis, beneficial effects of MSCs were absent in mice lacking monocytes and macrophages [25]. Similarly, in an *Escherichia coli (E.coli)* pneumonia

murine model, depletion of alveolar macrophages abrogated the beneficial effects of MSCs on bacterial clearance and cytokine regulation [40]. In sepsis models, MSC administration increases circulating neutrophils, however, less tissue and alveolar infiltration is seen [25,35,39,44,51,58,63]. Administration of MSCs in a murine LPS model reduced the formation of neutrophil extracellular traps (NETS), which have been implicated in neutrophil mediated tissue destruction in ARDS [39]. In murine models of CLP induced sepsis, MSCs increased circulating T-regulatory cells [62,63]. Laroye *et al*, 2019, demonstrate Wharton's Jelly MSCs significantly increased circulating T-regulatory cells compared to bone marrow MSCs, alongside improvements in bacterial clearance and survival [63]. Chao *et al*, 2014, demonstrate both umbilical cord and bone marrow derived MSCs increased circulating T-regulatory cells *in vivo*, and *in vitro* restored their immunosuppressive capacity. However, in this CLP model, while a trend towards improved survival was seen it was not significant and bacterial clearance was not evaluated [62]. Further, in a *Klebsiella* pneumonia murine model, in which MSC administration was associated with improved survival, there was reduced pro-inflammatory IL-17 and IFN-γ expressing CD4+ T cell subsets within the lungs without any increase in lung bacterial loads [50]. Decreased dendritic cell (DC) aggregation in lung tissue has been demonstrated in models of ARDS [50,61]. *In vitro* studies suggest MSCs induce DCs to a regulatory phenotype via hepatocyte-growth factor (HGF) dependent mechanisms [61]. These studies demonstrate MSCs modulate immune cell distribution and restore dysregulated immune cell function in ARDS and sepsis. Modulation of macrophage function appears particularly important in mediating MSC effects and is discussed further in relation to their antimicrobial effects.

3.2 Reparative effects

MSCs restore structure and function in multiple organs in models of ARDS and sepsis. Histological evidence of lung injury in models of ARDS is reduced by MSCs, with a reduction in alveolar haemorrhage, oedema, hyaline membrane formation, and collagen deposition [33,34,37-39,43,46,48,54,64-66]. In a murine CLP model, MSC administration reduced evidence of histological injury in the small bowel, kidney, and liver illustrated by a reduction in subepithelial spaces in small bowel villi, tubular epithelial cell swelling, degeneration in the kidney, and liver necrosis [57]. In a similar model, MSC administration reduced cardiac interstitial oedema and restored cardiac myocyte architecture [54]. In small and large animal models of ARDS, MSCs restore functional measures including oxygenation and lung compliance [44,51,64,65,67,68]. MSCs improve biochemical markers

of organ function in small and large animal models of sepsis including markers of renal dysfunction (urea, creatinine), liver injury (AST, ALT), and lactate [25,36,45,47,63,67-69]. The restorative mechanisms of MSCs include anti-apoptotic and anti-oxidant activity. A reduction in cellular apoptosis in the endothelium, epithelium, and organs has been demonstrated following MSC administration in models of ARDS and sepsis [25,33,36,41,48,66,69,70]. Sepsis induced acute kidney injury (AKI) has been attenuated by MSC administration in a rodent CLP model with improved tubular function, electrolyte excretion, free water clearance, and restoration of urinary osmolality [41]. Anti-oxidant activity, including upregulation of Klotho protein and restoration of endothelial nitric oxide synthesis (eNOS), in part mediated the protective effects of MSCs in this model [41].

Restoration of epithelial and endothelial function is a key target of MSC activity in models of ARDS and sepsis and mechanisms facilitating this include paracrine growth factor secretion by MSCs and transfer of messenger RNA (mRNA), micro RNA (miRNA), and mitochondria from MSCs and their extracellular vesicles (EVs). In models of ARDS, functional restoration of the alveolar epithelial-endothelial barrier has been demonstrated, indicated by a reduction in bronchioalveolar fluid (BALF) protein and measures of lung oedema [24,33,43,44,48,51,66,67,71,72]. Several growth factors, including keratinocyte growth factor (KGF), vascular endothelial growth factor (VEGF), and hepatocyte growth factor (HGF) have also been shown to mediate the reparative effects of MSCs on endothelial and epithelial cells [33,48,58,73,74]. In addition to paracrine secretion of KGF, Zhu *et al*, 2014, demonstrated KGF mRNA content in MSC microvesicles (MVs) contributed to the immunomodulatory and reparative effects of MSCs [72]. In this murine LPS model, the administration of MVs from MSCs pre-treated with KGF siRNA had diminished beneficial effects compared to control MSCs. In a human EVLP model, MSC MV administration reduced perfusate Syndecan-1, a marker of endothelial glycocalyx breakdown, and increased alveolar Angiopoietin-1 (Ang-1), a regulator of endothelial stability [73]. Tang *et al*, 2017, supported the role of Ang-1 mRNA content within MVs, demonstrating Ang-1 mRNA-deficient MVs have diminished protective effects in a murine LPS model [75]. *In vitro* studies have supported the ability of MSC EVs to shuttle mRNA to target cells, with subsequent translation of mRNA to its corresponding protein within the target cells [74].

Restoration of endothelial integrity requires the presence of intact adherens junctions and tight junctions and MSC administration has been shown to enhance the formation of key proteins in these structures, including

VE-cadherin, Occludin-1, Claudin-1, and B-catenin [33,48,71,76]. In sepsis endothelial dysfunction is believed to have a role in microvascular disturbance and MSCs may have a protective role in regulating this. In a murine intraperitoneal sepsis model, administration of IFN-γ preconditioned MSCs reduced leucocyte adherence to the endothelium, increased leucocyte flow through the microvasculature, and increased the velocity of red blood cell flow through the microvasculature [77]. In this model, plasma levels of E-selectin, a leucocyte-endothelial cell adhesion molecule, were reduced by naïve and IFN-γ preconditioned MSCs. Plasma ICAM-1, another adhesion molecule, was increased by IFN-γ preconditioned MSCs, and it is postulated this may have a protective effect on leucocyte adhesion by competing with membrane bound ICAM-1 [77].

Mitochondrial transfer from MSCs and their MVs has a role in the restoration of cellular function. Islam *et al*, 2012, demonstrated MSCs transfer functional mitochondria to alveolar type II (ATII) cells via MVs and nanotubules in a connexin-43 dependent manner [78]. Mitochondrial transfer to ATII cells by MSCs restored cellular bioenergetics and ATP dependent surfactant secretion in a murine LPS model [78]. In a murine CLP model, administration of MSCs in the early stages of sepsis restored functional mitochondria in muscle satellite cells, improved myofiber formation at 21 days, and improved functional parameters of muscle strength [42]. Thus, demonstrating mitochondrial transfer from MSCs has long term reparative effects on muscle regeneration in sepsis.

One caveat, and an as yet incompletely understood aspect of MSC biology, is the role of the microenvironment in driving MSC behaviour *in vivo*. Islam *et al*, 2019, have described both beneficial and detrimental MSC effects in varying murine models of acute lung injury [79]. In particular, MSCs, administered to animals injured with hydrochloric acid (causing high levels of IL-6, fibronectin, and low antioxidant capacity), promoted lung fibrosis. This was overcome in a model in which the lung microenvironment was corrected prior to the administration of MSCs by administering the antioxidant GPx-1. In a similar vein, Abru *et al*, 2019, have reported that MSCs exposed to non-ARDS BALF were superior in promoting an anti-inflammatory phenotype in monocytes than those exposed to ARDS BALF [80]. Further work is required to understand these phenomena and to allow for more targeted therapy.

3.3 Antimicrobial effects

MSCs improve bacterial clearance in microbial induced models of ARDS and sepsis [24,34,35,38,45-47,62-65,81-85]. MSCs have been shown to secrete antimicrobial peptides (AMPs) with direct antimicrobial effects, including LL-37, lipocalin-2, β defensin-2, and hepcidin [24,34,45,46,81]. Sung *et al*, 2015, demonstrated the antibacterial effects of MSCs against *E.coli in vitro* and *in vivo,* in a murine *E.coli* model, are mediated by secretion of β defensin-2 via toll-like receptor (TLR4) signalling [46]. TLRs recognise microbial ligands (known as pathogen associated molecular patterns; PAMPS) and have a key role in the activation of innate immune cells but are also found on MSCs [86]. *In vitro,* MSCs preconditioned with the TLR3 ligand, Poly (I:C), demonstrated enhanced production of soluble factors including COX-2, IDO, IL-6, and IL-8 and inhibited the release of pro-inflammatory cytokines, including TNF-α and IL-1β, from macrophages [85]. In a murine CLP model, Poly (I:C) preconditioned MSCs enhanced antimicrobial effects and reduced circulating pro-inflammatory cytokines IL-6, TNF-α, KC (the murine orthologue of IL-8), and CCL5 [85]. *In vivo*, MSC TLR activation by endogenous PAMPs may have a role in MSC activation and regulating their antimicrobial activity.

MSCs enhance the function of monocytes and macrophages, demonstrated by increased phagocytic activity and bacterial killing capacity [35,58,83,87]. MSCs also induce alternative state activation of macrophages [35,59,87,88]. Interestingly, Rabani *et al*, 2019, demonstrated MSCs can induce macrophage activation to both M1-like and M2-like phenotypes with distinct antimicrobial effects (M1) and anti-inflammatory (M2) effects [87]. This may in part explain why MSCs can both enhance bacterial clearance while also inducing immunoregulatory effects. The M1-like phenotype was dependent on PGE_2 and phosphatidylinositol 3-kinase and was associated with increased reactive oxygen species (ROS) which may induce bacterial killing via oxidative damage [87]. Song *et al*, 2016, demonstrated exosomal transfer of microRNA (miR-146a) to macrophages polarised to the M2 phenotype and was partially responsible for the beneficial effects of administering IL-1β primed MSCs in a CLP model of sepsis [89]. Others have demonstrated mitochondrial transfer from MSC EVs to macrophages and stimulation by MSC secreted factors, including PGE_2, IDO, and TSG-6, have a role in activation of the M2 anti-inflammatory phenotype [25,59,90,91]. In a rodent faecal sepsis model, induction of macrophage heme-oxygenase 1 (HO-1), an anti-oxidant, has been identified as a further pathway by which MSCs enhance macrophage phagocytosis and bacterial killing [83]. PGE_2 and lipoxin A4 mediated HO-1 expression in this model [83]. These studies identify PGE_2 as a key mediator facilitating the antimicrobial effects of MSCs. In contrast, in a model of *Pseudomonas Aerginosa* pulmonary infection, adipose

derived MSCs suppressed PGE_2 production and exogenous PGE_2 administration reduced the antimicrobial effects of MSCs [84], highlighting the complexity of PGE_2 pathways in MSC signalling.

