Stem Cell-based therapies for COVID-19-related acute respiratory distress syndrome

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Abstract
As the number of confirmed cases and resulting death toll of the COVID-19 pandemic continue to increase around the globe - especially with the emergence of new mutations of the SARS-CoV-2 virus in addition to the known alpha, beta, gamma, delta and omicron variants - tremendous efforts continue to be dedicated to the development of interventional therapeutics to mitigate infective symptoms or post-viral sequelae in individuals for which vaccines are not accessible, viable or effective in the prevention of illness. Many of these investigations aim to target the associated acute respiratory distress syndrome, or ARDS, which induces damage to lung epithelia and other physiologic systems and is associated with progression in severe cases. Recently, stem cell-based therapies have demonstrated preliminary efficacy against ARDS based on a number of preclinical and preliminary human safety studies, and based on promising outcomes are now being evaluated in phase II clinical trials for ARDS. A number of candidate stem cell therapies have been found to exhibit low immunogenicity, coupled with inherent tropism to injury sites. In recent studies, these have demonstrated the ability to modulate suppression of pro-inflammatory cytokine signals such as those characterizing COVID-19-associated ARDS. Present translational studies are aiming to optimize the safety, efficacy and delivery to fully validate stem cell-based strategies targeting COVID-19 associated ARDS for viable clinical application.

KEYWORDS
COVID-19, mesenchymal stem cells, stem cells, therapeutics

1 | COVID-19, ACUTE RESPIRATORY DISTRESS SYNDROME, AND MULTI-ORGAN INVOLVEMENT

The novel 2019 coronavirus disease (COVID-19) was first identified in Wuhan, China with the emergence of a cluster of pneumonia cases in December 2019 and was declared a pandemic by the World Health Organization (WHO) on 11 March 2020.1 As of 30 December 2021, there have been 53,795,407 confirmed cases and 820,355 deaths in the United States alone according to the Centers for Disease Control and Prevention, with a global total of 290,959,019 cases and 5,446,753 deaths according to the World Health Organization as of 4 January 2022.2 The causative pathogen for COVID-19 is the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), a
distinct strain of coronavirus related to those that resulted in the severe acute respiratory syndrome (SARS) pandemic in 2003 and the Middle East respiratory syndrome (MERS) pandemic in 2012. Because of its recent emergence, much remains to be elucidated regarding the pathophysiological mechanisms, sequelae and strength and duration of the host immune response in SARS-CoV-2, despite a tremendous amount of research worldwide. SARS-CoV-2 has demonstrated high genetic variability and a rapid mutation rate, and preliminary evidence suggests that immune protection may be limited, providing challenges to the development of vaccines and treatments. The persistent emergence of novel SARS-CoV-2 variants, highlighted by the Delta and Omicron examples, present continued concerns that novel strains capable of effective vaccine escape and/or heightened virulence will emerge, rendering present vaccines ineffective in preventing infection. Even where vaccines are effective, meeting global demands to provide vaccine accessibility to local populations has proven challenging. The emergence of a contradictory but sizeable body of evidence that efficacy of present vaccines against SARS-CoV-2 wanes after 4–6 months further complicates meeting this demand. Collectively, these issues underscore the clear need that persists for the development of effective therapies to address SARS-CoV-2 infection and its more severe clinical presentations.

COVID-19 is characterized by a diverse number of potential complications, both respiratory and non-respiratory. A recent study conducted in Wuhan, China examining 201 hospitalized COVID-19 pneumonia patients for example, demonstrated that 41.8% of the patients developed acute respiratory distress syndrome (ARDS) during COVID-19-related pneumonia, with the subset of patients progressing to ARDS exhibiting a mortality rate of 52.4%. With a median patient age of 51, this case study also underscored the elevated susceptibility of older subpopulation to ARDS and consequently a substantially higher risk of mortality. ARDS is a life-threatening lung pathology characterized by rapid onset and resulting from a massive and generalized pro-inflammatory immune response in the lungs, circulation and other tissues in COVID-19 patients; this cytokine storm represents the most life-threatening development in COVID-19 patients. In non-COVID patients, ARDS typically arises as a complication of pneumonia, systemic infection and major trauma, and is associated with elevated transport of fluid from lung capillaries to alveoli, the air sacs that are the site of gas exchange with the blood, resulting in pulmonary oedema, hypoxemia, and loss of lung compliance secondary to epithelial damage and pulmonary fibrosis.

The cytokine storm characterizing COVID-19 ARDS has also been implicated in tissue damage and embolus formation in multiple organ systems and to play a key role in the pathophysiology of extrapulmonary multiple organ failure secondary to ARDS; indeed, this process is hypothesized to be key in the development of a number of emergent chronic post-COVID-19 pathologies. For instance, emerging evidence investigating the development of ‘post-viral syndrome’ in a subset of post-recovery COVID-19 patients is examining possible corollaries with earlier SARS variants which produced a chronic pathological state resembling chronic fatigue/myalgic encephalomyelitis as a result of viral infiltration to select brain regions.

Histopathological examination of brain tissue in necropsied patients has also demonstrated neurological complications in a subset of COVID-19 patients implicating non-inflammatory neurovascular damage in clinical manifestations ranging from loss of olfaction/gustation to loss of involuntary control of breathing through medullary centres, with the virus hypothesized to spread to the brain from the upper respiratory tract via the transcribrial route, where angiotensin-converting enzyme 2 (ACE2)-expressing tissues enable viral internalization. Evidence from other coronaviruses in human case reports and in vivo models also suggest the possibility of brain lesioning and fatal encephalitis.

OVERVIEW OF COVID-19 ARDS PATHOPHYSIOLOGY

SARS-CoV-2 is a large, enveloped Betacoronavirus of the Coronaviridae family, order Nidovirales, which it shares with 5 other species and 6 other total strains of the coronavirus family known to infect humans to date. These include the Alphacoronaviruses HCoV-229E and HCoV-NL63; and the Betacoronaviruses HCoV-OC43, HCoV-HKU1, SARS-CoV (now often designated SARS-CoV-1 to avoid confusion), and MERS-CoV. SARS-CoV-1, with which SARS-CoV-2 exhibits 77.5% sequence homology, was causative of the 2002–2004 SARS outbreak, characterized by variable virulence with localized mortality rates as high as 17% and was noted for mutating to optimize viral-host binding and replication; the Middle East respiratory syndrome (MERS-CoV) virus, with which SARS-CoV-2 shares 50% sequence homology and which enters host cells via the DPP4 receptor, exhibits a mortality rate of 35% and was noted for mutating to optimize viral-host binding and replication; the Middle East respiratory syndrome (MERS-CoV) virus, with which SARS-CoV-2 shares 50% sequence homology and which enters host cells via the DPP4 receptor, exhibits a mortality rate of 35% and was noted for mutating to optimize viral-host binding and replication. Symptoms of the other human coronavirus strains are generally mild with low rates of mortality and morbidity and these infections are typically associated with the ‘common cold’, accounting for 10%–30% of all adult upper respiratory infections annually.

Coronaviruses are comprised of single-stranded, positive-sense RNA genomes of 28–32 kb in length, the largest genomes of all RNA viruses, and these are translated to non-structural proteins through two open-reading frames. SARS-CoV-2 displays a surface Spike S glycoprotein on its viral envelope that is critical for host receptor binding and invades host cells via interaction with the angiotensin-converting enzyme 2 (ACE2) receptor and is subsequently internalized where it integrates within the host genome and exploits its machinery for viral replication. ACE2 receptors are widely distributed in lung alveolar epithelia as well as nasopharyngeal and oral mucosa cells; extra-pulmonary expression is widely distributed among tissues spanning multiple physiological systems, however, including the liver, kidneys, gut, endothelial and vascular smooth muscle, and brain, providing a mechanism for multi-organ involvement.
3.1 Candidates for cell-based therapies

Despite years of efforts by multiple investigative teams aiming to develop viable treatments for ARDS, many candidate therapies have failed to show efficacy. While corticosteroidal anti-inflammatory drugs such as dexamethasone and hydrocortisone or interleukin receptor antagonists have been investigated for ARDS and COVID-associated ARDS with evidence of success for severely ill/ventilated patient outcomes in a large number of instances, study outcomes to date have also proven inconsistent with these interventions, with steroidal interventions in some cases even elevating mortality in related cases of ARDS with influenza, leading to hesitancy among many clinicians to rely on these—especially given the lack of clarity on risk factors in instances of contraindication.

In the case of broad immune-suppressant effects with cytokine-suppressant drugs for instance, it has been hypothesized that immunosuppression may worsen infection which in some cases outweighs the beneficial effects of cytokine storm suppression, depending on the stage of disease and pre-existing immune status of the patient.

More recently, certain stem cell-based therapies are beginning to show promising results in mitigating ARDS symptoms. Key examples of these candidate interventions are shown in Table 1. These carry a number of predicted advantages over corticosteroidal or receptor antagonist-based pharmacological interventions, including their intrinsic inflammatory-suppressant properties which combine with a milieu of added supportive therapeutic components which promote for instance cellular repair and normalization of function; their generally non-immunogenic properties as the molecular contents are protected in physiological settings within lipid bilayers, eluding immune recognition; elevated uptake of their secretory components, as carried in extracellular vesicles owing to their recognition as biological carriers, optimizing delivery efficiency compared with non-cellular/non-vesicular molecular therapies; and the sustained secretion of therapeutically relevant factors following administration, which allows for a more protracted delivery within the recipient following each dose while simultaneously removing the need for higher and less well-tolerated single-dose concentrations within a single dose to achieve efficacy.

Mesenchymal stem cells (MSCs) have been given particular attention in recent years as they do not require de-differentiation as is the case with pluripotent sources, yet can still be induced to lineage-specific, directed differentiation. MSCs can bypass the technical constraints presented by isolating cells from specific organs, or ethical concerns surrounding use of embryonic cells because, they can be harvested from both autologous and allogeneic sources of relatively accessible tissue sources including umbilical cord, bone marrow, adipose tissue, and placenta. Careful characterization of these cells, including matching major histocompatibility complex (MHC) and genetic stability testing, may allow the development of a clinical-grade, ready-for-use, allogeneic cell bank sourced from healthy donor stem cells to facilitate prompt administration of MSCs for acute diseases, circumventing time-sensitive restrictions and technical constraints on the extraction and processing of patient-derived tissues in point-of-care settings that would be required for autologous administration.

Another potential stem cell candidate for ARDS-targeted therapeutics is neural stem/progenitor cells (NSCs). NSCs have been widely investigated in studies of neurodegenerative diseases and cancers, but at present, studies utilizing NSCs in the context of ARDS are lacking. Human foetal telencephalon-derived NSCs have shown a positive clinical safety profile including low immunogenicity, low risk of tumourigenicity attributed to limited post-transplantation proliferative activity, demonstrated the absence of lung aggregation or embolus formation, and relative technical ease of isolation and expansion compared with other types of stem cells. Indeed, these cell lines have already been applied by the authors in a number of completed or ongoing approved clinical trials in the United States, including NCT01172964, NCT02015819, and NCT03072134 (completed); and NCT02192359, NCT05139056 (ongoing/recruiting). NSCs have also displayed high compatibility with a variety of therapeutic agents, such as pro-drug converting enzymes, oncolytic viruses, antibodies, oligonucleotides and nanoparticles, and they demonstrate inherent tropism migrating to sites of inflammation post-injection. Using an avian v-Myc transformation strategy, NSCs have also successfully been immortalized allowing for continued proliferation and expansion in culture, while

| Cell type       | Candidates                                      | Advantages                                      | Disadvantages                              |
|-----------------|------------------------------------------------|-------------------------------------------------|--------------------------------------------|
| Progenitor cells| Endothelial Progenitor Cells                    | High therapeutic efficacy                        | Difficult to Isolate                      |
|                 | Epithelial Progenitor Cells                     | High therapeutic efficacy                        | Difficult to Isolate                      |
| Stem cells      | Embryonic Stem Cells                            | Totipotent                                      | Tumorigenicity, ethical issues            |
|                 | Induced Pluripotent Stem Cell                   | Accessibility, low rejection                    | Tumorigenicity, low efficacy              |
|                 | Mesenchymal Stromal/Stem Cells                  | Accessibility, high therapeutic efficacy         | Questionable immunogenicity,              |
|                 | Neural Stem/Progenitor Cells                    | Administration convenience, high compatibility  | Controversial tumorigenicity              |
|                 |                                                 | with various treatment, low immunogenicity      | Related research has not been found        |
maintaining a non-tumourigenic and clinically safe profile as the v-
Myc-transformed cells cease proliferation upon introduction to a
host physiological environment, and are completely degraded by the
host within several days post-administration. This unique strategy
provides opportunities for the formation of a therapeutically viable
neural stem cell bank for wide and rapid allogeneic use.

More rigorous and comprehensive comparative studies of MSCs
and NSCs evaluating their therapeutic contents, biodistribution, cy-
tokine profiles, safety profiles and efficacy for central and periph-
eral pathological states will be needed to further investigate their
potential as therapeutic interventions.43,44,55,56 A summary of key
examples from the basic science/preclinical literature investigating
efficacy of MSC therapies in in vivo models of ARDS is presented in
Table 2. Summarily, these collective studies provide a compelling
body of preclinical evidence supporting the efficacy of therapeutic
benefit in the attenuation and/or reversal of ARDS-induced inflam-
lation and lung histological damage in response to stem cell-based
therapeutic intervention. An important exception is noted in the case
of severe influenza-associated ARDS, for which reported outcomes
are variable (see i.e. Darwish et al. 2013, Gotts et al. 2014). It is noted
that this may be attributed to the inherent limitations of murine se-
vere influenza models, which involve a short duration of pathology
and generally do not permit assessment of long-term histological and
functional recovery. It is also noted in these studies however that
MSC-secreted factors TSG-6 and PGE2 may be deleterious in severe
influenza specifically owing to its effect in the upregulation of COX-
2, a positive correlate of morbidity and mortality in severe influenza.

### 3.2 Therapeutic benefits of MSCs

The initiating events for the progression of acute lung injury and
ARDS, whether caused by trauma, infection, or other mecha-
nisms of immune dysregulation, involve the infiltration of neu-
rophils and macrophages to the alveolar space.9 These produce
pro-inflammatory cytokines, including TNF-α and IL-6, IL-1β, and IL-
8, factors which amplify the production of ROS and severely damage
endothelial and epithelial tissues by decreasing lung barrier function
while increasing vascular permeability.57

MSCs act via secretion of multiple paracrine factors, either
transported in extracellular vesicles or in a free state, including
microRNAs, mRNAs, peptides58 and even mitochondrial DNA.59
Currently identified MSC secretory factors include the following:
keratinocyte growth factor (KGF), interleukin-1 receptor antagonist
(IL1-ra), TNF-α-stimulated tumour necrosis factor inducible gene 6
(TSG-6), insulin-like growth factor-1 (IGF-1), lipoxin A4 (LXA4), an-
giopoietin-1 (Ang1), prostaglandin E2 (PGE2) and fibroblast growth
factor 7 (FGF-7).54,60–71 These paracrine factors, following uptake by
recipient host cells, facilitate a variety of functions including alveolar
fluid clearance, restore cell permeability, enhance resident immune
cell phagocytosis, and enhance tissue repair in the lungs through
anti-inflammatory and anti-apoptosis effect (Figure 1).59

#### 3.2.1 Anti-apoptotic, anti-oxidative, and anti-
inflammatory effects

MSCs demonstrate inflammation- and oxidative-suppressive functions.58–61,69,70 Hypoxic preconditioning has been shown to
enhance this effect when transplanted to hypoxic sites, in addition
to improving the survival, retention, proliferation and tissue forma-
tion of the damaged tissue.72–74 MSCs that overexpress cytopro-
tective factors such as heme oxygenase-1 (HO-1), nuclear factor
erythroid 2-related factor 2 (Nrf2), and hypoxia-inducible factor 1-
alpha (HIF-1α) also reduce oxidative stress-induced cytotoxicity and
apoptosis.75–78 In addition to reducing oxidative stress, MSCs retain
the chemotaxis and phagocytic activity of neutrophils, which facili-
tate pathogen clearance with reduced cytotoxicity to other immune
cells and pulmonary cells in sites of inflammation sites.69 Patients
with pathogen-induced ARDS may especially benefit from this activ-
ity. In short, MSCs integrate neutrophil inhibition and reduction of
oxidative stress by modulating secretion of both anti-apoptotic and
anti-oxidative factors.

Suppression of the inflammatory response is by definition a form
of immune-suppression and would thus typically represent a contra-
indication for patients susceptible to or who have recently acquired
a viral infection. Paradoxically, however, in COVID-19 patients who
have progressed to pneumonia and/or acute respiratory distress
syndrome (ARDS), there is evidence that suppression of at least key
aspects of an excessive immune/inflammatory response is condu-
cive to improved survival. Indeed, some success has been found in
COVID-19 associated ARDS using the steroidal drug methylprednis-
olone, which also attenuates the inflammatory response.79

Mesenchymal stem cells have also paradoxically been shown to
secrete a number of cytokines with anti-apoptotic properties (e.g.
IL-6, IL-8, GM-CSF).80,81 The oxidative stress results from persistent
ROS production and ROS-dependent NETosis (or neutrophil extrac-
tellular trap, where extracellular fibres are released to bind the
pathogens, during the time of cell death) of activated neutrophils that
inhibited from apoptosis because of the presence of anti-apoptotic
cytokines.59,70,71 These aspects of the MSC secretome might be
hypothesized to promote ARDS pathogenesis by contributing to
increased oxidative stress, and thus to act counter-productively as
an ARDS therapeutic. However, the demonstrated preclinical effi-
cacy of MSCs in ARDS models suggests that the net effect of MSCs
is overall suppression of inflammatory-ARDS; further, it has been
noted that these anti-apoptotic cytokines may exhibit other thera-
apeutically beneficial activities independent of inflammatory/oxida-
tive modulation, such as prolonging the metabolic life of beneficial
immune cells.60

The inflammation-suppressing effects of MSCs are well demon-
strated in in vivo literature. Adipose MSC-derived paracrine factors
have been demonstrated to inhibit T-cell differentiation and activation
as well as suppressing production of IFN-γ in vitro.69 Their secreted
vesicles are already being tested in five currently registered clinical tri-
als for respiratory distress via aerosolized inhalation (NCT04602104,
| Injury type | Reference | Experimental model | MSC source | Dose; route of administration; timing of treatment | Outcomes |
|-------------|-----------|--------------------|------------|-------------------------------------------------|----------|
| LPS, E. coli, Bleomycin, P. aeruginosa | Cardenes et al. (2019) | Adult Dorsett Cross sheep weighing 30–40 kg, IV | hMAPCs | $10 \times 10^6$ cells/kg IV or $1 \times 10^6$ cells/kg EB; 1 h after LPS infusion | Improvement in arterial oxygenation. Broad systemic distribution via IV route, localized distribution via EB route |
| | Chien et al. (2012) | Male BALB/C mice, intratracheal 25 μg LPS | Orbital fat-derived stem/stromal cells | $3 \times 10^5$ cells; IV; 20 min after injury | Significant reduction in pulmonary inflammation, decrease in total protein concentration and neutrophil counts in alveolar fluid, reduced endothelial and alveolar epithelial permeability, reduced neutrophil and macrophage infiltration |
| | Danchuk et al. (2011) | 8–10-week-old female BALB/C mice, oropharyngeal 1 mg/kg LPS from E. coli 0111:B4 | Adult hMSCs from the Center for the Preparation and Distribution of Adult Stem Cells | $2.5 \times 10^5$ cells; oropharyngeal aspiration; 4 h after LPS infusion and 30 min after first dose | Significantly reduced lung inflammation, expression of pro-inflammatory cytokines, neutrophil counts and total protein in bronchoalveolar region. Reduction in pulmonary edema |
| | Gupta et al. (2007) | 6–8-week-old male C57BL/6 mice, intratracheal 5 mg/ml LPS from E. coli 055:B5 b(Sigma-Aldrich) | mMSCs from GFP+ C57BL/6 mice | $7.5 \times 10^5$ cells; intratracheal; 4 h after LPS infusion | Increased survival. Significant decrease in pulmonary edema and bronchoalveolar protein/endothelial and alveolar epithelial permeability |
| | Horie et al. (2020) | Adult male Sprague Dawley rats, intratracheal $2 \times 10^9$ E. coli ES162 (serotype: O9 K30 H10) in a 300-μL PBS suspension | Bone marrow and umbilical cord-derived hMSC and UC-derived CD362+ hMSC | $1 \times 10^9$ cells/kg; IV; 30 min after E. coli instillation | Improved oxygenation. Reduced acute lung/histological injury, bacterial load, and inflammatory marker levels |
| | Jung et al. (2019) | 7-week-old male C57BL/6 mice weighing 21–23 g, intratracheal 5 mg/kg LPS from E. coli 055:B5 (Sigma-Aldrich, MO, USA) | Human adipose-derived stem cells (hASCs) (StemPRO Human Adipose-Derived Stem Cells; Thermo Fisher, MA, USA); | $2 \times 10^5$ cells; IV; 4 h after LPS infusion | Reduced neutrophil infiltration and myeloperoxidase levels. Reduced alveolar hemorrhage/congestion, lung injury scores, and collagen deposition around the vessels. Reduced levels of fibrosis accompanied by alveolar septal or interstitial thickening |
| | Li et al. (2019) | 6–8-week-old male C57BL/6 mice weighing 20–25 g, intratracheal 50μl 2mg/ml LPS from E. coli O111:B4 and 30 μl PBS 4 h after LPS infusion | mMSCs from Cyagen Biosciences, Inc. (Santa Clara, CA, USA), mMSCs-sh hairpin RNA (sh)control, mMSCs-shLats1 | $5 \times 10^4$ cells; airway; 4 h after LPS infusion | Reduced lung wet weight/body weight ratio, total bronchoalveolar fluid protein and albumin concentrations, and evidence of pulmonary fibrosis and pathological changes in lungs. Reductions in levels of proinflammatory factors, and increased levels of anti-inflammatory factors. MSC differentiation toward alveolar type-II epithelial cells observed |