Macrophage function may be further enhanced by the anti-apoptotic effects of MSCs. *In vitro,* MSCs inhibited LPS induced apoptosis of alveolar macrophages, prolonging their lifespan, in part by suppressing Wnt/B-catenin signalling pathways [60]. In addition to paracrine signalling, Jackson *et al*, 2016, demonstrated the importance of mitochondrial transfer in enhancing phagocytosis by alveolar macrophages [40]. *In vitro* studies demonstrated mitochondrial transfer to macrophages via tunnelling nanotubes (TNT). The consequent enhanced bioenergetic profile of the macrophages was associated with improved phagocytic uptake of bacteria. Inhibition of TNT formation reduced the antimicrobial effects of MSCs. This group have also demonstrated mitochondrial transfer to human monocyte derived macrophages (MDMs) can occur via EVs [59].

4.0 Preclinical Studies of MSCs and MSC-derived products in ARDS and Sepsis

4.1 Preclinical investigations of MSCs in ARDS and Sepsis: Small animal models

Small animal models provide evidence that MSCs are efficacious in ARDS and sepsis. McIntyre *et al,* 2016, conducted a systematic review of preclinical models of ARDS and reported a meta-analysis of mortality outcomes following MSC administration compared with diseased control groups [92]. The majority of studies were conducted in rodent models and injury was induced by a variety of methods including direct and indirect infection, inflammation, trauma, and ventilation. MSC origin was described as syngenic (54%), xenogenic (37%), allogenic (9%), and autologous (3%). In total, 70 studies were reviewed, however, only 17 studies reported mortality outcomes and were included in the meta-analysis (n = 612 in MSC group, n = 1361 in control group). MSCs were reported to reduce the overall risk of death (Odds Ratio (OR) 0.24, 95% confidence interval (CI) 0.18 to 0.34) [92]. A subgroup analysis reported similar protective effects of MSC administration regardless of injury model, MSC source, route of administration, or MSC preparation.

Lalu *et al,* 2016, conducted a systematic review of MSC therapy in preclinical models of sepsis and 41 relevant studies were identified, of which 18 studies reported mortality outcomes and were included in a meta-analysis (n = 504 in MSC group, n = 446 in control group) [93]. All were conducted in rodent models and methods to induce sepsis or sepsis-like injury included caecal ligation and puncture (CLP, 50%), live bacterial injection (10%), and bacterial component injection (40%). MSC origin included xenogenic (50%), syngeneic (40%), allogeneic (5%), and autologous (5%) and sources included bone marrow (60%), adipose tissue (20%), and umbilical cord (20%). A similar reduction in the risk of death was reported following MSC administration as was reported in the preclinical ARDS meta-analysis (OR 0.27, 95% CI 0.18 to 0.4) [93]. Subgroup analysis suggested MSC therapy in sepsis was effective regardless of dosing ($< 1.0 \times 10^6$ cells/kg vs $> 1.0 \times 10^6$ cells/kg), source or timing of administration (< 1 hour vs 1-6 hours following disease induction) of administration. The protective effect of MSC administration was maintained in sepsis models also administering resuscitation fluids and/or antibiotics. In both the ARDS and sepsis meta-analysis, a subgroup analysis of MSC origin did not support a protective effect of autologous MSCs, however, this was derived from a single study evaluating autologous adipose derived MSCs administered in a rodent model via an intraperitoneal route.[94]

Small animal models have provided considerable insight regarding the mechanisms of action of MSCs in models of ARDs and sepsis and consistently demonstrate the immunomodulatory, antimicrobial, and reparative effects, as previously discussed. More recently, small animal models have explored methods of optimising MSC function, which are explored further in Section 6. Limitations of preclinical models should also be considered. Both of the systematic reviews discussed here reported methodological limitations among included studies, including inadequate reporting of randomisation processes and blinding. None of the included studies were considered low risk of bias [92,93]. Furthermore, Lalu *et al*, 2016, reported that publication bias exists, with an overestimation of effect size by 27%; although MSCs remained significantly associated with reduced mortality following adjustment for this (OR 0.34, 95% CI 0.22 to 0.52) [93].

4.2 Preclinical investigations of MSCs in ARDS and Sepsis: Large animal models

Large animal studies of MSC administration in models of ARDS and sepsis have facilitated study of systemic and pulmonary haemodynamics and pulmonary physiology following MSC administration [67,68,95]. MSC administration (10×10^6 cells/kg) 1 hour post injury in an ovine ARDS model, induced by cotton smoke insufflation and live bacterial instillation, attenuated increased pulmonary arterial pressure, reduced lung oedema, improved oxygenation, and increased arterial blood pressure at 24 hours [67]. While there is a theoretical risk of MSCs lodging in the pulmonary circulation, this model demonstrated no difference in pulmonary vascular resistance or pulmonary airway pressures between 5 or 10×10^6 cells/kg doses and controls at 24 hours. Similarly, in a swine oleic acid induced ARDS model, MSC administration (2×10^6 cells/kg) 1 hour post injury demonstrated no early physiological differences following MSC administration, although follow up was limited to 4 hours [95]. Favourable outcomes at 48 hours following adipose derived MSC administration (2×10^6 cells) in an ovine smoke inhalation model have been demonstrated, with a reduction in lung oedema, increased oxygenation, and a reduction in airway pressures [96]. In a swine model of polymicrobial sepsis, umbilical cord MSC administration (1×10^6 cells/kg) improved survival (0% untreated vs 60% MSCs, n = 6 per group, p = 0.08), lowered vasopressor requirements, and improved oxygenation [68]. Furthermore, an improvement in mixed venous oxygen saturations (SvO_2) and a reduction in lactate following MSC administration suggested a reduction in tissue hypoxia. Thus, large animal models of MSC administration in models of ARDS and sepsis have demonstrated safety and improved outcomes.

MSC administration during extracorporeal membrane oxygenation (ECMO) has been investigated in large animal models of ARDS. Millar *et al*, 2020, demonstrated that endobronchial MSC administration in an ovine model of ARDS during ECMO improved indices of shock (vasopressor requirements, arterial pressure, lactate) and histological lung injury, although interestingly did not improve oxygenation or pulmonary mechanics [97]. An earlier study by Kocyildirim *et al*, 2017, had investigated MSC administration during ECMO in a swine LPS model [98]. Similarly, they demonstrated a trend towards reduced histological lung injury in a small study group (n = 3), however, no effects on oxygenation or haemodynamics were reported during a 4 hour period of follow up [98]. Of concern, the study by Millar *et al*, 2020, demonstrated the function of the membrane oxygenator was significantly impaired following MSC administration, with an increase in trans-oxygenator pressure gradient from 4 hours post MSC delivery [97]. MSCs by definition are plastic adherent and cells consistent with MSCs were found to be adherent to membrane oxygenator fibres following their administration during ECMO in this model [97]. This supported previous findings in an *ex-vivo* ECMO model, which similarly demonstrated that MSC avidity for binding to plastic surfaces impaired the function of a membrane oxygenator [99]. The authors concluded that MSC administration could not be recommended during ECMO. In an ongoing trial of MSC therapy in patients with COVID-19 ARDS, patients receiving ECMO are excluded [100].

4.3 Preclinical investigations of MSCs in ARDS and Sepsis: Human ex-vivo lung perfusion (EVLP) models

In human EVLP models of ARDS, induced by live bacteria and endotoxin, administration of human MSCs has demonstrated an ability to restore AFC, reduce lung oedema, and histological lung injury [58,101]. Lee *et al*, 2013, further demonstrated that MSC administration in an *E.coli* EVLP model increased bacterial clearance, mediated by increased alveolar macrophage phagocytosis [58]. In an EVLP model by McAuley *et al*, 2014, MSC administration restored AFC in lungs rejected for transplantation with an impaired baseline AFC < 10% [102].

4.4 Preclinical investigations of MSCs in respiratory viral infection: insights for COVID-19

MSCs have not been investigated in preclinical models of COVID-19. MSC administration in viral models of lung injury may provide insight, however, they have reported conflicting findings (Table 1).

In terms of lung inflammation, injury, and effect on survival, Chan *et al*, 2016, demonstrated in H5N1 infected aged mice, MSC administration 5 days post infection reduced histological lung injury, lung oedema, and pro-

inflammatory cytokines in BALF [103]. In this model survival benefit up to day 18 was demonstrated in aged mice but not in young mice. Loy *et al*, 2019, also investigated MSC administration 5 days post infection in a *H5N1* murine model and demonstrated a reduction in body weight loss, pulmonary vascular permeability, and proinflammatory cytokines [104]. A trend towards improved survival was seen following MSC administration in this model, however, it did not achieve statistical significance [104]. Li *et al*, 2016, investigated early administration of MSCs in a murine *H9N2* influenza model and reported reduced lung oedema, improved gas exchange, and a non-significant trend towards improved survival at day 3 [105]. In contrast, *in vivo* studies of murine and human MSC administration in small animal models of *H1N1* infection have failed to demonstrate benefit in survival, histological lung injury, or inflammatory response [106,107]. However, administration of MSC derived extracellular vesicles (MSC-EVs), as a cell free alternative, has been demonstrated to be beneficial in a large animal swine model of *H1N1* influenza [108]. In this study by Khatri *et al*, 2018, MSC EVs were administered 12 hours post infection and reduced histological lung injury was demonstrated, with only minor inflammatory cell infiltration compared to extensive lung lesions, characterised by inflammatory cell infiltrate, alveolar thickening, and alveolar collapse, which were seen in control *H1N1* infected swine.

Similarly, the effect of MSCs on viral load in these *in vivo* models is conflicting and is summarised in Table 1. Small animal models of *H5N1* or *H9N2* influenza have not supported antiviral activity of MSCs, with no difference in lung viral titres, despite a reduction in inflammation, lung oedema, and trends towards improved survival following MSC administration [104,105,109]. In contrast, Gotts *et al*, 2014, demonstrated a modest reduction in lung viral titres following MSC administration in *H1N1* infected mice, despite the absence of any beneficial effects on histological or biochemical markers of lung injury [107].

In the swine MSC-EV model described above, Khatri *et al*, 2016, demonstrated MSC-EV administration lowered nasal and lung viral titres 100-fold [108]. *In vitro* studies also support the antiviral activity of MSCs. In swine lung epithelial cells (LECs) infected with *H1N1* influenza, MSC-EVs reduced viral replication and viral induced LEC apoptosis [108]. Mechanisms underlying the antiviral mechanisms of MSCs require further investigation. The antiviral activity of MSC-EVs was reduced by pre-treatment of MSC-EVs with Rnase enzyme suggesting antiviral activity is mediated in part by the RNA content of MSC-EVs [108]. Antimicrobial peptides secreted by MSCs may also have a role in their antiviral activity, LL-37 for instance has been shown to have *in vitro* antiviral activity against *influenza A* [110]. MSCs are known to regulate immune cell responses in models of ARDS and

sepsis, including induction of T-regulatory cells which aid viral clearance, but regulation of the immune cell response in respiratory viral models requires further investigation [62]. Importantly, despite the immunomodulatory actions of MSCs, *in vivo* studies have not demonstrated trends towards increased viral activity [103-108].

Variation in the efficacy of MSCs in preclinical viral models may in part be explained by the response of MSCs and the host to different viral strains. *In vitro* studies have demonstrated that viral infection of human alveolar epithelial cells (AECs) with *H5N1* and *H7N9* influenza increased their protein permeability and decreased their fluid clearance, but this did not occur in *H1N1* influenza infected human AECs [103]. Further, *in vitro* studies have demonstrated MSCs possess influenza virus receptors and are susceptible to influenza viral infection, which may alter their functional properties [111]. The absence of such receptors on cell free alternatives, such as MSC-EVs, may contribute to their antiviral activity in the swine *H1N1* model [108]. *SARS-COV-2*, the viral pathogen causing COVID-19, enters host cells by binding with Angiotensin Converting Enzyme-2 (ACE2) receptors on cell surfaces. It is therefore of interest that in a study of clinical grade MSCs for administration to COVID-19 patients the MSC population lacked ACE2 receptors [112]. Preclinical mouse models have limitations in the study of viral infections due to differences in cellular receptors for viral entry and innate immune responses, resulting in reduced susceptibility to human viruses and reduced disease severity [113]. Mice are not natural hosts for influenza virus: the virus requires repeated laboratory passage and adaptation before being able to initiate infection and replication in the murine respiratory tract [114]. This limits the use of murine models to study influenza pathogenesis.

Transgenic mice expressing human ACE2 have been found to be a useful model for studying *SARS-COV-2* and the study of MSCs in this model will provide interesting insights regarding their mechanisms and efficacy in COVID-19 [115].

5.0 Clinical trials of MSCs and MSC derived products in ARDS, sepsis and viral respiratory infections

5.1: Safety in clinical trials

Clinicaltrials.gov (accessed July 2020) records over 1100 registered clinical trials involving MSC therapy. MSC products have been approved for clinical use in Crohn's perianal fistulae and graft versus host disease following successful Phase 3 clinical trials [116,117]. A recent systematic review and meta-analysis of intravascular MSC therapy between 1980 and April 2019 included 55 randomised control trials (RCTs) conducted in 12 different countries with a total of 2696 patients [118]. Conditions included cardiovascular, neurological, renal, liver, respiratory, endocrine, haematological/oncological malignancies, immune deficient and inflammatory conditions, general frailty, and sepsis. Overall, it was demonstrated MSC therapy compared to controls was associated with an increased risk of fever (Relative Risk (RR) = 2.48, 95% CI 1.27 to 4.86). There was no difference between MSC therapy compared to controls in non-fever acute infusion-related toxicity, infection, thrombotic/embolic events, or malignancy (RR = 1.16, 0.99, 1.14, 0.93; 95% CI 0.70 to 1.91, 0.81 to 1.21, 0.67 to 1.95, 0.60 to 1.45). Furthermore, the risk of death was significantly lower in the MSC treated group compared to controls (pooled analysis 40 studies, n = 1991 patients, RR 0.78, 95% CI 0.65 to 0.94), thus demonstrating the potential benefits and limited risks of MSC therapy. Theoretical concerns regarding the tumorgenicity of MSCs have been raised but no signals of tumorigenic potential have been reported in clinical studies [118]. There is emerging evidence of anti-tumorigenic properties of MSCs, and MSC therapy is currently being investigated as a cancer therapy in clinical trials [119]. MSCs are reported to have pro-coagulant effects in *in vitro* and *in vivo* studies, mediated in part through expression of tissue factor, however, this risk has not translated to clinical trials [118,120,121]. Ongoing safety evaluation, including long term follow up, remains important in MSC clinical trials.

An interesting approach to this challenge is by the use of healthy volunteer disease simulating models. MSC administration has been studied in a human *in vivo* model of LPS endotoxaemia. Perlee *et al*, 2018, evaluated the effects of intravenous administration of adipose derived MSCs on the early inflammatory response to intravenous LPS in healthy male subjects in a randomised placebo-controlled study. Participants were randomised to receive either placebo or 0.25, 1, or 4 x 10^6 cells/kg (n = 32, n = 8 per study group) [121]. MSCs were generally well tolerated with no difference in haemodynamic or respiratory parameters. An enhanced febrile response was demonstrated in subjects treated with the highest dose of MSCs (4 x 10^6 cells/kg). Six

adverse events (AEs) were reported, two of which were related to MSC administration, including throat irritation and pruritis. MSC administration modulated inflammatory cytokine responses, with increased IL-8, IL-10 and TGF-β release. The administration of high dose MSCs (4 x 10^6 cells/kg) was associated with a transient increase in markers of coagulation activation, including thrombin-antithrombin complexes (TATc) and d-dimer. LPS administration resulted in a fibrinolytic response, with increased plasma concentration of tissue type plasminogen activator (tPA), which was attenuated by administration of high dose MSCs (4 x 10^6 cells/kg) [121]. *In vitro* studies of the MSCs used in these experiments suggested procoagulant effects were tissue factor dependent. Despite these procoagulant effects there was no evidence of clinically relevant thromboembolic events in this study [121].

5.2 MSCs in ARDS and Sepsis clinical trials (Summary Table 2 and Table 3)

Zheng *et al*, 2014, conducted a small Phase 1 randomised placebo-controlled trial of adipose derived MSC therapy in patients with moderate to severe ARDS. Patients received either a single infusion of MSCs (1 x 10^6 cells/kg) or placebo (n = 6 in each group) within 48 hours of enrolment. No infusion related toxicity or serious adverse events (SAEs) were reported. SP-D levels, a marker of epithelial cell injury, were significantly decreased in the MSC group at day 5 compared to baseline, but did not differ significantly from the control group [122]. Yip *et al*, 2020, conducted a Phase 1 dose escalation study of umbilical cord derived MSCs in patients with moderate to severe ARDS who had failed to respond to conventional therapy at 5 days [123]. Patients received a single intravenous infusion of either 1, 5, or 10 x 10^6 cells/kg (n = 3 per dose). Three mild adverse events (AEs) related to MSC infusion were reported (n = 2 transient desaturation and hypotension; n = 1 generalised rash), however, no dose limiting toxicity or SAEs were reported. Lv *et al*, 2020, conducted an open label, single arm study administering a single intravenous infusion of umbilical cord MSCs (1 x 10^6 cells/kg) in patients with sustained moderate to severe ARDS after 24 hours of conventional therapy (n = 22). No infusion associated events within 24 hours were reported, however, there is a lack of event reporting beyond this [124].

Matthay *et al*, have undertaken Phase 1 and Phase 2 studies of allogeneic bone marrow derived MSC therapy in patients with moderate to severe ARDS (START 1 and START 2) [125,126]. Cryopreserved bone marrow derived MSCs were thawed and washed to remove the cryoprotectant dimethylsulfoxide (DMSO) prior to dilution for administration. In Phase 1, an open label dose escalation trial, no infusion associated AEs were

reported and doses up to 10 x 10^6 cells/kg predicted body weight (PBW) were well tolerated. In Phase 2, a randomised double-blind placebo controlled trial, patients received either a single intravenous infusion of MSCs (10 x 10^6 cells/kg PBW) or placebo (n = 40 MSC group, n = 20 control group) [126]. Patients were excluded if ARDS had been present for more than 96 hours. No infusion related AEs were reported. Mortality at day 28 and day 60 was non-significantly higher in the MSC group compared to the placebo group (Day 28: 30% vs 15%, OR 2.5, p = 0.34; Day 60: 38% vs 25%, OR 1.8, p = 0.40). Baseline APACHE III score was higher in the MSC group, suggesting increased physiological disturbance, and adjustment for APACHE III score reduced the hazard ratio (HR) for mortality (D28 Adjusted HR 1.43, Unadjusted HR 2.15; D60 Adjusted HR 1.19, Unadjusted HR 1.68). A post-hoc analysis revealed cell viability following the wash process was lower than had been expected (range 36% to 85%). Viability dependent effects were seen with angiopoietin-2 (Ang-2), a marker of endothelial damage, which significantly decreased after administration of MSCs with a greater viability. Similarly, an association between improved oxygenation index and greater MSC viability was evident. The START investigators are currently recruiting to a Phase 2b RCT of MSC therapy in moderate to severe ARDS (STAT trial, NCT03818854) aiming to recruit 120 patients to receive 10 x 10^6 cells/kg or placebo within 120 hours following ICU admission.

MultiStem® bone marrow derived multipotent adult progenitor cells (MAPC) have been investigated in the Phase 1/2 MUST-ARDS clinical trial, an open label dose escalation study followed by randomised placebo-controlled trial (NCT02611609). MAPC meet the minimal defining criteria for MSCs but are reported to have a greater proliferation capacity than traditional MSCs [22,127]. Preliminary results report MultiStem® treatment reduced 28-day mortality (from 40 to 25%), increased ventilator free days (from 9.2 to 12.9 days) and increased ICU-free days (from 9.2 to 10.3 days). However, the trial has only been reported in abstract form to date and is underpowered to assess clinical outcomes [128].

Chen *et al*, 2020, published an open label study of menstrual blood-MSC therapy for H7N9 viral induced moderate to severe ARDS [129]. MSCs at a dose of 1 x 10^6 cells/kg were administered at varying stages of ARDS (defined as early or late) and patients received multiple infusions (either 3 or 4). No infusion related toxicity or SAEs were reported. A control group of 44 patients with H7N9 viral induced ARDS was included in the analysis, however, no detail on selection of the control group is provided. Though survival was reported to be higher in the experimental group compared to the control group (82.4% vs 45.5).