(Continues)
| Injury type                  | Reference               | Experimental model                                      | MSC source                  | Dose; route of administration; timing of treatment | Outcomes                                                                 |
|-----------------------------|-------------------------|---------------------------------------------------------|-----------------------------|--------------------------------------------------|--------------------------------------------------------------------------|
| Mao et al. (2015)           | 8–10-week-old male C57BL/6 mice, intratracheal 2 × 10^6 CFU P. aeruginosa | mASCs                      | 1 × 10^5 cells or 1 × 10^6 cells; intratracheal; 1 h after injury | Reduced bacterial burden, alveolar neutrophil accumulation, and reduced levels of myeloperoxidase, macrophage inflammatory protein-2 and total proteins in broncho-alveolar fluid. Reduced evidence of lung injury |
| Martinez-Gonzalez et al. (2012) | 10–12-week-old male BALB/C mice, intranasal 8 mg/kg LPS from E. coli 055:B5 (Sigma-Aldrich) | hASCs or hASCs-sST2         | 1 × 10^6 cells; IV; 6 h after injury         | Reduced lung airspace inflammation and vascular leakage, and evidence of preserved alveolar architecture. Significant reductions in protein content, differential neutrophil count, and proinflammatory cytokine concentrations in bronchoalveolar fluid. Absence of apoptosis and minimal inflammatory cell infiltration |
| Rojas et al. (2005)         | 6–8 week old C57BL/6 mice; intratracheal 4 U/kg bleomycin | Bone marrow-derived mMSCs  | 5 × 10^5 cells; IV; 6 h after bleomycin administration | Differentiation of stem cells into specific and distinct lung cell phenotypes, increase in circulating levels of G-CSF and GM-CSF (known for their ability to promote the mobilization of endogenous stem cells), decrease in inflammatory cytokines |
| Rojas et al. (2014)         | Adult Dorsett Cross sheep weighing 36.5 to 65 kg, IV 3.5 μg/kg E. coli endotoxin LPS from E. coli 055:B5 (Sigma, St. Louis, MO, USA) | MultiStem (human bone marrow derived multipotent adult progenitor cells (hMAPCs)) | 4, 10, or 40 × 10^6 cells; EB; 30 min after LPS infusion | Restoration of blood oxygen levels, improvement in carbon dioxide (CO2) clearance and pulmonary vascular pressure. Reduction in lung edema, reduced markers of inflammation |
| Zhang et al. (2013)         | 8–10-week-old female C57BL/6 mice, oropharyngeal 15 mg/kg LPS from E. coli 055:B5 (Sigma-Aldrich) | hASCs or mASCs isolated from inbred transgenic C57Bl/6-Tg(UBC-GFP)30Scha/J mice (Jackson Laboratories, Bar Harbor, ME, USA) | 3.5 × 10^5 cells; oropharyngeal aspiration; 4 h after injury and 30 min after first dose | Reductions in total protein and albumin concentrations in bronchoalveolar fluid and myeloperoxidase activity. Reduced leukocyte including neutrophil migration into alveoli, reduced expression of proinflammatory cytokines/increased anti-inflammatory cytokine (IL-10) |
Patient case studies/trials in Wuhan, China suggest that intravenously injected mesenchymal stem cells (MSCs) specifically may exhibit efficacy in attenuating symptoms once patients diagnosed with COVID-19 have progressed to pneumonia. In a study by Leng and colleagues, this therapeutic approach was found to produce marked symptom improvements in seven hospitalized COVID-19/pneumonia patients within days. In a related case study, a similar treatment approach was found to generate comparable improvements within two weeks in a 65-year-old patient after other mainstay therapies.

**TABLE 2**

| Injury type | Reference | Experimental model | MSC source | Dose; route of administration; timing of treatment | Outcomes |
|-------------|-----------|-------------------|------------|-----------------------------------------------|----------|
| Virus       | Chan et al. (2016) | 6–8-week-old female Balb/C mice, intranasal 106 TCID50 of H5N1 Influenza A/HongKong/486/97 Bone marrow-derived hMSCs from the Texas A&M Health Science Center | 5 × 10^5 cells; IV; 5 days post-infection | Reversed infection-induced downregulation of sodium and chloride transporter proteins associated with alveolar fluid clearance disruption. Reduced wet-to-dry lung weight ratio, vascular protein leakage/alveolar protein permeability. Reductions in inflammatory cytokine/chemokine levels and invading macrophages/monocytes in lung |
|             | Darwish et al. (2013) | 7–10-week-old male C57BL/6 mice, intranasal 425 EID50 or 150 EID50 influenza A/Mexico/410B/2009 (mouse-adapted H1N1) or 1000 EID50 influenza A/Mexico/410B/2009 (swine-origin pandemic H1N1) bone marrow-derived murine MSCs (mMSCs) and allogeneic hMSCs | 2 × 10^5 cells; IV; 4 h prior to infection and 2 days post-infection or 2 and 5 days post-infection | Negative outcome: Failure to improve survival or decrease pulmonary inflammation/inflammatory cell counts in influenza virus-infected mice with or without combination with oseltamivir |
|             | Gotts et al. (2014) | 8-week-old female C57BL/6, intranasal 100 foci-forming units of influenza A/H1N1/PR8 mMSCs and hMSCs from the National Institutes of Health repository in Temple, TX | 5 × 10^5 cells; retro-orbital; 5 and 6 days after infection | Negative outcome: Failure to improve weight loss, lung water measures, markers bronchoalveolar inflammation, or histological pathological markers. However, prevention of influenza-induced thrombocytosis modest reduction in lung viral load were observed |
|             | Li et al. (2016) | 6–8-week-old C57BL/6, intranasal 1 × 104 MID50 of A/HONG KONG/2108/2003 [H9N2 (HK)] H9N2 virus bone marrow-derived murine MSCs (mMSCs) | 1 × 10^5 cells; IV; 30 min pot-infection | Significantly reduced proinflammatory chemokine and cytokine levels in lung. Reduced invading/inflammatory immune cell invasion in lungs. Improvements in lung histopathology and arterial blood gas observed |
|             | Loy et al. (2019) | 6–8-week-old female BALB/C mice, intranasal 106 log TCID50 of A/Hong Kong/486/1997(H5N1) UC-MSCs | 5 × 10^5 cells; IV; 5 days post-infection | Restored alveolar fluid clearance, protein permeability measures. Modest improvement in survival |
MSC therapies are already being tested in COVID-19 patients on this pretext in a number of currently registered clinical trials (NCT04493242, NCT04384445, NCT04376987, NCT04371393, NCT04611256, NCT04905836, NCT04615429, NCT04525378, NCT04355728, NCT04269525, ChiCTR2000031494, NCT04355728, ChiCTR2000029990, NCT04252118, NCT04269525, NCT04273646, NCT04288102, NCT04313322, NCT04302519, NCT04315987).

Hypoxic preconditioning has also been shown to enhance the anti-oxidative capacity of MSCs when they are transplanted to hypoxic sites, in addition to improving the survival, retention, proliferation and tissue formation of the damaged tissue.72–74 MSCs that overexpress cytoprotective factors such as heme oxygenase-1 (HO-1), nuclear factor erythroid 2-related factor 2 (Nrf2) and hypoxia-inducible factor 1-alpha (HIF-1α) are effective in reducing oxidative stress-induced cytotoxicity and apoptosis.75–78 Moreover, MSCs have been shown to promote the chemotactic and phagocytic activity of neutrophils, which selectively facilitate pathogen clearance while avoiding cytotoxicity to host immune and pulmonary cells in sites of inflammation.89–91 Thus, MSCs integrate neutrophil inhibition and reduction of oxidative stress by modulating secretion of both anti-apoptotic and anti-oxidative factors.

The therapeutic efficacy of mesenchymal cells such as those of placental origin is not limited to suppression of inflammation. For example, secretory signals from trophoblast-derived cells, in particular therapeutically relevant microRNAs, have been demonstrated to exhibit potent antiviral properties in in vitro assays.84

### 3.2.2 Alveolar fluid clearance and restoration of cell permeability

Infiltration of neutrophils and pro-inflammatory immune responses cause endothelial and epithelial cells to become permeable, resulting in pulmonary oedema.97 In the presence of damaged pulmonary tissues, MSCs secrete the keratinocyte growth factor FGF-7, which contributes to restoring amiloride-dependent sodium transport and enhancing alveolar fluid clearance.67,68 Also contributing to clearing alveolar fluid, MSCs secrete cell ligands KGF and Ang-1 that repair the membrane channels and their permeability.65,66 Ang-1 also acts as a stabilizing, anti-inflammatory, and anti-permeable agent to restore the plasma leakage and membrane insulation during the time of lung injury.85–88 An in vitro study of introducing damaged alveolar epithelial cells (AECs) in different culturing environment showed that MSCs produce drastic increase of the IL-1 receptor antagonists and prostaglandin E2 in the presence of the injured AECs and these factors re-established the normal permeability of the epithelial cells.54 This experiment demonstrates the possible route of action of MSCs when administered into patients with injured lung. Thus, administration of MSCs has potential to re-establish normal alveolar fluid level in patients with pulmonary oedema by removing fluid and restoring cell permeability.

### 3.2.3 Anti-inflammatory responses

High levels of pro-inflammatory cytokines, such as TNFα, TGFβ1, IL-1β, and IL-8, have been reported in pulmonary oedema fluid from ARDS patients.89 TNFα and IL-1β are among a cocktail of cytokines released from macrophages after the immune system is activated.90 Once released, they act as specific cell-membrane bound receptors to activate a signalling cascade to increase production of pro-inflammatory cytokines, lipid mediators, ROS and cell adhesion molecules.90,91 The increased production of cytokines, lipid mediators and cell adhesion molecules facilitates migration of the inflammatory cells into tissues and worsens the lung injury as a result.90 In an in vitro study, the MSCs secreted cytokine IL-1RA, which dramatically lessens the inflammation effect of TNFα and IL-1β expressed by macrophages.54 IL-1RA acts as a competitive inhibitor to IL-1β by blocking IL-1β’s binding site and the production of TNFα by macrophages.54 MSCs also release...
substances such as TSG-6, IGF-1 and LXA4, which induce anti-inflammatory responses in murine acute lung injury models by directly acting on the cells inducing inflammation to undergo phenotypic transition. In addition to abrogating the effect of TNF-α, IL-6, and IL-1β, MSCs have also been shown to inhibit recruitment of neutrophils and protein formulation within the inner alveolar space.