McIntyre *et al*, 2018, reported the Phase 1 Cellular Immunotherapy for Septic Shock (CISS) open label, dose escalation trial of bone marrow derived MSC therapy. Patients received a single intravenous infusion of either 0.3, 1 or 3 x 10^6 cells/kg (n= 3 per dose) within 30 hours of admission to ICU. No MSC infusion associated AEs or SAEs were reported [130]. No signals of safety concerns or efficacy were detected in serial biomarker measurements up to 72 hours following MSC administration. Similarly, He *et al*, 2018, conducted an open label dose escalation trial of umbilical cord derived MSCs in patients with sepsis [131]. Patients with onset of severe sepsis within the previous 24 hours were enrolled to receive a single intravenous infusion of either 1, 2 or 3 x 10^6 cell/kg (n = 5 per dose). No SAEs related to the MSC infusion within 24 hours following MSC therapy were reported. Mortality was comparable to a historical case matched control group (20%, 3/15 MSC group vs 26%, 4/15 historical group). Pro-inflammatory biomarkers (including IL-6, IL-8, TNF-α, and CRP) declined between baseline and day 8 but no significant differences were identified between dose cohorts. Surviving patients were followed up for 18 months and no AEs were reported during this time. Thirdly, Galstyan *et al*, 2018, reported an open label, randomised controlled trial of bone marrow derived MSC therapy for septic shock in neutropenic patients (WCC < 0.5 x 10^9/l). Patients received either conventional therapy for septic shock (CT group n = 15) or conventional therapy plus a single intravenous infusion of bone marrow derived MSCs at a dose of 1 x 10^6 cells/kg within 10 hours of onset of septic shock (CT + MSCs group n = 15) [132]. MSC therapy was well tolerated with no infusion related reactions or respiratory or cardiovascular compromise. MSC therapy was associated with increased 28-day survival (CT + MSC 60% (9/15) vs CT only 20% (3/15), $p < 0.05$), however, no between group difference in survival was seen at 3 months and given the small sample size, conclusions regarding efficacy are limited.

5.3 MSCs in COVID-19 trials and reports (Summary Table 4)

MSC therapy in COVID-19 patients has been reported in open label, uncontrolled studies or on a compassionate use basis. Leng *et al*, 2020, reported administration of 1 x 10^6 MSCs/kg to 7 patients with COVID-19 pneumonia who had failed to improve with conventional therapy [133]. A placebo control group (n = 3) were enrolled in the study once all 7 patients in the MSC group had received the MSC intervention. Symptoms were reported to improve following MSC administration and no infusion related AEs were reported. The authors suggest an increase in peripheral blood regulatory T cells and dendritic cells in patients with more severe COVID-19, and reduction in circulating IL-10 and TNF-α levels, provide evidence of immunomodulation

[133]. Chen *et al*, 2020, reported a retrospective review of 25 patients who received MSC therapy (between 1 and 3 infusions of 1 x 10^6 cells/kg) for COVID 19 pneumonia [134]. Data reported is extremely limited, with no detail of baseline physiological status or COVID-19 severity. All patients are reported to have made a clinical improvement and 64% of patients had evidence of radiological improvement, though the definition of what constituted an improvement was not provided and there was no comparator group. Infusion related side effects were reported in 3 patients, including liver dysfunction, heart failure, and an allergic reaction, however, further detail of these events is absent [134]. Sengupta *et al*, 2020, have reported an open label cohort study investigating exosomes derived from bone marrow MSCs (ExoFlo™) as a treatment for severe COVID 19 pneumonia [135]. Eligible patients had been symptomatic for more than 72 hours and had evidence of clinical deterioration, indicated by a decreasing PaO_2/FiO_2 ratio. A single intravenous infusion of ExoFlo™ (15 ml in 100ml saline) was administered to 24 patients with severe COVID 19 pneumonia, including patients who required supplemental oxygen or non-invasive ventilation (n = 21) and patients who required invasive mechanical ventilation due to hypoxaemic respiratory failure (n = 4). No infusion related events or treatment related AEs were observed. Improvement in oxygenation was reported in 80% of patients following ExoFlo™ administration. Acute phase reactants, including CRP, ferritin, and D-dimer were significantly reduced from baseline to day 5 following ExoFlo™ administration [135].

Sanchez-Guijo *et al,* 2020, report administration of adipose derived MSCs to mechanically ventilated patients with COVID-19 pneumonia on a compassionate use basis (n = 13) [136]. Severity of illness at baseline (and whether patients fulfil ARDS criteria) is unclear, however, patients were eligible to receive treatment when there was radiological evidence of > 50% disease progression over the previous 24 to 48 hours. Patients received between 1 and 3 intravenous infusions of a target dose of 1 x 10^6 cell/kg with the total number of infusions dependent on clinical response. No adverse events related to MSC infusion were reported. Of note, patients who were successfully extubated at follow up at 16 days (n = 7, 53%) had received cells earlier than those who were not extubated (median time from intubation to MSC administration, 5 days vs 10 days).

While these studies do not highlight safety concerns regarding the use of MSCs in COVID-19, their small sample size, potential for selection bias, and absence of an appropriate control group limit the conclusions that can be drawn and at present there is no conclusive evidence of efficacy for MSCs in COVID-19. Importantly, after a number of case series, a range of randomised controlled clinical trials of MSC therapy for

COVID-19 has emerged (Table 4, including 8 studies investigating MSC administration in COVID-19 ARDS). Investigation in robust clinical trials is required to determine efficacy of MSC therapy in COVID-19 and it is hoped the rapid emergence of planned clinical trials will address this.

6.0 Optimising therapeutic potential of MSC therapy in ARDS, Sepsis and COVID-19

6.1 Manufacturing Considerations

MSCs are a heterogenous cell population which vary in their phenotype and functional characteristics depending on their source and methods of isolation and expansion, including the passage number (number of subdivisions in culture). Clinical studies in ARDS and sepsis have investigated MSCs from various sources (including adipose, umbilical cord (UC), and bone marrow (BM)), however, the optimal source is yet to be determined. *In vitro* studies have demonstrated UC tissue (Wharton's Jelly) derived MSCs exhibit superior immunosuppressive properties compared to BM-MSCs, adipose-MSCs, and placental MSCs [137,138]. Alcayaga *et al*, 2015, demonstrated menstrual blood-MSCs have enhanced antimicrobial properties *in vitro* compared to BM-MSCs [45]. In a murine CLP model comparing UC-MSCs to BM-MSCs, UC-MSCs exhibited an enhanced anti-inflammatory profile with increased T-regulatory cells within the lungs [139]. UC-MSCs but not BM-MSCs increased bacterial clearance and survival but differences between UC-MSCs and BM-MSCs were not significant. In rodent *E.coli* models, BM-MSCs demonstrated equivalence to UC-MSCs in improving oxygenation, lung compliance, bacterial clearance, BALF neutrophilia, and pro-inflammatory BALF cytokines [64,140]. MSCs from UC or placental sources may be practically advantageous as they are isolated from tissue sources that are readily available and usually biological waste products. They have been reported to have greater MSC density, providing a greater MSC yield, and have enhanced proliferation capacity therefore can be more rapidly expanded than other MSC sources [141].

MSCs are traditionally isolated by plastic adherence, however, advanced isolation techniques using specific cell surface markers may be favoured in future ATMP regulatory requirements. Cell surface markers investigated as potential candidates for advanced MSC isolation include Stro-1, CD271, stage-specific embryonic antigen-4 (SSEA-4), CD246, and CD362 (Syndecan-2). Unfortunately, cell surface markers identified to date have not provided functional advantages and limitations include the lack of universal expression by all MSCs and a lack of correlation with proliferation capacity, multipotency, and functionality [65,142]. CD362 (Syndecan-2) enriched MSCs have been investigated in bacterial and ventilator induced lung injury (VILI) models and demonstrated comparable reparative properties to traditionally isolated MSCs [64,65]. CD362 enriched MSCs are currently undergoing investigation in a Phase 1/2 clinical trial of ARDS and COVID-19 ARDS (NCT03042143) [100]. MSC expansion is essential to achieve sufficient therapeutic cell doses, however, Horie *et al*, 2020, have

demonstrated expansion beyond passage 3 was associated with reduced therapeutic efficacy in a rodent *E.coli* model [64]. Traditionally, foetal bovine serum (FBS) has been used as culture medium for MSC expansion. Xeno-free culture mediums are an alternative which negate theoretical immune and infective risks and may confer functional advantages [143]. In a rodent *E.coli* model, UC-MSCs cultured in xeno-free medium retained their typical MSC phenotype and demonstrated efficacy [140]. Further investigation is required to determine the optimal culture conditions in models of ARDS and sepsis.

Cryopreservation of allogeneic MSC therapies facilitates availability of 'off the shelf' products which are necessary for timely MSC administration in critical illness. Cryopreserved MSCs exhibit preserved immunomodulatory, reparative, and antimicrobial properties in models of ARDS and sepsis [47,64]. If clinical trials of MSCs in patients with sepsis, ARDS, and COVID-19 demonstrate efficacy, and approval for clinical use is granted, upscaling of MSC manufacturing will be required to facilitate patient access on a larger scale. Automated methods to aid large scale MSC production are currently being developed, including AUTOSTEM, a European commission funded research and innovation project [144].

6.2 Preconditioning to enhance MSC activity (Summary Table 5)

Preconditioning (or priming) during the manufacturing process involves exposure of MSCs to stimuli during culture and expansion to enhance their biological function, survival, and therapeutic efficacy. In models of ARDS and sepsis preconditioning with various agents has been investigated but has yet to translate to clinical trials. Horie *et al*, 2020, demonstrated in a rodent VILI model, MSCs primed with a combination of cytokines (IL-1β, TNF-α and IFN-γ) enhanced resolution of histological evidence of VILI, restored oxygenation, improved lung compliance, and reduced lung oedema [145]. Baudry *et al*, 2019, demonstrated that IFN-γ preconditioned MSCs, in a murine sepsis model, had beneficial effects on the microcirculation by increasing the flow of leucocytes, decreasing leucocyte adhesion, and increasing red blood cell velocity [77].

Hypoxic preconditioned MSCs, *in vitro,* demonstrate increased migration, enhanced survival, pro-angiogenic and anti-apoptotic mechanisms [146-148]. Alcayaga *et al*, 2015, have demonstrated MSCs exposed to hypoxia *in vitro* have reduced expression of the antimicrobial peptide hepcidin, though the *in vivo* consequences on antimicrobial activity were not investigated [45]. Li *et al*, 2015, provide limited evidence of the benefit of

hypoxic preconditioned MSCs in a murine LPS model with a reduction in BALF leucocyte and neutrophil infiltration, however, no data were reported on functional outcomes or cytokine analysis [149].

Tsoyi *et al*, 2016, investigated carbon monoxide (CO) preconditioned MSCs (CO-MSCs) in a murine model of CLP sepsis and demonstrated enhanced survival, bacterial clearance, and improved liver and kidney function in comparison to nonpreconditioned MSCs [150]. CO-preconditioning appeared to enhance the therapeutic window for administration with benefits persisting with delayed administration of CO-MSCs. Mechanistic studies by Tsoyi *et al*, 2016, suggested CO-preconditioning enhanced MSC function by enhancing their lipid mediator production, particularly D series resolvins. Silencing of lipoxygenase pathways, involved in lipid mediator biosynthesis, diminished the enhanced efficacy of CO-MSCs *in vitro* and *in vivo* [150]. Eicosapentaenoic acid (EPA), a polyunsaturated fatty acid and precursor of pro-resolving lipid mediators, has also been investigated as a preconditioning agent for MSCs in a murine CLP model [54]. EPA preconditioning enhanced expression of PGE_2, IL-10, resolvin-D1, and TGF-β1 by MSCs *in vitro*. *In vivo*, EPA preconditioned MSCs, compared to nonpreconditioned MSCs, enhanced survival, improved lung mechanics, reduced clinical severity scores, and further reduced evidence of distal organ injury.

Endotoxin from strains of gram negative bacteria (*Pseudomonas, Acinetobacter,* and *Acinetobacter* inactivated lipid A LPS) and *Staphylococcal* enterotoxin B have been investigated as MSC preconditioning agents in murine peritoneal sepsis models [151,152]. Enhanced survival and improved bacterial clearance were demonstrated *in vivo* in mice treated with LPS-MSCs and *Staphylococcal* enterotoxin B-MSCs in comparison to naïve MSCs. In vitro studies suggested *Staphylococcal* enterotoxin B-MSCs and LPS-MSCs enhanced antimicrobial activity in part by enhanced expression of the antimicrobial peptides, Hepcidin and LL-37 [151,152].

6.3 Gene transfection to enhance MSC activity (Summary Table 6)

MSC transgenic studies have demonstrated the potential to enhance MSC function in models of ARDS and sepsis. Downregulation of hippo signalling pathways (via LATS2 knockdown) enhanced the efficacy of MSC administration in a murine LPS model [153]. Hippo signalling regulates cell proliferation and differentiation and its downregulation was shown to enhance survival of MSCs and increase differentiation to ATII cells. Enhanced regenerative properties were illustrated by increased resolution of histological lung injury and a reduction in lung fibrosis indicated by reduced collagen deposition [153]. Overexpression of the Wnt/B-catenin pathway

has also been demonstrated to enhance the reparative effects of MSCs in a murine LPS model with increased MSC retention in the lung and differentiation to ATII cells [76]. Functional benefits of B-catenin overexpressing MSCs included decreased histological lung injury, decreased pulmonary vascular permeability, and a reduction in collagen deposition within the lungs. Increased occludin protein expression, indicating enhanced formation of endothelial tight junctions, was demonstrated with administration of both B-catenin overexpressing MSCs and Hippo downregulated MSCs [76,153]. In contrast, MSCs with knockdown of Stanniocalcin-2 (STC2), a regulator of calcium metabolism with known anti-apoptotic and anti-oxidant properties, diminished the beneficial effects of MSCs in a murine LPS model [154]. STC2 knockdown MSCs *in vitro* demonstrated increased expression of ROS, increased apoptosis, and were less effective modulators of alveolar macrophage activity [154]. STC2 knockdown MSCs had reduced expression of nuclear factor erythroid-2 related factor 2 (Nrf2) and HO-1 genes, which are involved in oxidative stress responses. Nrf2 and HO-1 overexpression by MSCs has been demonstrated to enhance their beneficial effects in LPS models of ARDs [155,156].

The benefit of MSCs overexpressing growth factors, including KGF and HGF, has been demonstrated in models of ARDS. Chen *et al*, 2013, investigated KGF expressing MSCs in a murine LPS model and demonstrated increased KGF mRNA expression and KGF protein levels in lung tissues [157]. KGF expressing MSCs significantly improved lung oedema and microvascular permeability at 24 and 72 in comparison to both naïve MSCs and controls and demonstrated enhanced the immunomodulatory actions, with a reduction in BALF neutrophils and pro-inflammatory cytokines (IL-1β and TNF-α). Surfactant protein production was enhanced by KGF expressing MSCs leading the authors to postulate the enhanced benefit of KGF expressing MSCs is mediated by enhanced proliferation of ATII cells. Furthermore, in a rodent LPS model, the beneficial effects seen with naive MSCs were attenuated by KGF knockdown MSCs [158]. In a murine LPS model, HGF overexpressing MSCs enhanced the beneficial effects of MSCs, reducing lung oedema and reducing histological lung injury, while MSCs underexpressing HGF had reduced beneficial effects compared to naïve MSCs [61]. Administration of MSCs overexpressing Angiopoietin 1 has been shown to be protective in murine LPS models restoring pulmonary vascular permeability to levels comparable to control mice [70,159].

Overexpression of the anti-inflammatory cytokine IL-10 enhances the immunosuppressive and antimicrobial potential of MSCs in live bacteria and endotoxin models of ARDS [160,161]. Jerkic *et al*, 2019, demonstrated IL-10 expressing MSCs enhanced survival, bacterial clearance, and phagocytic activity of alveolar macrophages in

a rodent *E.coli* pneumosepsis model [160]. Wang *et al*, 2018, similarly demonstrated IL-10 expressing MSCs enhanced survival and reduced markers of pulmonary vascular permeability in a murine LPS model [161]. Treatment with IL-10 expressing MSCs produced a persistent increase in serum IL-10 levels, in comparison to treatment with IL-10 alone, which only produced a transient peak in serum IL-10 levels. Thus, demonstrating altered gene expression by MSCs can enhance MSC function and produce sustained modulation of their immunoregulatory effects.

6.4 MSC-derived products as cell free alternatives

MSC extracellular vesicles (EVs), including microvesicles (MVs), nanovesicles (NVs) and exosomes, and MSC conditioned medium (CM), are cell free alternatives, which demonstrate efficacy in models of ARDS and sepsis [32,75,89,162-167]. MVs are small (100-1,000 nm) cell-derived particles containing proteins, mRNA, miRNA, and lipids, which arise from the plasma membrane and are formed by its outward blebbing. Exosomes are similar, however, arise from intraluminal vesicles, are smaller (30-100 nm), and are formed by fusion of intravesicular bodies with the plasma membrane. Both are secreted at a relatively slow rate, while nanovesicles, which share many similarities can be produced by serial extrusions of cells. MSC EVs and MVs are associated with survival benefit in small animal models of *E.coli* pneumonia [165,166]. Similar to MSCs, MSC derived products have demonstrated immunomodulatory, antimicrobial, and reparative effects. Park *et al*, 2019, demonstrate in a model of peritoneal sepsis, that MSC NVs are capable of attenuating the systemic response to sepsis with maintenance of body temperature, a reduction in circulating cytokines, and attenuation of leucocyte responses [163]. In an *E.coli* EVLP model MSC MV administration restored AFC, reduced histological lung injury, and demonstrated antimicrobial properties with enhanced bacterial clearance [168]. While MSC MVs appear to harness many of the beneficial effects of MSCs in models of ARDS and sepsis, they may not be as efficacious as MSCs. Silva *et al*, 2019, demonstrated MSCs are superior to MSC EVs in their ability to restore markers of histological lung injury, modulate alveolar leucocyte infiltration, and reduce lung oedema [164]. In contrast, Monsel *et al*, 2015, demonstrated MSCs and MVs derived from MSCs were similarly efficacious [166]. Similar to MSCs, the potency of MSC MVs can be enhanced by priming approaches. Varkouhi *et al*, 2019, demonstrated MSC EVs from IFN-γ primed MSCs have enhanced efficacy in comparison to MSC EVs from unprimed MSCs [165]. Song et al, 2016, demonstrated MSC exosomes from IL-1β primed MSCs significantly improved survival compared to exosomes from naïve MSCs in a model of CLP sepsis [89].

Several studies in models of ARDS have demonstrated MSC CM provides similar efficacy to MSCs alone [32,44,101]. However, in a rodent VILI model of ARDS, MSCs demonstrated superior efficacy to MSC CM alone during the early recovery from VILI [169]. Nonetheless, MSC CM presents a potential novel cell free alternative to MSCs and further investigation is required to determine if its therapeutic potential could be enhanced by dosing alterations, increased frequency of administration, or priming approaches. Nebulised MSC CM has been shown to maintain its antimicrobial activity *in vitro* and provides a direct route of administration, however, it has not been investigated in *in vivo* models [170]. Potential disadvantages to MSC CM include the challenge of delivering to the site of injury/inflammation and avoiding first pass metabolism in the liver, but also the absence of cells in a CM product means the product cannot respond to the environment, unlike MSCs themselves, which have been likened to a "living drug" [171].

6.5 Optimal dosing regimens

The optimal dosing regimen for clinical administration of MSCs has not been determined. Clinical trials conducted to date in ARDS and sepsis have administered a single intravenous infusion of MSCs, with doses ranging from 0.3 x 10^6 cells/kg to 10 x 10^6 cells/kg. Dose dependent effects have been demonstrated in preclinical models of ARDS and sepsis [34,67,83]. Jerkic *et al*, 2020, have reported beneficial effects in a dose-dependent manner with greatest benefit at a dose of 10 x 10^6 cells/kg (compared to placebo, 2 x 10^6 cells/kg and 5 x 10^6 cells/kg) in rodent models of polymicrobial systemic sepsis [83]. In a human LPS model, dose dependent adverse effects were demonstrated at the highest dose investigated (4 x 10^6 cells/kg) with enhanced febrile response and coagulation activation [121]. In clinical trials, doses up to 10 x 10^6 cells have been well tolerated without infusion related toxicity [125,130]. Dose dependent effects on measures of efficacy have not been demonstrated in clinical trials, however, the primary aim of dose escalation studies using different doses of MSCs has been to determine safety and the numbers of patients (n = 3 to 5) in each dose cohort has been too small to detect clinically significant differences in measures of efficacy [122,123,125,130,131]. It is noteworthy in the START Phase 2a trial, trends towards improvement in oxygenation index and biomarkers of endothelial damage (Ang-2) were greatest in patients receiving the highest number of viable cells, pointing towards potential dose dependent effects.[126] .

Similarly, the efficacy of MSC EVs appears to be dose dependent. In a murine endotoxin model, doubling the MV dose from 15 microliters (uL) (equivalent to MVs released from 1.5 x 10^6 MSCs over 48 hrs) to 30 uL

increased measures of efficacy, however, further doubling to 60 uL conferred no additional benefit [167]. MSC and MSC derived products, such as EVs and CM, are not equivalent in their functional activity. The MSC secretome within a given volume of CM will depend on both the quantity of MSCs it is exposed to and the duration of exposure. MSC EV preparations may vary in their composition, concentration, and activity depending on the nature of the MSCs they are derived from and the protocols used in their manufacture and separation [31]. It is therefore difficult to compare dosing regimens between studies. An effective potency assay to predict therapeutic efficacy would be an important development to allow direct comparison of MSC products, however, at present this is not available.