MSCs can also act to reduce cell attraction and migration as mediators of inflammatory processes, as in lung injury settings. For example, TGFβ1 has an essential role in lung repair and fibroproliferation by promoting collagen synthesis. However, overexpression of TGFβ1 has been shown to facilitate the migration of fibroblasts from the extracellular space into the intracellular alveolar space, and to activate the human procollagen I promoter to induce inflammation of lung tissues. IL-8 is also involved in regulating the migration of endothelial cells into the alveolar space. Moreover, the anti-IL-8 autoantibody, which bind to IL-8 with a very high affinity and prevents IL-8 from attracting and binding to neutrophils, forms a complex with IL-8 and immunoglobulin G, recruiting an inflammatory response from the periphery.

3.2.4 | Genetic modification of stem cell therapies

Because inflammatory pathway induction is the key underlying cause of ARDS, extensive studies involving genetic modifications of MSCs have been conducted to improve the anti-inflammatory properties of MSCs. One system that has been a focus for genetic modification approaches in the context of cardiovascular diseases and is receiving increasing attention in the study of ARDS, is the renin-angiotensin system (RAS). Within the RAS, there is substantial interest arises in the angiotensin-converting enzyme (ACE). The ACEs cleave the peptide hormone angiotensin-I (Ang I) into Ang II. Ang II interacts with angiotensin II type 1 receptor (AT1R) and induces pulmonary vasoconstriction and increases vascular permeability, leading to oedema. Ang II also exerts pro-inflammatory effects and promotes fibroproliferation. On the other hand, ACE2 degrades Ang II to Ang-(1–7), which interacts with the Mas receptor to mediate anti-inflammatory responses. Because the type II alveolar epithelial cells, where ACE2 is produced, are severely damaged in ARDS, the ACE2/Ang-(1–7)/Mas axis is an interesting target for genetically modifying MSCs to overexpress ACE2 to counter the aggravated effects of the ACE/AngII/AT1R axis in ARDS. MSCs that overexpress ACE2 have shown therapeutic benefits in reducing neutrophil influx and pro-inflammatory cytokine production in preclinical studies, and hence promoting endothelial repair and resolving pulmonary functions.

Relevant to COVID-19 and ARDS, transmembrane ACE2, together with the cellular serine protease TMPRSS2, mediates cell entry of SARS-CoV-2 and its in vivo replication. This raises the question of whether ACE2 expression by administered stem cells could complicate treatment of SARS-CoV-2 by allowing for infection of administered cells. A clinical trial conducted in China showed that transplanted MSCs express minimal gene expression of ACE2 and TMPRSS2, the two main routes of infection for SARS-CoV-2. These MSCs also secrete high levels of immunomodulatory factors (e.g. IL-10, IP-10, TNF-α, TGF-β, HGF, LIF, GAL, NOA1, FGF, VEGF, EGF, BDNF, NGF) after transplantation and stimulate lung repair, improving clinical outcomes for patients. These contradictory findings regarding cytokine production in ACE2-overexpressing versus ACE2-negative MSCs indicate the urgent need to identify targets other than the ACE2 axis to maximize the benefits of genetically modified MSCs in treating ARDS, whether induced by SARS-CoV-2 or not. In addition to ACE2 overexpression or knockout MSCs, MSCs coadministered with human recombinant soluble ACE2 (hrsACE2) have been evaluated. A recent cryoelectron microscopic study of SARS-CoV-2 claimed that these MSCs inhibit SARS-CoV-2 infections in host cells, and they can prevent downstream effects of COVID-19 during an early stage. Although the therapeutic benefits of hrsACE2-cotreated or overexpressing MSCs are not known during later stages of COVID-19, hrsACE2-expressing MSCs may still show effects similar to those of ACE2-negative MSCs in terms of blocking SARS-CoV-2 cell entry and replication and stimulating lung repair through undiscovered pathways.

Substantial emphasis has also been put on another inflammatory signalling pathway, the IL-33/ST2 pathway. IL-33, a recently discovered member of the IL-1 cytokine family, is abundantly present in endothelial and epithelial cells in the skin, gastrointestinal tract and lungs. It is released as an alarmin during injury and interacts with its receptor, suppression of tumourigenicity 2 (ST2). ST2 is expressed in two isoforms, a transmembrane receptor (ST2L) and a soluble decoy receptor (sST2). Interaction of IL-33 with these isoforms triggers opposing inflammatory signalling pathways. IL-33/ST2L initiates acute inflammation through Th2-dependent immune responses, while IL-33/sST2 attenuates Th2-dependent inflammation as sST2 lacks the intracellular Toll/Interleukin-1 receptor domain to induce the signalling pathway. The sST2-overexpressing MSCs showed therapeutic benefit in mice in attenuating acute LPS-induced pulmonary inflammation. In addition, the plasma concentration of sST2 can be used as a diagnostic factor to distinguish ARDS from acute heart failure and serves as a prognostic biomarker to assess the severity of ARDS to determine how supportive treatments and weaning practices should be implemented.

In addition to their anti-inflammatory properties, the therapeutic efficacy of MSCs depends on several other factors such as homing, tissue restoration and protective effects against apoptosis and oxidation. Genetic modification can thus serve as an excellent tool to improve the therapeutic benefits of MSCs. MSCs have shown tropism to injury sites, but they may lose homing receptors after the large-scale expansion needed to produce enough cells for therapeutic doses. MSCs genetically engineered to overexpress the C-X-C motif chemokine receptor 4 (CXCR4) on their cell surface showed improved homing to sites of tissue injury. CXCR4 interacts with its ligand, stromal cell-derived factor-1 (SDF-1), which has elevated expression at sites of tissue injury. Similarly, MSCs that overexpress the E-prostanoid 2 (EP2) receptor, which interacts with prostaglandin E2, have shown
| Identifier       | Status                        | Phase | Treatment                                                                 | Dose                                                                 | Regimen (total number of doses; frequency) | Route | Country         |
|------------------|-------------------------------|-------|---------------------------------------------------------------------------|----------------------------------------------------------------------|---------------------------------------------|-------|-----------------|
| NCT05127122     | Not yet recruiting            | I/II  | ExoFlo                                                                    | 10 ml or 15 ml                                                        | Single dose                                |       | USA             |
| NCT04347967     | Not yet recruiting            | I     | UMC119-06                                                                 | Low, medium, and high                                                | Single dose                                | IV    | Taiwan          |
| NCT04371393     | Recruiting                    | III   | Remestemcel-L                                                            | 2 × 10^6                                                            | Two doses; four days apart                 | IV    | USA             |
| NCT04366063     | Recruiting                    | II/III| MSCs or MSCs +MSC-EVs                                                   | 100 × 10^6 (±10%) w/o EVs                                            | Two doses; Day 0 and 2 (MSCs), Day 4 and 6 (MSC-EVs) | IV    | Islamic Republic of Iran |
| NCT04367077     | Recruiting                    | II/III| MultiStem                                                                  | N/A                                                                | Single dose                                | IV    | USA             |
| NCT02804945     | Completed                     | II    | Allogeneic Human MSCs                                                     | N/A                                                                | Single dose                                | IV    | USA             |
| NCT02112500     | Unknown (previously recruiting)| II    | MSCs cultured and extracted from bone marrow of enrolled patients       | N/A                                                                | IV                                           | Korea |                 |
| NCT04348461     | Not yet recruiting            | II    | Allogeneic and expanded adipose tissue-derived mesenchymal stromal cells| 1.5 × 10^6                                                          | Two doses; N/A                              | IV    | Spain           |
| NCT03807804     | Recruiting                    | II    | HLCM051 (MultiStem)                                                      | 900 × 10^6 (±20%)                                                    | Single dose                                | IV    | Japan           |
| NCT04377334     | Not yet recruiting            | II    | Allogeneic BM-MSCs                                                       | N/A                                                                | N/A                                         | N/A   | Germany         |
| NCT02444455     | Unknown (previously recruiting)| I/II  | Human Umbilical-Cord-Derived MSCs (UCMSC)                                | 0.5 × 10^6                                                          | Three doses; once a day                     | IV    | China           |
| NCT04289194     | Active, not recruiting        | I/II  | HCR040 (whose active substance is HC016, allogeneic adipose-derived adult mesenchymal stem cells expanded and pulsed with H2O2) | -                                                                   | Phase I: Single dose; dose escalation Phase II: Single dose of 2 × 10^6 cells/kg | IV    | Spain           |
| NCT02095444     | Unknown (previously recruiting)| I/II  | Menstrual blood stem cells                                               | 10 × 10^5                                                           | Four doses; two doses per week and two weeks| IV    | China           |
| NCT04355728     | Recruiting                    | I/II  | Umbilical Cord Mesenchymal Stem cells (UCMSC)                            | 100 × 10^6                                                          | Two doses; within 24 and 72 h               | IV    | USA             |
| NCT03042143     | Recruiting                    | I/II  | Realist Orbcel-C (Human umbilical cord derived CD362 enriched MSCs)      | 100 × 10^6 200 × 10^6 400 × 10^6                                   | Phase I: Single dose; Dose escalation Phase II: Single dose of 400 × 10^6 cells | IV    | USA             |
| Identifier | Status             | Phase | Treatment                                                                 | Dose Cells | Regimen (total number of doses; frequency) | Route | Country |
|------------|--------------------|-------|---------------------------------------------------------------------------|------------|-------------------------------------------|-------|---------|
| NCT04331613 | Recruiting        | I/II  | CASTem (immunity- and matrix-regulatory cells (IMRCs), also named M cells, differentiated from clinical-grade human embryonic stem cells (hESCs)) | -          | Single dose; dose escalation              | IV    | China   |
| NCT04390139 | Recruiting        | I/II  | XCEL-UMC-BETA (Wharton-Jelly MSCs)                                       | -          | Two doses; Day 1, 3                       | IV    | Spain   |
| NCT02611609 | Completed         | I/II  | MultiStem (adult stem cell)                                               | Low High   | Single dose, dose escalation              | N/A   | USA     |
| NCT04333368 | Recruiting        | I/II  | Umbilical cord Wharton's jelly-derived human                              | -          | Three doses; Day 1, 3, 5                  | IV    | France  |
| NCT02175303 | Unknown           | I/II  | Placenta-derived decidual stromal cell therapy                           | -          | One or more doses; Weekly                 | IV    | Sweden  |
| NCT01775774 | Completed         | I     | Allogeneic Bone Marrow-Derived Human MSCs                                | -          | Single dose; dose escalation              | IV    | USA     |
| NCT04390152 | Not yet recruiting | I     | Wharton's jelly derived Mesenchymal Stem cells                           | 50 × 10^6  | Two doses; N/A                           | IV    | Colombia |
| NCT01902082 | Unknown (previously recruiting) | I     | Allogeneic Adipose-derived MSCs                                           | -          | Single dose                              | IV    | China   |
| NCT04347967 | Not yet recruiting | I     | Human umbilical cord-derived MSCs (UMC 119–06)                           | Low Medium | N/A                                       | IV    | Taiwan  |
| NCT04400032 | Not yet recruiting | I     | BM-MSCs                                                                   | 25 × 10^6  | Three doses, consecutive days (dose escalation) | IV    | Canada  |
| NCT04345601 | Not yet recruiting | I     | BM-MSCs                                                                   | -          | Single dose                              | IV    | USA     |
| NCT03608592 | Recruiting        | N/A   | Human Umbilical Cord MSCs                                                | -          | Single dose                              | IV    | China   |

Note: Search parameters: Condition or disease: Acute Respiratory Distress Syndrome. Other terms: Stem cell. Excluded studies: Observational studies, non-stem cell interventions.
| Identifier   | Status              | Phase | Treatment                              | Dose                      | Regimen (total number of doses; frequency) | Route | Country       |
|--------------|---------------------|-------|----------------------------------------|---------------------------|---------------------------------------------|-------|---------------|
| NCT05132972 | Recruiting          | II/III| UCMSCs                                 |                           | Three doses; Day 0, Day 3, and Day 6        | IV    | Indonesia     |
| NCT04490486 | Not yet recruiting  | I      | UCMSCs                                 | $100 \times 10^6$        | Two doses; Day 0, 3                        | IV    | USA           |
| NCT04371393 | Recruiting          | III    | MSCs (Remestemcel-L)                   | $2 \times 10^6$          | Two doses; Four days apart                  | IV    | USA           |
| NCT04366063 | Recruiting          | II/III | MSCs or MSCs +MSC-EVs                 | $100 \times 10^6$ (±10%) w/o EVs
$100 \times 10^6$ (±10%) w/ EVs | Two doses; Day 0 and 2 (MSCs), Day 4 and 6 (MSC-EVs) | IV    | Islamic Republic of Iran |
| NCT04367077 | Recruiting          | II/III | MultiStem                              | N/A                       | N/A                                         | IV    | USA           |
| NCT04416139 | Recruiting          | II     | Mesenchymal Stem cells                 | $2 \times 10^6$          | Single dose: Day 1                          | IV    | Mexico        |
| NCT04315987 | Not yet recruiting  | II     | NestCell® Mesenchymal Stem Cell        | $20 \times 10^6$         | Three doses; Day 1, 3, 5                    | IV    | Brazil        |
| NCT04348435 | Enrolling by invitation | II  | Hope Biosciences Allogeneic Adipose-derived Mesenchymal Stem Cell Therapy (allogeneic HB-adMSCs) | $50 \times 10^6$
$100 \times 10^6$
$200 \times 10^6$ | Five doses; Week 0, 2, 6, 10, 14 | IV    | USA           |
| NCT04349631 | Enrolling by invitation | II  | Autologous HB-adMSCs                  | N/A                       | Five Doses; N/A                             | IV    | USA           |
| NCT04288102 | Recruiting          | II     | MSCs                                  | $40 \times 10^6$         | Three doses; Day 0, 3, 6                    | IV    | China         |
| NCT04348461 | Not yet recruiting  | II     | Allogeneic and expanded adipose tissue-derived mesenchymal stromal cells | $1.5 \times 10^6$ | Two doses; N/A                             | IV    | Spain         |
| NCT04362189 | Not yet recruiting  | II     | HB-adMSCs                              | $100 \times 10^6$        | Four doses; Day 0, 3, 7, 10                 | IV    | USA           |
| NCT04299152 | Not yet recruiting  | II     | Stem Cell Educator-Treated Mononuclear Cells Apheresis (autologous human multipotent cord blood stem cells (CB-SG)) | N/A                       | Single (extra dose if needed; a week apart) | IV    | USA           |
| NCT04377334 | Not yet recruiting  | II     | Allogeneic BM-MSCs                     | N/A                       | N/A                                         | IV    | Germany       |
| NCT04389450 | Not yet recruiting  | II     | PLX-PAD (allogeneic ex vivo expanded placental mesenchymal-like adherent stromal cells) | Interval high dose
High dose
Low dose | Interval high dose: 15 doses; 1 week apart
High dose: Single dose
Low dose: Single dose | Intramuscular | USA and Israel |
| NCT04361942 | Recruiting          | II     | Allogeneic MSCs                        | $1 \times 10^6$          | Single dose                                | IV    | Spain         |
| Identifier      | Status                  | Phase | Treatment                                                                 | Dose                  | Regimen (total number of doses; frequency) | Route | Country |
|-----------------|-------------------------|-------|---------------------------------------------------------------------------|-----------------------|--------------------------------------------|-------|---------|
| NCT04269525     | Recruiting              | II    | UC-MSCs                                                                  | $99 \times 10^6$      | Four doses; Day 1, 3, 5, 7                 | IV    | China   |
| NCT04336254     | Recruiting              | I/II  | Allogeneic human dental pulp MSCs (BSD BTC & Utooth BTC)                 | $30 \times 10^6$      | Three doses; Day 1, 4, 7                   | IV    | China   |
| NCT04366323     | Recruiting              | I/II  | Allogeneic and expanded adipose tissue-derived MSCs                      | $80 \times 10^6$      | Two doses; N/A                            | IV    | Spain   |
| NCT04382547     | Enrolling by invitation | I/II  | Allogenic Pooled Olfactory Mucosa-derived Mesenchymal Stem Cells          | N/A                   | N/A                                        | IV    | Belarus |
| NCT04346368     | Not yet recruiting      | I/II  | Bone marrow-derived MSCs                                                 | $1 \times 10^6$       | Single dose                               | IV    | China   |
| NCT04390152     | Not yet recruiting      | I/II  | WI-MSCs                                                                  | $50 \times 10^6$      | Two doses; N/A                            | IV    | Colombia|
| NCT04390139     | Recruiting              | I/II  | XCEL-UMC-BETA (Wharton-Jelly mesenchymal stromal cells)                  | $1 \times 10^6$       | Two doses; Day 1, 3                        | IV    | Spain   |
| NCT04341610     | Withdrawn               | I/II  | Allogeneic adipose tissue-derived MSCs                                   | $100 \times 10^6$     | N/A                                        | N/A   | Denmark |
| NCT04398303     | Not yet recruiting      | I/II  | ACT-20-MSC (allogeneic human umbilical derived mesenchymal stem cells) or ACT-20-CM (ACT-20-MSC conditioned medium) | $1 \times 10^6$ cells/kg in 100 mL CM 100 mL CM only | N/A | IV | USA |
| NCT03042143     | Recruiting              | I/II  | Realist Orbcel-C (Human umbilical cord derived CD362 enriched MSCs)      | $400 \times 10^6$     | Single dose                               | IV    | UK      |
| NCT04333368     | Recruiting              | I/II  | umbilical cord Wharton's jelly-derived mesenchymal stromal cells (UC-MSC) | $1 \times 10^6$       | Three doses; Every other day               | IV    | France  |
| Identifier       | Status                  | Phase | Treatment                                                                 | Dose | Regimen (total number of doses; frequency) | Route | Country |
|------------------|-------------------------|------|---------------------------------------------------------------------------|------|------------------------------------------|-------|---------|
| NCT04313322     | Recruiting              | I    | Wharton’s Jelly Mesenchymal stem cells (WJ-MSCs) derived from cord tissue of newborns | -    | $1 \times 10^6$                      | Three doses; three days apart | IV     | Jordan  |
| NCT04252118     | Recruiting              | I    | MSCs                                                                      | $30 \times 10^6$ | -                                        | Three doses; Day 0, 3, 6 | IV     | China   |
| NCT04302519     | Not yet recruiting      | I    | Dental pulp mesenchymal stem cells                                        | -    | $1 \times 10^6$                      | N/A; Day 1, 3, 7 (Dose escalation) | IV     | China   |
| NCT04371601     | Active, not recruiting  | I    | UC-MSCs                                                                  | -    | $10^6$                                  | Four doses; every 4 days | IV     | China   |
| NCT04397796     | Not yet recruiting      | I    | Allogeneic bone marrow-derived MSCs (CD73+, CD90+, CD105+, CD14-, CD34-, CD45-, HLA-DR-) | N/A  | N/A                                     | N/A | USA     |
| NCT044000032    | Not yet recruiting      | I    | BM-MSCs                                                                  | $25 \times 10^6$ | $50 \times 10^6$ | $90 \times 10^6$ | Three doses; Three consecutive days (24±4 h apart) (dose escalation) | IV | Canada |
| NCT04345601     | Not yet recruiting      | I    | Allogeneic blood-derived MSCs                                             | $1 \times 10^6$ | -                                       | Single dose | IV | USA     |
| NCT04273646     | Not yet recruiting      | N/A  | Human Umbilical Cord MSCs                                                | -    | $0.5 \times 10^6$                    | Four doses; Day 1, 3, 5, 7 | IV | China   |
| NCT04393415     | Not yet recruiting      | N/A  | Cord blood stem cells or platelet rich plasma (PRP)                      | N/A  | N/A                                     | N/A | Egypt   |
| NCT04293692     | Withdrawn               | N/A  | UC-MSCs                                                                  | -    | $0.5 \times 10^6$                    | Four doses; Day 1, 3, 5, 7 | IV | China   |

*Note: Search parameters: Condition or disease: COVID; Other terms: Stem cell. Excluded studies: Observational studies, non-stem cell interventions*
improved homing and retention. EP2-overexpressing MSCs have also shown additional benefits in terms of tissue restoration by reducing pulmonary vascular permeability and improving histopathology. Alveolar restoration and oedema clearance have been improved by use of MSCs that overexpress KGF. KGF plays a significant role in stimulating proliferation of alveolar type II cells and surfactant synthesis for pulmonary epithelial repair. To further enhance lung repair and restore pulmonary functions, genetic modifications involving anti-apoptosis and anti-oxidation pathways, for example overexpression of heme oxygenase-1, are also being extensively investigated.

These studies of diverse genetic modifications to MSCs have shown that a single modification can yield multiple benefits and suggest promise of this approach. Given the rapid advance of genetic engineering technology to date, we may expect that the huge capacity of MSCs will allow multiple modifications, raising the possibility of synergistic benefits to treat ARDS and increased numbers of genetically modified MSCs translated to clinical studies.