The frequency of MSC administration is a further consideration. In a rodent *E.coli* model, multiple dosing at 6 hours and 12 hours conferred additional efficacy to MSC administration compared to administration at 6 hours alone [64]. A clear safety signal for MSCs in human subjects is required prior to proceeding to multiple dosing regimens and some investigators have proceeded to this approach based on the safety information currently available. The SEPCELL Phase 2 randomised placebo controlled trial of two intravenous infusions of allogeneic adipose derived MSCs (160 x 10^6 cells per dose at days 1 and 3) in patients with severe community acquired bacterial pneumonia has indicated it completed enrolment (n = 84) in March 2020 and study completion, including follow up to day 90, is expected in July 2020 (NCT 03158727, status available at https://clinicaltrials.gov/ct2/show/NCT03158727, last accessed July 2020). In clinical trials registered to investigate COVID-19 there is variation in both the MSC dose and frequency of administration (see Table 4). The rationale for variation in dosing regimens in this population is unclear and lacks the evidence base usually required during the clinical development process.

7.0 Conclusion

Preclinical investigations provide compelling evidence for the potential immunomodulatory, reparative, and antimicrobial effects of MSCs in models of ARDS and sepsis. Preclinical investigation has shown antiviral and reparative effects in some but not all animal models of influenza. These preclinical insights provide a therapeutic rationale for MSC treatment in patients with ARDS, sepsis, and COVID-19 ARDS. The mechanisms by which MSCs exert their effects are diverse and include paracrine signalling, direct antimicrobial actions, and transfer of mRNA, miRNA, and cellular organelles, including mitochondria, via direct cell contact and extracellular vesicles. Where other therapies in ARDS and sepsis targeting specific pathways have failed to show efficacy, the multiple mechanisms of actions of MSC therapy are hypothesised to address multiple complex pathogenic mechanisms. Clinical trials of MSC therapy in ARDS and sepsis to date have been small, early phase studies with the primary aim of evaluating safety, with reassuring safety data to date. Reports of MSC therapy for COVID-19 have been in the setting of small, uncontrolled studies with methodological limitations. Clinical trials of MSC therapy in ARDS, sepsis, and COVID-19 are ongoing and will provide further insights into the safety and efficacy of MSC therapy in these conditions. There is much potential to optimise the therapeutic efficacy of MSC products through manufacturing conditions, preconditioning and altered gene expression. Furthermore, MSC derived products such as extracellular vesicles and conditioned medium offer promise as cell free alternatives to MSCs.

8.0 Expert opinion

Despite an abundance of pre-clinical evidence and early clinical evidence supporting the role of MSCs in ARDS and sepsis, a number of challenges remain in optimising the therapeutic potential of MSCs in these conditions. Determination of the optimal therapeutic product requires consideration to be given to optimising the cell source, method of isolation, and culture conditions. While preclinical investigations suggest dose dependent effects and potential benefits of multiple dosing regimens, this remains to be determined in clinical trials. It is recognised that many factors can enhance MSC function including growth medium, preconditioning agents, and altered gene expression. Cell-free alternatives, including extracellular vesicles and conditioned medium, also have promise. Preclinical investigations support each of these approaches individually, however, few have translated into clinical practice. At present, there is no effective potency assay to predict the therapeutic efficacy of MSCs. Development of a potency assay would help identify the most effective strategies to optimise MSC therapeutic function and aid translation of these strategies into clinical product development. The heterogenicity of MSC products presents challenges in their clinical development, with each individual MSC product requiring robust evaluation in a clinical trial setting. The failure of one MSC product to demonstrate efficacy in a clinical trial does not discount all MSC products. Determining the optimal characteristics of MSC products specifically for ARDS and sepsis is essential to optimise clinical trial outcomes and strive towards successful clinical development.

Numerous early phase clinical trials of MSC products are underway in the setting of ARDS and sepsis, with many COVID-19 studies emerging. As ATMPs, clinical trials of MSCs present regulatory burdens in excess of other investigational medicinal products. The additional infrastructure required to facilitate MSC manufacture and delivery further complicates clinical trials. It is unsurprising therefore that many registered MSC clinical trials are not completed. Nonetheless, several completed MSC trials in ARDS and sepsis have demonstrated delivery of this therapy to critically ill patients is feasible in small numbers of patients. Larger, methodologically robust, clinical trials are required to evaluate the safety, efficacy, and feasibility of MSC products in these patient populations. If clinical trials in ARDS, sepsis, and COVID-19 demonstrate efficacy, advancement of MSC products to clinical implementation will provide further logistical challenges. Ideally, potency assays which predict the therapeutic efficacy of MSCs should be developed and assessed within the remit of an MSC clinical trial. Upscaling of manufacturing will be required to ensure ease of availability; unique infrastructure and

trained personnel will also be required to deliver an effective cell therapy service. Consideration of these challenges during the clinical development of MSC products is required to ensure future implementation and delivery of this therapy to critically ill patients with ARDS and sepsis is achievable.

In relation to COVID-19, preclinical investigations in ARDS, sepsis, and respiratory viral infections provide insight into the therapeutic potential of MSCs for this novel pathogen. It is biologically plausible the immunomodulatory, reparative, and antiviral properties elicited in these models could translate to COVID-19. The scale, rapidity, and poor outcomes of the COVID-19 pandemic have resulted in the rapid emergence of clinical trials of MSC therapy in COVID-19. Despite the rapid advancement to clinical trials, evaluation of MSC therapy within models of COVID-19 ARDS is essential and should be prioritised to further understand the actions and potential efficacy of MSCs in COVID-19.

Table 1: *In vivo* studies of MSCs in viral respiratory models

Ref.	Author	Year	Species	Viral Strain	Intervention	Outcomes	Antiviral effects
104	Loy	2019	Murine	H5N1	UC hMSC IV 5 x 10^5 cells 5 days PI	⇑ survival (not significant) ⇑ body weight ⇓ vascular permeability ⇔ histological lung injury ⇓ IP-10 (CXCL10), MCP-1 (CCL2), RANTES (CCL5), IL-6, TNFα, IL-1β, IL-8	⇔ Lung viral titres (day 7 & day 10 PI)
108	Khatri	2018	Swine	H1N1	BM sMSC-EVs IT 80 ug/kg 12 hours PI	⇓ histological lung injury ⇓ vascular permeability ⇓ TNFα ⇓ CXCL10 ⇑ IL-10 (not significant)	⇓ Nasal or lung viral titres (nasal day 1 and 3 PI, lung day 3 post EV administration)
103	Chan	2016	Murine	H5N1	BM hMSC IV 5 x 10^5 cells Day 5 PI	In aged but not young mice: ⇑ survival ⇓ lung oedema and vascular permeability ⇓ histological lung injury In BAL fluid: ⇓ CD4+ T cells and NK cells ⇑ macrophages ⇓ IP-10 (CXCL10), MCP-1 (CCL2), MCP-3(CCL7), MIP-1, RANTES (CCL5), IL-4, IL-17, TNFα	⇔ Lung viral titres (day 7 & day 10 PI)
105	Li	2016	Murine	H9N2	BM mMSC IV 1 x 10^5 cells 30 mins or Day 1 PI	⇑ survival (not significant) ⇓ lung oedema ⇓ histological lung injury ⇑ oxygenation In serum and/or BAL fluid: ⇓ GM-CSF, MIG (CXCL9), IL-1α, IL-6, TNFα, IFY-y ⇑ IL-10	⇔ Lung viral titres (day 3 PI)
107	Gotts	2014	Murine	H1N1	mMSC or hMSC IV 5 x 10^5 cells Day 5 and Day 6 PI	⇔ weight loss ⇔ vascular permeability ⇔ lung oedema ⇔ histological lung injury	⇓ Lung viral titres (day 7 PI)
106	Darwish	2013	Murine	H1N1	BM mMSC or hMSC IV 2.5 or 5 x 10^5 cells Day 0, 2 or day 5 PI	⇔ survival ⇔ weight loss ⇔ chemokine/cytokines ⇔ BAL inflammatory cells	⇔ Lung viral titres (day 6 PI)

hMSC – human Mesenchymal Stromal Cells; mMSC – murine Mesenchymal Stromal Cells; sMSC – swine Mesenchymal Stromal Cells; MSC-EVs – Mesenchymal Stromal Cell derived extracellular vesicles; BM – Bone Marrow; UC – Umbilical cord; IV – Intravenous infusion; IT – Intrathecal; PI – post infection; BAL – bronchioalveolar lavage; ⇑ increased; ⇓ decreased; ⇔ no effect

Table 2: Clinical trials MSCs in ARDS

Ref.	Author, Year or Registration	Trial design	Patient recruitment	MSC source	MSC route/dose/ number of infusions	Main findings
129	Chen, 2020	Cohort study	N = 17 (MSCs) N = 42 (control)	Allo menstrual blood	IV, 1 x 10^6/kg, 3 or 4	No MSC infusion related acute toxicity Mortality 17.6 % vs 54.5% (MSCs vs control p = 0.006)
124	Lv, 2020	Single arm, open label	N = 22	Allo UC	IV, 1 x 10^6 cells/kg, 1	No infusion associated events D60 Mortality 45%
123	Yip, 2020	Open label, dose escalation	N = 9	Allo UC	IV, 1, 5 and 10 x 10^6 cells/kg, 1	3 mild adverse events related to treatment In-hospital mortality 33%
126	Matthay, 2019	RCT	N = 60 (40 MSC : 20 control)	Allo BM	IV, 10 x 10^6 cells/kg PBW, 1	No infusion associated events D28 mortality 30% vs 15% (MSCs vs control; OR 2.4, CI 0.5 – 15.1)
128	Bellingan, 2019 NCT02611609	Phase 1: Open label, dose escalation Phase 2: RCT	Phase 1: N = 9 Phase 2: N = 30 (20 MSC : 10 control)	Multistem® Allo BM MAPC	Phase 1: IV, 300 and 900 x 10^6 cells, 1 Phase 2: IV 900 x 10^6 cells, 1	Single grade 1 possible infusion related reaction No serious adverse events D28 mortality 25% vs 40% (MSC vs control)
125	Wilson, 2015	Open label, dose escalation	N = 9	Allo BM	IV, 1, 5 and 10 x 10^6 cells/kg PBW, 1	No infusion associated events or treatment related adverse events D60 mortality 22%
122	Zheng, 2014	RCT	N = 12 (6 MSC : 6 control)	Allo AT	IV, 1 x 10^6 cells/kg, 1	No serious adverse events related to study drugs
	NCT02804945	Single arm, open label	N = 20	Unknown	IV, 3 x 10^6 cells/kg, 1	Completed, not published
	REALIST NCT020421143	Phase 1: Open Label Dose escalation Phase 2: RCT	Phase 1: N = 9 Phase 2: N = 60 (30 MSC : 30 control)	Allo CD362+ UC MSC	Phase 1: IV, 100, 200 and 400 x 10^6 cells, 1 Phase 2: IV, 400 x 10^6 cells, 1	Phase 1 Complete Phase 2 Recruiting
	STAT trial NCT03818854	RCT	N = 120	Allo BM MSC	IV, 10 x 10^6 cells/kg, 1	Recruiting