4 | CURRENT CLINICAL TRIALS USING MSCS TO TREAT ARDS

Due to the relative ease of tissue/cell sourcing and the consequently higher volume of preclinical work investigating clinical viability, MSC therapies have been the subject of a relatively higher number of approved clinical trials compared with other stem cell-based therapeutics. As of 5 January 2022, there are 65 and 122 interventional stem cell clinical trials for ARDS and COVID-19, respectively, listed on clinicaltrials.gov. Han and colleagues note the key limitation that many of the present studies in this area are limited by small sample sizes and lack of a well-defined time-response relationship between MSC administration and patient performances.

Current registered trials using stem cell interventions for COVID-19 associated and other presentations of ARDS are summarized in Tables 3 and 4.

5 | CELL-FREE THERAPIES DERIVED FROM THE STEM CELL SECRETOME: EXTRACELLULAR VESICLES AND miRNA

In light of the key role of secretory factors in conferral of therapeutic benefits from MSC therapy, there is increasing interest in studying the therapeutic benefits of MSC-derived extracellular vesicles (MSC-EVs) as compared to MSCs themselves. As MSC-EVs are a cell-free treatment, they offer multiple advantages over cell-based treatments. First, as they are non-nucleated and acellular, they cannot proliferate, and thus, there is minimal risk of tumorigenicity. Second, as they do not express HLA antigens, they pose much lower risks of immunogenicity, and graft-versus-host disease than their source cells, and thus are safer for allogeneic transplantation as they pose a lower risk of host immunorejection. Third, MSC-EVs are smaller than cells, which allows for better penetration into target tissues. In contrast, because of their larger dimensions cell therapies are restricted from penetrating certain membrane barriers or extravasating from capillaries to key tissues relative to their much smaller secretory vesicles, and they carry a higher risk of embolus formation.

Furthermore, storage of MSC-EVs does not require cryopreservatives, such as DMSO, which are necessary for long-term storage of MSCs but are detrimental to their viability. In addition, MSC-EVs are less affected by repeated freeze and thaw cycles as compared to MSCs.

The secretome of MSCs and other stem cells includes small soluble proteins such as cytokines, chemokines, growth factors, and anti-inflammatory factors. MSC-EVs contain microRNAs (miRNAs) and messenger RNAs (mRNAs) that interact with the target cell's endogenous mRNAs and facilitate the production of proteins with therapeutically relevant including inflammation-suppressing niches within the cells (Figure 2). These various EV contents affect a variety of cell functions to bring desired effects in different disease models. EV-based therapies have also been artificially engineered to incorporate key therapeutic components for delivery.

There is ample evidence that extracellular vesicles and their factors derived from a variety of cell sources are also able to directly impart
**TABLE 5** Interventional MSC-EV Clinical Trials as of January 05, 2022

| Identifier     | Status                     | Phase | Treatment                  | Dose                  | Regimen (total number of doses; frequency) | Route      | Country     |
|----------------|----------------------------|-------|----------------------------|-----------------------|------------------------------------------|------------|-------------|
| NCT04602442    | Enrolling by invitation    | II    | MSC-EVs                    | 0.5-2 × 10^10 EVs /3 mL | Twenty doses; twice a day                | Inhalation | Russia      |
| NCT04491240    | Completed                  | I/II  | MSC-EVs                    | 0.5-2 × 10^10 EVs /3 mL | Twenty doses; twice a day                | Inhalation | Russia      |
| NCT04276987    | Completed                  | I     | MSC-EVs                    | 2.0 × 10^8 EVs /3 mL   | 5 doses; once a day                      | Inhalation | China       |
| NCT04493242    | Completed                  | II    | MSC-EVs                    | 1X @ 8 or 12 × 10^{11} / 100 ml | Single dose                            | IV         | USA         |
| NCT04798716    | Not yet recruiting         | I/II  | MSC-EVs                    | 2 × 10^9 or 4 × 10^9 or 8 × 10^9 EVs /mL | Three doses; once every other day | IV         | USA         |
| NCT04366063    | Recruiting                 | II/III| MSCs or MSCs +MSC-EVs     | 100 × 10^6 (±10%) w/o EVs / 100 ml | -                                       | IV         | Islamic Republic of Iran |
| NCT04398303    | Not yet recruiting         | I/II  | ACT-20-MSC (allogeneic human umbilical derived mesenchymal stem cells) or ACT-20-CM (ACT-20-MSC conditioned medium) | - | 1 × 10^6 in 100 ml CM / 100 ml CM only | N/A        | IV USA      |
| NCT04276987    | Not yet recruiting         | I     | Allogenic adipose mesenchymal stem cells derived exosomes (MSCs-Exo) | 2 × 10^8 nano vesicles | -                                       | Five doses; Day 1, 2, 3, 4, 5 | Aerosol inhalation | China |
| NCT04313647    | Recruiting                 | I     | A Tolerance Clinical Study on Aerosol Inhalation of Mesenchymal Stem Cells Exosomes In Healthy Volunteers | 1X @ 2.0 × 10^8 - 8.0 × 10^8 exosomes / dose | Single dose                            | Aerosol inhalation | China |
immune-modulatory effects on recipient cells and physiologic systems through their therapeutically relevant intrinsic contents. For example, dendritic cell (DC) exosomes reduced inflammation and associated arthritis in a murine model.161 Exosomes within the secretome of multiple immune cell types have themselves been found to participate in host immunity against invading viral pathogens by transporting antiviral factors between cells and activating antiviral mechanisms.162 Directly inhibiting pathogen proliferation and infection as well as inducing humoral and cytotoxic immunity.163

It has been demonstrated for instance that murine bone marrow DCs pulsed with diphtheria toxoid (DT) are induced to generate exosomes promoting a DT-specific immunoglobulin response.164 Treatment with exosomes derived from Toxoplasma gondii antigen (Ag)-pulsed DC cells was similarly shown to induce anti-T. gondii Ag antibodies in association with elevated humoral response and symptom improvement in infected mice.165 Infection with Mycobacterium tuberculosis promoted release of exosomes containing M. tuberculosis MHC-II complexes associated with antimicrobial activity from murine macrophages.166 Exosomeanchoring protein-fused DNA vectors expressing antigens specific to Human Papilloma Virus (HPV)167 as well as a broad spectrum of other viruses including Influenza, Hepatitis C, Crimean-Congo Hemorrhagic Fever, and the flaviviruses Ebola and West Nile Virus168 were effective in promoting an antigen-specific cytotoxic T lymphocyte (CTL) response in mice. Moreover, exosomes loaded with ovalbumin antigens from DC pulsing have been shown to not only augment T-cell responsiveness, IFN-γ and IgG production but to mediate a Th1 shift.169

There is also evidence of secreted exosomes exhibiting directly antiviral properties through their intrinsic contents. Exosomes secreted by macrophages in response to IFN-α have been demonstrated to utilize Hepatitis A receptors to deliver antiviral substances to hepatocytes.170 Exosomes isolated from human trophoblasts have also been found to exhibit directly antiviral properties in vitro, associated with miRNA cargoes derived from the chromosome 19 cluster as well as a unique peptide and phospholipid repertoire.84 Intriguingly, Herpes Simplex 1 (HSV-1) viral microRNAs miR-H28 and miR-H29 transmitted via exosomes have also been found to restrict viral cell-cell transmission via IFN-γ upregulation, postulated by the authors to represent a mechanism of limiting viral spread to uninfected cells in favour of maximizing transmission to an alternate host.171 Exosomes containing the spike S protein derived from other variants of SARS-associated coronavirus (SARS-CoV) have also been found to successfully induce the generation of neutralizing antibodies in a murine model.172

The investigation of the therapeutic benefits of MSC-EVs in comparison to MSCs is a rapidly developing field. As of 5 January 2022, there are nine MSC-EV clinical trials, summarized in Table 5.

6 | CONCLUSION

The persistent emergence of novel SARS-Cov-2 variants, and with these fears of novel strains capable of effective vaccine escape and/or heightened virulence, combined with continued struggles in meeting global demands of accessibility for vaccines to local populations, underscore the clear need that remains for effective therapies to address SARS-Cov-2 infection and severe COVID-19 clinical manifestation. Stem cell-based therapy has great potential for treating COVID-19 associated ARDS, a condition presenting a high mortality risk for which there remains a substantial unmet need for effective treatment interventions. Stem cell-based therapies have immunomodulatory effects and multiple preclinical and clinical studies have supported their proposed efficacy against ARDS.124,127,159,171,172 Among the many types of stem cells currently being evaluated for ARDS and/or COVID-19, MSC-based therapies are gaining popularity because of their ease of isolation and convenient culturing.43 MSC-EVs are also of increasing interest as an acellular alternative modality able to exert many of the key therapeutically relevant benefits without presenting the risks of cell therapies such as immunogenicity or tumourigenesis. Further clinical studies incorporating larger patient sample sizes are needed to more definitively establish optimal dose schedules and dose-response times, and to better evaluate comparative efficacy with cell therapies derived from specific optimized culture conditions to maximize therapeutic contents and functional benefit, and from varying cell/tissue sources both mesenchymal and potentially investigating non-mesenchymal alternatives of equal or superior therapeutic value for this indication. Moreover, stem cell-derived secretory factors, as contained in extracellular vesicles, should be given greater attention as an acellular alternative therapeutic niche presenting much of the benefit demonstrated preclinically and clinically with their source cells, but circumventing the technical and safety concerns presented by administration of cell as opposed to cell-secretome based therapies.

CONFLICT OF INTEREST

CDC is affiliated with StemXO Inc., a private biotechnology company with an interest in stem cell therapeutic development. The other authors declare no conflicts of interests.

AUTHOR CONTRIBUTIONS

Gary Ngai: Investigation (equal); Writing – original draft (equal); Writing – review & editing (equal). Dae Hong Kim: Formal analysis (equal); Writing – review & editing (equal). Mohamed Ahammad: Formal analysis (equal); Writing – review & editing (equal). Margarita Gutova: Formal analysis (equal); Writing – review & editing (equal). Karen Aboody: Conceptualization (equal); Writing – review & editing (equal). Christopher D Cox: Conceptualization (equal); Formal analysis (equal); Writing – original draft (equal); Writing – review & editing (equal).

DATA AVAILABILITY STATEMENT

Data sharing not applicable to this article as no data sets were generated or analyzed during this study.

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REFERENCES

1. WHO Timeline - COVID-19. https://www.who.int/news-room/detail/27-04-2020-who-timeline-covid-19. Accessed February 24, 2022.

2. Coronavirus Disease 2019. Centers for Disease Control and Prevention. 2020. https://www.cdc.gov/coronavirus/2019-ncov/cases-updates/cases-in-us.html. Accessed February 24, 2022.

3. de Wit E, van Doremalen N, Faalzaran D, Munster VJ. SARS and MERS: recent insights into emerging coronaviruses. Nat Rev Microbiol. 2016;14:523-534.

4. Phan T. Genetic diversity and evolution of SARS-CoV-2. Front Cell Neurosci. 2021;15(1):e199.

5. Goldberg Y, Mandel M, Bar-On YM, et al. Waning immunity after 8 months of BNT162b2 vaccine in Israel. N Engl J Med. 2021;385(24):e85.

6. Levin EG, Lustig Y, Cohen C, et al. Waning immune humoral response to BNT162b2 Covid-19 vaccine over 6 months. N Engl J Med. 2021;385(24):e84.

7. Wu C, Chen X, Cai Y, et al. Risk factors associated with acute respiratory distress syndrome and death in patients with Coronavirus Disease 2019 Pneumonia in Wuhan, China. JAMA Intern Med. 2020;180(7):934. 10.1001/jamaintermed.2020.0994

8. Coperchini F, Chiovato L, Croce L, Magri F, Rotondi M. The cytokine storm in COVID-19: an overview of the involvement of the chemokine/chemokine-receptor system. Cytokine Growth Factor Rev. 2020;19:25-32.

9. Bellani G, Matthan MBT, Law A, et al. Epidemiology, patterns of care, and mortality for patients with acute respiratory distress syndrome in intensive care units in 50 countries. JAMA. 2016;315(7):788-800.

10. Stevens JP, Law A, Giannakoulis J. Acute respiratory distress syndrome. BMJ. 2018;319:732.

11. Ware LB, Matthay MA. The acute respiratory distress syndrome. N Engl J Med. 2000;342:1334-1349.

12. Wang H, Ma S. The cytokine storm and factors determining the sequence and severity of organ dysfunction in multiple organ dysfunction syndrome. Am J Emerg Med. 2008;26(6):711-715.

13. Moldofsky H, Patcai J. Chronic widespread musculoskeletal pain, fatigue, depression and disordered sleep in chronic post-SARS syndrome; A case-controlled study. BMC Neuro. 2011;11:1-7.

14. Perrin R, Riste L, Hann M, et al. Interleukin-1 blockade on days alive and ventilator-free in patients with moderate or severe acute respiratory distress syndrome and COVID-19: the CoDEX randomized clinical trial. JAMA. 2020;324(13):1307-1316.

15. Kim JM, Chung YS, Jo HJ, et al. Identification of Coronavirus CoV-2 infection—more than just the common cold. JAMA. 2020;323(8):707-708.

16. Peiris JS, Yuen KY, Osterhaus AD, Stöhr K. The severe acute respiratory syndrome (SARS) coronavirus. Nature. 2003;423(6939):395-400.

17. Coperchini F, Chiovato L, Croce L, Magri F, Rotondi M. The cytokine storm in COVID-19: an overview of the involvement of the chemokine/chemokine-receptor system. Cytokine Growth Factor Rev. 2020;19:25-32.

18. Perrin R, Riste L, Hann M, et al. Interleukin-1 blockade on days alive and ventilator-free in patients with moderate or severe acute respiratory distress syndrome and COVID-19: the CoDEX randomized clinical trial. JAMA. 2020;324(13):1307-1316.

19. Hamming I, Timens W, Bulthuis ML, Lely AT, Navis GV, van Goor H. Tissue distribution of ACE2 protein, the functional receptor for SARS coronavirus. A first step in understanding SARS pathogenesis. J Pathol. 2004;203(2):631-637.

20. Fan E, Brodie D, Slutsky AS. Acute respiratory distress syndrome: advances in diagnosis and treatment. JAMA. 2018;320(21):698-710.

21. Tomazini BM, Maia IS, Campolante AB, et al. Effect of dexamethasone on days alive and ventilator-free in patients with moderate or severe acute respiratory distress syndrome and COVID-19: the CoDEX randomized clinical trial. JAMA. 2020;324(13):1307-1316.

22. Kasereka MC, Hawkes MT. Neuroinvasive potential of human coronavirus OC43: role of SARS-CoV-2 infection and COVID-19: Lessons from viral RNA neurotropism and possible relevance to Parkinson's disease. Front Cell Neurosci. 2021;15(15):199.

23. Kim JM, Chung YS, Jo HJ, et al. Identification of Coronavirus CoV-2 infection—more than just the common cold. JAMA. 2020;323(8):707-708.

24. Wang MD, Jolly AM. Changing virulence of the SARS virus: the epidemiological evidence. Bull World Health Organ. 2004;82:547-548.

25. Peiris JS, Yuen KY, Osterhaus AD, Stöhr K. The severe acute respiratory syndrome (SARS) coronavirus. N Engl J Med. 2003;349(25):2431-2441.

26. Zaki AM, Van Boheemen S, Bestebroer TM, Osterhaus AD, Fouchier RA. Isolation of a novel coronavirus from a man with pneumonia in Saudi Arabia. N Engl J Med. 2012;367(19):1814-1820.

27. Letko M, Miazgowicz K, McMinn R, et al. Adaptive evolution of MERS-CoV to species variation in DPP4. Cell Rep. 2018;24(7):1730-1737.

28. Kim D, Lee JY, Yang JS, Kim JW, Kim VN, Chang H. The architecture of SARS-CoV-2 transcriptome. Cell. 2020;181(4):914-921.

29. de Wilde AH, Snijder EJ, Kikkert M, van Hemert MJ. Host factors in coronavirus replication. In: Tripp RA, Tompkins SM, eds. Roles of Host Gene and Non-coding RNA Expression in Virus Infection. Springer; 2017:1-42.

30. Kim JW, Chung YS, Jo HJ, et al. Identification of Coronavirus CoV-2 infection—more than just the common cold. JAMA. 2020;323(8):707-708.

31. Tomazini BM, Maia IS, Campolante AB, et al. Effect of dexamethasone on days alive and ventilator-free in patients with moderate or severe acute respiratory distress syndrome and COVID-19: the CoDEX randomized clinical trial. JAMA. 2020;324(13):1307-1316.

32. Prescott HC, Rice TW. Corticosteroids in COVID-19 ARDS: evidence and hope during the pandemic. JAMA. 2020;324(13):1292-1295.

33. Matthay MA, Thompson BT. Dexamethasone in hospitalised patients with COVID-19: addressing uncertainties. Lancet Respir Med. 2020;8(12):1170-1172.

34. King A, Vail A, O’Leary C, et al. Anakinra in COVID-19: important considerations for clinical trials. Lancet Rheumatol. 2020;2(7):e379-e381.

35. Cavalli G, De Luca G, Campochiaro C, et al. Interleukin-1 blockade with high-dose anakinra in patients with COVID-19, acute respiratory distress syndrome, and hyperinflammation: a retrospective cohort study. Lancet Rheumatol. 2020;2(6):e325-e331.

36. Huet T, Beaussier H, Voisin O, et al. Anakinra for severe forms of COVID-19: a cohort study. Lancet Rheumatol. 2020;2(7):e393-e400.

37. Toniati P, Piva S, Catalina M, et al. Tocilizumab for the treatment of severe COVID-19 pneumonia with hyperinflammatory syndrome and acute respiratory failure: a single center study of 100 patients in Brescia, Italy. AutoImmum Rev. 2020;19(7):102568.

38. Khiali S, Khani E, Entezari-Maleki T. A comprehensive review of tocilizumab in covid-19 acute respiratory distress syndrome. J Clin Pharmacol. 2020;60(9):1131-1146.

39. Leach JK, Whitehead J. Materials-directed differentiation of mesenchymal stem cells for tissue engineering and regeneration. ACS Biomater Sci Eng. 2018;4:1115-1127.

40. Kilian KA, Bugarija B, Lahn BT, Mrksich M. Geometric cues for directing the differentiation of mesenchymal stem cells. Proc Natl Acad Sci U S A. 2010;107:4872-4877.

41. Guillam-Prats R, Artigas A. Current status of stem cell therapy for sepsis and acute respiratory distress syndrome. In: Loewy Z, ed. Innovations in Cell Research and Therapy. IntechOpen; 2020:3.

42. Pourrajab F, Forouzannia SK, Tabatabaei SA. Molecular characteristics of bone marrow mesenchymal stem cells, source of regenerative medicine. Int J Cardiol. 2013;163:125-131.
44. Zheng G, Huang L, Tong H, et al. Treatment of acute respiratory distress syndrome with allogeneic adipose-derived mesenchymal stem cells: a randomized, placebo-controlled pilot study. Respir Res. 2014;15:39.

45. Hofibbaum AM, Moe S, Marshak-Rothstein A. Opposing effects of transmembrane and soluble Fas ligand expression on inflammation and tumor cell survival. J Exp Med. 2000;191:1129-1200.

46. Sakhistswary R, Raymond AA. Stem cell therapy in neurodegenerative diseases. Nat Rev Neurol. 2012;7:1822-1831.

47. Bagni JR, Sheets KT, Hingtgen SD. Neural stem cell therapy for cancer. Methods. 2016;99:37-43.

48. Coffelt SB, Marini FC, Watson K, et al. The pro-inflammatory peptide LL-37 promotes ovarian tumor progression through recruitment of multipotent mesenchymal stromal cells. Proc Natl Acad Sci U S A. 2009;106:3806-3811.

49. Liu S, Ginestier C, Ou SJ, et al. Breast cancer stem cells are regulated by mesenchymal stem cells through cytokine networks. Cancer Res. 2011;71:614-624.

50. Khakoo AY, Pati S, Anderson SA, et al. Human mesenchymal stem cells exert potent antitumorigenic effects in models of Kawasaki's syndrome. J Exp Med. 2006;203:1235-1247.

51. Ballagamba BC, Abreu BR, Grivich I, et al. Human mesenchymal stem cells are resistant to cytotoxic and genotoxic effects of cisplatin in vitro. Genet Mol Biol. 2016;39:129-134.

52. Ortiz LA, Dutreil M, Fattman C, et al. Interleukin 1 receptor antagonist mediates anti-inflammatory and anti-fibrotic effects of mesenchymal stem cells during lung injury. Proc Natl Acad Sci U S A. 2007;104:11002-11007.

53. Wilson JG, Liu KD, Zhuo H, et al. Mesenchymal stem (stromal) cells for treatment of ARDS: a phase 1 clinical trial. Lancet Respir Med. 2015;3:24-32.

54. Simonon OE, Mougiakakos D, Helderin N, et al. In vivo effects of mesenchymal stromal cells in patients with severe acute respiratory distress syndrome. Stem Cells Transl Med. 2015;4:1109-1213.

55. Fanelli V, Vlahou A, Ghanadrian S, Simonietti U, Slutsky AS, Zhang H. Acute respiratory distress syndrome: new definition, current and future therapeutic options. J Thorac Dis. 2013;5:326-334.

56. Khubbutiya MS, Vagabov AV, Temnov AA, Skifas AN. Paracrine mechanisms of proliferative, anti-apoptotic and anti-inflammatory effects of mesenchymal stromal cells in models of acute organ injury. Cytotherapy. 2014;16:579-585.

57. Huppert LA, Liu KD, Matthay MA. Therapeutic potential of mesenchymal stromal cells in the treatment of ARDS. Transfusion. 2019;59:869-875.