RCT – Randomised controlled trial; Allo – Allogeneic; BM – Bone Marrow; UC – Umbilical cord; AT – Adipose tissue; IV – Intravenous; PBW – predicted body weight

Table 3: Clinical trials MSCs in Sepsis

Ref	Author, Year or Registration	Trial design	Patient recruitment	MSC source	MSC route/dose/ number of infusions	Main findings
131	He, 2018	Open label, dose escalation	N = 15	Allo UC MSCs	IV 1, 2 or 3 x 10^6 cells/kg, 1	No MSC infusion associated events or SAEs
130	McIntyre, 2018	Open label, dose escalation	N = 9	Allo BM MSCs	IV 0.3, 1 or 3 x 10^6 cells/kg, 1	No MSC infusion associated adverse events or SAEs
132	Galstyan, 2018	Open label RCT	N = 30 MSC group = 15 Control = 15	BM MSCs	IV, 1 x 10^6 cells/kg, 1	28-day survival 20% vs 60% (MSC vs control, $p < 0.05$)
	SEPCELL NCT03158727	RCT	N = 84	Allo AT MSCs	IV, 160 x 10^6 cells/dose, 2	Completed July 2020
	CISS 2 NCT03369275	RCT	N = 114	Allo BM MSCs	IV, 300 x 10^6 cells, 2	Not yet recruiting
	CHOCMSC NCT02883803	RCT	N = 66	MSCs (source unknown)	IV 1 x 10^6 cells/kg, 1	Not yet recruiting

RCT – Randomised controlled trial; Allo – Allogeneic; BM – Bone Marrow; UC – Umbilical cord; AT – Adipose tissue; IV – Intravenous; PBW – predicted body weight

Table 4: Clinical reports & trials MSCs in COVID-19

Ref	Author, Year or Registration	Population	Trial design	Patient recruitment	Cell source	MSC route/dose/ number of infusions	Main findings
136	Sanchez-Guijo, 2020	COVID 19 Pneumonia IMV	Retrospective review	N = 13	Allo AT MSCs	IV, 1×10^6 cells/kg, 1-3	No adverse events
134	Chen, 2020	COVID 19 pneumonia	Retrospective review	N = 25	Unknown	IV, 1×10^6/kg, 1-3	3 treatment related adverse events reported
135	Sengupta, 2020	COVID 19 pneumonia	Open label study	N = 24	Exoflo™ (BM MSC derived exosomes)	IV, 15 ml Exoflo™, 1	No infusion related reactions or adverse events
112	Leng, 2020	COVID 19 pneumonia	Case series	N = 7	Unknown	IV, 1×10^6/kg, 1	No acute infusion related or allergic reactions
	NCT04366063	COVID 19 ARDS	Open label RCT	N = 60	Unknown	IV, 100×10^6 cells/dose, 2	Recruiting
	NCT04355728	COVID 19 ARDS	Randomised, blinded outcome assessor	N = 24	UC MSCs	IV, 100×10^6 cells/dose, 2	Recruiting
	NCT04348461	COVID 19 ARDS	RCT	N = 100	Allo AT MSCs	IV, 1.5×10^6 cells/kg, 2	Not yet recruiting
	NCT04371393	COVID 19 ARDS	RCT	N = 300	Mesoblast Remestemcel-L BM MSCs	IV, 2×10^6 cells/kg, 2	Recruiting
	NCT04345601	COVID 19 ARDS	Open label	N = 30	BM MSCs	IV, 1×10^8 cells/dose, 1	Not yet recruiting
	NCT03042143 COVID 19 REALIST	COVID 19 ARDS	RCT	N = 60	REALIST Orbcel-C Allo UC CD362+ MSCs	IV, 400×10^6 cells/dose, 1	Recruiting
	NCT04377334	COVID 19 ARDS	Open label RCT	N = 40	Allo BM MSCs	Unknown	Not yet recruiting
	NCT04333368 STROMA-CoV2	COVID 19 ARDS	RCT	N = 60	UC WJ MSCs	IV, 1×10^6 cells/kg, 3	Recruiting

	NCT04269525	COVID 19 pneumonia in ICU	Open label	N = 10	UC MSCs	IV, 9.9 x 10^7 cells/dose, 4	Recruiting
	ACTRN12620000478910	COVID 19 pneumonia	RCT	N = 24	UC MSCs	IV, 5 x 10^6 cells/kg, 1	Not yet recruiting
	NCT04315987	COVID 19 pneumonia	Open label	N = 66	NestCell® (source N/S)	IV, 2 x 10^7 cells, 3-4	Not yet recruiting
	NCT04252118	COVID 19 pneumonia	Open label	N = 20	MSCs N/S	IV, 3 x 10^7 cells/dose, 3	Recruiting
	NCT04366323	COVID 19 pneumonia	Open label RCT	N = 26	Allo AT MSCs	IV, 80 x 10^6 cells/dose, 2	Not yet recruiting
	NCT04313322	COVID 19	Open label	N = 5	Allo WJ MSCs	IV, 1 x 10^6 cells/kg, 3	Recruiting
	NCT04336254	COVID 19 pneumonia	RCT	N = 20	Allo DP MSCs	IV, 3 x 10^7 cells/dose, 3	Recruiting
	NCT04288102	COVID 19 pneumonia	RCT	N = 90	MSCs N/S	IV, 4 x 10^7 cells/dose, 3	Recruiting
	NCT04346368	COVID 19 pneumonia	RCT	N = 20	BM MSCs	IV, 1 x 10^6 cells/kg, 1	Not yet recruiting
	NCT04273646	COVID 19 pneumonia	Open label RCT	N = 48	UC MSCs	IV, 0.5 x 10^6 cells/kg, 4	Not yet recruiting
	NCT04348435	Risk of occupational exposure COVID 19	RCT	N = 100	Allo AT MSCs	IVI, 50, 100 or 200 x 10^6 cells/dose, 5	Enrolling by invitation
	NCT04349631	Risk of occupational exposure COVID 19	Unknown	Unknown	Auto AT MSCs	IV, Dose unknown, 5	Enrolling by invitation
	NCT04339660	COVID 19 pneumonia	RCT	N = 30	UC MSCs	IV, 1 x 10^6 cells/kg, 2	Recruiting
	NCT04302519	COVID 19 pneumonia	Open label	N = 24	DP MSCs	IV, 1 x 10^6 cells/kg, 3	Not yet recruiting
	NCT04352803	Hospitalized COVID 19	Open label RCT	N = 20	Auto AT MSCs	IV, 0.5 x 10^6 cells/kg, 1	Not yet recruiting
	NCT04371601	COVID 19 pneumonia	Open label RCT	N = 60	UC MSCs	IV, 10 x 10^6 cells/kg, 4	Active, not recruiting
	NCT04366271 MESCEL-COVID19	COVID 19 pneumonia	Open label RCT	N = 106	UC MSCs	IV, Dose unknown, 1	Not yet recruiting
	NCT04362189	Hospitalised COVID 19	RCT	N = 110	Allo AT MSCs	IV, 100 x 10^6/dose,	Not yet recruiting

						4	
	NCT04361942	COVID 19 pneumonia in ICU	RCT	N = 24	Allo MSCs	IV, 1 x 10^6/kg, 1	Recruiting
	NCT04341610 ASC COVID-19	COVID 19 pneumonia	RCT	N = 40	AT MSCs	IV, 100 x 10^6 cells/dose 1	Not yet recruiting
	NCT04276987	COVID 19 pneumonia	Unknown	N = 30	Exosomes from Allo AT MSCs	Nebulised, 2 x 10^8 nano vesicles/3mL, 5	Not yet recruiting

IV – Intravenous; RCT – Randomised controlled trial; Allo – Allogeneic; BM – Bone Marrow; UC – Umbilical cord; AT – Adipose tissue; PBW – predicted body weight; IMV – invasive mechanical ventilation

Table 5: Studies of MSC pre-conditioning

Ref	Author	Year	Model	Preconditioning	MSC source	Main findings
64	Horie	2020	Rodent VILI model	Cytokines (IL-1B, TNFα, IFN-y)	BM hMSC	⇑enhanced resolution of histological lung injury ⇑oxygenation ⇑lung compliance by cytokine preactivated MSCs ⇓lung oedema ⇓BALF protein ⇓ BALF cell counts
77	Baudry	2019	Murine sepsis model	IFN-y	BM hMSC	Beneficial effect on microcirculation by increasing flow of WBCs, decreasing adhesion of WBCs, and increasing the average red blood cell velocity
164	Silva	2019	Murine LPS model	ARDS serum	BM mMSC or EVs	⇓alveolar collapse – MSCs > EVs ⇓neutrophil infiltration – MSCs > EVs ⇓interstitial oedema and collagen deposition – MSCs not EVs ⇓BALF cell count – MSCs = EVs ⇓BALF protein MSCs = EV Improved lung mechanics – MSCs not EVs In pARDS: ⇓TNFα, IL-6, KC, VEGF, TGFβ– MSCs = EVs In Non-pARDS: ⇓TNFα, IL-6 (MSCs=EVs), ⇓KC (MSCs>EVs), VEGF (MSCs only) ⇔ TGFβ
54	Silva	2019	Murine CLP model	Eicosapentaenoic acid	Adipose mMSC	EPA MSCs ⇑ expression PGE-2, IL-10, TGFβ, RvD1 ⇑survival ⇓Clinical severity score Improved lung mechanics ⇓alveolar collapse, interstitial oedema, alveolar septal inflammation ⇓neutrophil infiltration(lung) ⇓collagen deposition ⇓IL1-B, KC, TFGβ ⇑VEGF in EPA-MSCs ⇔IL-10 ⇓peripheral cell counts ⇓injury to distal organs (liver, kidney, heart, spleen, small bowel)
151	Saeedi	2019	Murine sepsis model	LPS, oxidative stress, serum deprivation	BM mMSC	⇑ survival ⇓ bacterial load ⇓IL-4 & TNFα ⇑IL-10 (⇓AST/ALT by LPS preconditioned MSCs