58. Matthay MA. Therapeutic potential of mesenchymal stromal cells for acute respiratory distress syndrome. Ann Am Thorac Soc. 2015;12:554-557.

59. Danchuk S, Ylostalo JH, Hossain F, et al. Human multipotent stromal cells attenuate lipopolysaccharide-induced acute lung injury in mice via secretion of tumor necrosis factor-α-induced protein 6. Stem Cell Res Ther. 2011;2:27.

60. Foskett AM, Bazhanov N, Ti XY, et al. Phase-directed therapy: TSG-6 targeted to early inflammation improves bleomycin-injured lungs. Am J Physiol Lung Cell Mol Physiol. 2014;306:L120-L131.

61. Ionescu L, Byrne RN, van Haaf ten T, et al. Stem cell conditioned medium improves acute lung injury in mice: in vivo evidence for stem cell paracrine action. Am J Physiol Lung Cell Mol Physiol. 2012;303:L967-L977.
82. Leng Z, Zhu R, Hou W, et al. Transplantation of ACE2-mesenchymal stem cells improves the outcome of patients with COVID-19 pneumonia. Aging Dis. 2020;11(2):216.

83. Liang B, Chen J, Li T, et al. Clinical remission of a critically ill COVID-19 patient treated by human umbilical cord mesenchymal stem cells: a case report. Medicine. 2020;99(31):e21429.

84. Ouyang Y, Bayer A, Chu T, et al. Isolation of human trophoblastic stem cells improves the outcome of patients with COVID-19. Nat Biotech. 2020;38(7):879-891.

85. Thurston G, Rudge JS, Ioffe E, et al. Anti-interleukin 8 autoantibody: Interleukin 8 co-mediate the endothelial cell migration through the activation of PKCζ. J Cell Physiol. 2004;198:53-61.

86. Sánchez E, Alquicer A, Martínez- González I, et al. Human mesenchymal stem cells overexpressing the IL-33 antagonist soluble IL-1 receptor–like–1 attenuate endotoxin-induced acute lung injury. J Cell Physiol. 2018;233:572-580.

87. Verdecchia P, Cavallini C, Spanevello A, Angeli F. The pivotal link between ACE2 deficiency and SARS-CoV-2 infection. Crit Care Med. 2020;76:14-20. doi:10.1016/j.ccm.2020.04.037

88. Kuba K, Imay I, Yao S, et al. A crucial role of antitussin converting enzyme 2 (ACE2) in SARS coronavirus–induced lung injury. Nat Med. 2005;11:875-879.

89. Hoffmann M, Kleine-Weber H, Schroeder S, et al. SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. Cell. 2020;181:271-280.e8.

90. Ge X-Y, Li J, Yang XL, et al. Isolation and characterization of a bat SARS-like coronavirus that uses the ACE2 receptor. Nature. 2013;503:535-538.

91. Lange Z, Zhu R, Hou W, et al. Transplantation of ACE2-mesenchymal stem cells improves the outcome of patients with COVID-19 pneumonia. Aging Dis. 2020;11(2):216.

92. Montgomery S, Gruber P, et al. Anti-interleukin 8 autoantibodies induce the endothelial cell migration through the activation of phosphoinositide 3-Kinase-Rac1/RhoA pathway. Int J Biol Sci. 2011;7:782-791.

93. Kurdowska A, Noble JM, Steinberg KP, Ruzinski JT, Hudson LD, Martin TR. Anti-interleukin 8 autoantibody: Interleukin 8 complexes in the acute respiratory distress syndrome. Am J Respir Crit Care Med. 2001;163:463-468.

94. Kurdowska A, Miller EJ, Noble JM, et al. Anti-IL-8 autoantibodies in alveolar fluid from patients with the adult respiratory distress syndrome. J Immunol. 1996;157:2699-2706.

95. Kurdowska A, Noble JM, Grant IS, Robertson CR, Haslett C, Donnelly SC. Anti-interleukin-8 autoantibodies in babies at risk for acute respiratory distress syndrome. Crit Care Med. 2002;30:2335-2337.
124. Curley GF, Hayes M, Ansari B, et al. Mesenchymal stem cells enhance recovery and repair following ventilator-induced lung injury in the rat. Thorax. 2012;67:496-501.

125. Liu L, Chen JX, Zhang XW, et al. Chemokine receptor 7 overexpression promotes mesenchymal stem cell migration and proliferation via secreting Chemokine ligand 12. Sci Rep. 2018;8:204.

126. Morrison T, McAuley D, Krasnodembskaya A. Mesenchymal stem cell stromal cells for the treatment of the acute respiratory distress syndrome: the beginning of the story. J Intensive Care Soc. 2015;16:320-329.

127. Abraham A, Krasnodembskaya A. Mesenchymal stem cell- derived extracellular vesicles in acute respiratory distress syndrome: a review of current literature and potential future treatment options. Stem Cells Transl Med. 2019;8:2503.

128. Yang J, Zhang N, Wang HW, Gao P, Yang QP, Chen JX, Zhang XW, et al. Chemokine receptor 7 overexpression promotes mesenchymal stem cell attenuated lung injury. Hum Gene Ther. 2016;27:621-630.

129. Shyamsundar M, McCauley DF, Ingram RJ, et al. Keratinocyte growth factor promotes epithelial survival and resolution in a human model of lung injury. Am J Respir Crit Care Med. 2014;189:1520-1529.

130. Han J, Lu X, Zou L, Xu X, Qiu H. E- Prostanoid 2 receptor overexpression in mesenchymal stem cells facilitates treatment of acute lung injury in rats. J Biol Chem. 2015;290:1994-2006.

131. Shi M, Li J, Liao L, et al. Regulation of CXCR4 expression in human mesenchymal stem cells by cytokine treatment: role in homing efficiency in NOD/SCID mice. Haematologica. 2007;92:897-904.

132. Lu X, Han J, Xu X, et al. PGE2 promotes the migration of mesenchymal stem cells through the activation of FAK and ERK1/2 pathway. Stem Cells Int. 2017;2017:1-11.

133. Han J, Lu X, Zou L, Xu X, Qiu H. E-Prostanoid 2 receptor overexpression promotes mesenchymal stem cell attenuated lung injury. Hum Gene Ther. 2016;27:621-630.

134. Lerner T, Gimona M, Aigner L, et al. Applying extracellular vesicles based therapeutics in clinical trials—an ISEV position paper. J Extracellular Vesicles. 2015;4(1):30087.

135. McCulloch CJ, Olson JK, Wang Y, et al. Treatment of experimental nercrotizing enterocolitis with stem cell-derived exosomes. J Pediatr Surg. 2018;53(6):1215-1220.

136. Khatri M, Richardson LA, Meuli T. Mesenchymal stem cell-derived extracellular vesicles attenuate influenza virus-induced acute lung injury in a pig model. Stem Cell Res Ther. 2018;9:17.

137. Abraham A, Krasnodembskaya A. Mesenchymal stem cell-derived extracellular vesicles for the treatment of acute respiratory distress syndrome. Stem Cells Transl Med. 2019;9:28-38.

138. Andaloussi SE, Lakhal S, Mager I, Wood MJ. Exosomes for targeted siRNA delivery across biological barriers. Adv Drug Deliv Rev. 2013;65(3):391-397.

139. Alvarez-Erviti L, Seow Y, Yin H, Betts C, Lakhal S, Wood MJ. Delivery of siRNA to the mouse brain by systemic injection of targeted exosomes. Nat Biotechnol. 2011;29(4):341-345.

140. Wood MJ, O’Loughlin AJ, Lakhal S. Exosomes and the blood–brain barrier: implications for neurological diseases. Therapeutic Deliv. 2011;2(9):1095-1099.

141. Yang T, Martin P, Fogarty B, et al. Exosome delivered anticancer drugs across the blood-brain barrier for brain cancer therapy in Danio rerio. Pharm Res. 2015;32(6):2003-2014.

142. Morales-Prieto DM, Stojilkovic M, Diezel C, Streicher PE, Roestel F, Lindner J, Weis S, Schmeer C, Marz M. Peripheral blood exosomes pass blood-brain-barrier and induce glial cell activation. bioRxiv. doi:10.1101/471409

143. Shah TG, Predescu D, Predescu S. Mesenchymal stem cell-derived extracellular vesicles in acute respiratory distress syndrome: a review of current literature and potential future treatment options. Clin Transl Med. 2019;8:25.

144. L PK, Kandori S, Misra R, S V, K R, Verma RS. The mesenchymal stem cell secoretome: a new paradigm towards cell-free therapeutic mode in regenerative medicine. Cytokine Growth Factor Rev. 2019;46:1-9.
165. Beauvillain C, Ruiz S, Guiton R, Bout D, Dimier-Poisson I. A vaccine based on exosomes secreted by a dendritic cell line confers protection against T. gondii infection in syngeneic and allogeneic mice. *Microbes Infect.* 2007;9(14-15):1614-1622.

166. Ramachandra L, Qu Y, Wang Y, et al. Mycobacterium tuberculosis synergizes with ATP to induce release of microvesicles and exosomes containing major histocompatibility complex class II molecules capable of antigen presentation. *Infect Immun.* 2010;78(12):5116-5125.

167. Bonito PD, Ridolfi B, Columba-Cabezas S, et al. HPV-E7 delivered by engineered exosomes elicits a protective CD8+ T cell-mediated immune response. *Viruses.* 2015;7(3):1079-1099.

168. Anticoli S, Manfredi F, Chiozzini C, et al. An exosome-based vaccine platform imparts cytotoxic T lymphocyte immunity against viral antigens. *Biotechnol J.* 2018;13(4):1700443.

169. Qazi KR, Gehrmann U, Domange Jordö E, Karlsson MC, Gabriëlssoon S. Antigen-loaded exosomes alone induce Th1-type memory through a B cell-dependent mechanism. *Blood.* 2009;113(12):2673-2683.

170. Yao Z, Qiao Y, Li X, et al. Exosomes exploit the virus entry machinery and pathway to transmit alpha interferon-induced antiviral activity. *J Virol.* 2018;92(24):e01578.

171. Huang R, Wu J, Zhou X, Jiang H, Zhou GG, Roizman B. Herpes simplex virus 1 MicroRNA miR-H28 exported to uninfected cells in exosomes restricts cell-to-cell virus spread by inducing gamma interferon mRNA. *J Virol.* 2019;93(21):e01005-19.

172. Kuate S, Cintal J, Doerr HW, Überla K. Exosomal vaccines containing the S protein of the SARS coronavirus induce high levels of neutralizing antibodies. *Virology.* 2007;362(1):26-37.

173. Gupta N, Su X, Popov B, et al. Intrapulmonary delivery of bone marrow-derived mesenchymal stem cells improves survival and attenuates endotoxin-induced acute lung injury in mice. *J Immunol.* 2007;179:1855-1863.

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