152	Saeedi	2019	Murine peritoneal sepsis model	Staphylococcal Enterotoxin B	BM mMSC	⇑ survival ⇓ bacterial load ⇓IL-4 & TNFα ⇑IL1-0 ⇓AST/ALT
150	Tsoyi	2016	Murine CLP model	Carbon monoxide	BM mMSC	⇑ survival ⇓cell death in spleen of CO-MSCs ⇓ALT/AST & creatinine ⇓bacterial load ⇑clearance of apoptotic neutrophils (efferocytosis)
149	Li	2015	Murine LPS model	Ischaemic/hypoxic preconditioning (30, 60 or 90 mins)	BM hMSC or Exosome	⇓BAL WCC/neutrophil count MSC exosome ⇓BAL MIP2, BAL Protein
85	Zhao	2014	Murine CLP model	TLR 3 ligand, poly (I:C)	UC hMSC	Poly (I:C) enhanced expression of COX2, IDO, IL-6 and IL-8 and reduced expression of TGFβ ⇑ survival ⇓ bacterial load ⇓IL-6, TNFα, KC, CCL5 with P-MSCs ⇓Creatinine, Amylase, BUN, ALT

hMSC – human Mesenchymal Stromal Cell; mMSC – murine Mesenchymal Stromal Cell; EV –Extracellular vesicle; BM – Bone Marrow; UC – Umbilical cord; BALF – Bronchoalveolar lavage fluid; CLP – Caecal ligation and puncture; VILI – Ventilator induced lung injury; WBCs – White blood cells; WCC – White cell count; BUN – Blood urea nitrogen; EPA - Eicosapentaenoic acid; CO – Carbon monoxide; LPS - Lipopolysaccharide ⇑ increased; ⇓ decreased; ⇔ no effect

Table 6: studies of MSC genetic modification

Ref	Author	Year	Model	Gene expression	MSC source	Main Findings
154	Lv	2020	Murine LPS model	Stanniocalcin-2 (STC-2) knockdown (lentiviral transfection)	BM hMSC	⇓histological lung injury ⇓lung vascular permeability ⇓BALF leucocyte count ⇓TNFα & IL-17A ⇑antioxidant activity
160	Jerkic	2019	Rodent *E.coli* pneumosepsis model	IL-10 expression (IL-10 recombinant adenovirus)	UC hMSCs	⇑survival ⇑lung compliance IL-10 MSCs ⇑ oxygenation ⇓alveolar fluid protein ⇓bacterial load/⇑ phagocytosis IL-10 MSCs ⇓ alveolar neutrophils and ⇑ macrophages ⇓TNFα & IL-6 Rodent IL-10 not ⇑ Human IL-10 ⇑ in IL-10 MSC group
153	Li	2019	Murine LPS model	Lats 1 downregulation (lentiviral transfection)	mMSC	⇓histological lung injury ⇓lung fibrosis score/collagen deposition ⇓lung oedema ⇓BALF TP/Albumin ⇑occludin expression ⇓IL-1β and IL-6 ⇑IL-4 and IL-10
61	Lu	2019	Murine LPS model	HGF under- or over-expression (lentiviral transfection)	mMSC	⇓Lung injury ⇓Dendritic cell aggregation in lung
155	Chen	2019	Rodent LPS model	Heme-oxygenase-1 expression (HO-1 lentivirus transfection)	BM rMSC	⇑HO-1 mRNA and protein expression (enhanced by HO1+MSCs) ⇑survival ⇓histological lung injury ⇓BALF neutrophils ⇓TNFα, IL-1β, NF-κB ⇑HGF, KGF, IL-10
161	Wang	2018	Murine LPS model	IL-10 overexpression (retroviral transfection)		⇑ survival ⇓weight loss ⇓BALF protein ⇓BALF TNFα ⇑ IL-10 ⇑ IL-10 producing T cells and B cells in spleen and lung
156	Zhang	2018	Murine LPS model	Nrf2 overexpression (lentiviral transfection)	Adipose hMSC	⇓histological lung injury ⇓lung epithelial cell apoptosis ⇓W:D ratio ⇓collagen deposition ⇓IL-1β, IL-6 ⇑IL-10 ⇑Nrf2 mRNA and protein exp in lungs
75	Tang	2017	Murine LPS model	Ang-1 under expression (Ang-1 siRNA lentivirus transfection)	MV (from BM-hMSC)	⇑BALF Ang-1 ⇓BALF neutrophils ⇓BALF MIP2 ⇓BALF Albumin

						⇓Evan's blue dye leakage ⇓histological lung injury
76	Cai	2015	Murine LPS model	B-catenin expression	BM mMSC	⇓histological lung injury MSCs still detectable in lungs at 14 days ⇓BALF protein ⇓KGF at day 3 but ⇑ day 14 ⇓IL-1β and IL-6 BALF ⇑IL-10 BALF ⇓collagen deposition at day 14
158	Li	2015	Rodent LPS model	KGF underexpression (transfection KGF siRNA)	BM MSC	⇓histological lung injury (attenuated in KGF knockdown MSCs) ⇓W:D ratio
157	Chen	2013	Murine LPS model	KGF overexpression		⇓microvascular permeability ⇓lung injury ⇓IL-1β, TNFα ⇑Increased IL10
159	Xu	2008	Murine LPS model	Ang-1 overexpression	BM mMSC	⇑Ang-1 expression ⇓BAL protein ⇓TNFα ⇓MPO activity ⇓histological lung injury
70	Mei	2007	Murine LPS model	Ang-1 overexpression	BM mMSC	⇓BALF total/neutrophil count ⇓ histological lung injury ⇓IFN-γ, TNFα, IL-6, IL-1β ⇓BALF protein, albumin, IgM Evidence of apoptosis reduced (caspase 3/7)

hMSC – human Mesenchymal Stromal Cell; mMSC – murine Mesenchymal Stromal Cell; EV –Extracellular vesicle; BM – Bone Marrow; UC – Umbilical cord; BALF – Bronchoalveolar lavage fluid; CLP – Caecal ligation and puncture; VILI – Ventilator induced lung injury; WBCs – White blood cells; WCC – White cell count; BUN – Blood urea nitrogen; TP – Total protein; ALB - Albumin; W:D – Lung wet:dry ratio: LPS - Lipopolysaccharide ⇑ increased; ⇓ decreased; ⇔ no effect

Annotated references

*[1] Berlin definition of ARDS

*[2] Sepsis 3 Consensus definition of Sepsis and Septic Shock

*[17] Comprehensive review of clinical trials of pharmacological interventions in ARDS

*[18] Emerging evidence of benefit of dexamethasone in ARDS

*[22] Defining criteria of MSCs

*[40] Mitochondrial transfer via tunnelling nanotubules enhances macrophage phagocytosis *in vitro* and *in vivo* models of ARDS

*[35] In vivo model demonstrating antimicrobial effects of MSCs in gram-negative sepsis.

*[41] In vivo model demonstrating reparative effects of MSCs in a CLP model of sepsis.

*[43] In vivo model demonstrating reparative effects of MSCs in ventilator induced lung injury

*[73] Reparative and immunomodulatory effects of MSC microvesicles in human ex vivo lung perfusion model.

*[58] Reparative, antimicrobial and immunomodulatory effects of MSCs in human ex vivo lung perfusion model with lung injury induced by live bacteria.

*[121] Intravenous MSC administration in healthy human subjects in an LPS endotoxaemia model.

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**[130] An open label Phase 1 dose escalation trial of Bone Marrow derived MSCs in septic shock.

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Mesenchymal stromal cells for acute respiratory distress syndrome (ARDS), sepsis and COVID 19 infection: optimising the therapeutic potential

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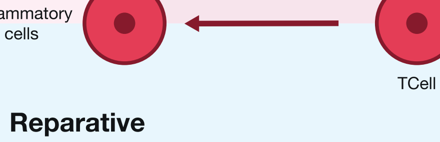

Immunomodulation
MSCs modulate the inflammatory environment:
↓Pro inflamatory Cytokines
↑Anti inflamatory Cytokines
↑Neutrophil apoptosis
↑Neutrophil tissue infiltration
Neutrophil
Modulates cytokine release towards anti-inflammatory phenotype
↑ROS
↑PGE2/PI3
↑HO-1
↑Lipoxin A4
Monocyte / Macrophage
Enhanced macrophage phagocytosis and bacterial killing
↑Regulatory DC cells
↓DC tissue infiltration
↑T-Regulatory cells
Dendritic Cell
↓Pro-inflammatory CD4+ T cells
TCell
Soluble Factor Secretion:
IL-6
TGF-β
TSG6
IL-10
IDO
PGE2
Antimicrobial
MSCs modulate immune cell response to pathogens
Enhanced bacterial phagocytosis and bacteria killing
↓ Bacterial load
Bacteria
↓Viral replication
↓Viral load
Virus
Antimicrobial peptide (AMP) secretion:
LL37
Hepcidin
βdefensin
Lipocalin 2
Reparative
MSCs transfer mitochondria to target cells via tunnelling nanotubules
Extracellular vesicles released from MSCs transport mitochondria, mRNA and miRNA
Growth factor, adhesion proteins and vascular stabilising protein secretion:
KGF
HGF
VEG
Ang-1
ICAM
Kidney
↓ Tubular injury
↑ Free water clearance
↑ Electrolyte excretion
↑ eNOS
↑ Klotho protein
↓ Apoptosis
Heart
↓ Interstitial oedema
Restore myocyte architecture
Small Intestine
↓ Subepthelial spaces in villi
Skeletal Muscle
↑ Myofiber formation
↑ Muscle Strength
Lungs
↑ Alveolar Fluid Clearance
↓ Lung oedema
↑ Compliance
↑ Oxygenation
↓ Apoptosis
Vascular Endothelium
Alveolar Epithelium
The alveolar epithelium-vascular endothelium interface.
In ARDS (left side) epithelial and endothelial dysfunction occur with resultant neutrophil infiltration, loss of barrier integrity, alveolar inflammatory exudate and later deposition of collagen.
MSC administration (right side) restores integrity of alveolar endothelial – epithelial interface. Transfer of mitochondria, mRNA, and miRNA from MSCs and their EVs contributes to restoration of normal architecture and function. Modulation of the inflammatory environment occurs with a reduction in immune mediated tissue damage and enhanced bacterial clearance.
mRNA
miRNA
Mitochondria
Extracellular Vesicle
Cytokines
Growth Factors
AMPs
MSC
Macrophage
Neutrophil
Red Blood Cells
Tunnelling Nanotubes
Platelet
Collagen
Bacteria
Virus