



Current status of cell-based therapies for respiratory virus infections: applicability to COVID-19

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It is imperative to better comprehend the rationale and underlying data that both support and refute effectiveness of MSCs in respiratory virus infections, and to define the targeted patient population and potential cell therapy approaches for COVID-19 <https://bit.ly/2VbmYXs>

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ABSTRACT The severe respiratory consequences of the coronavirus disease 2019 (COVID-19) pandemic have prompted urgent need for novel therapies. Cell-based approaches, primarily using mesenchymal stem (stromal) cells (MSCs), have demonstrated safety and possible efficacy in patients with acute respiratory distress syndrome (ARDS), although they are not yet well studied in respiratory virus-induced ARDS. Limited pre-clinical data suggest that systemic MSC administration can significantly reduce respiratory virus (influenza strains H5N1 and H9N2)-induced lung injury; however, there are no available data in models of coronavirus respiratory infection.

There is a rapidly increasing number of clinical investigations of cell-based therapy approaches for COVID-19. These utilise a range of different cell sources, doses, dosing strategies and targeted patient populations. To provide a rational strategy to maximise potential therapeutic use, it is critically important to understand the relevant pre-clinical studies and postulated mechanisms of MSC actions in respiratory virus-induced lung injuries. This review presents these, along with consideration of current clinical investigations.

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Introduction

The coronavirus disease 2019 (COVID-19) pandemic, originating in Wuhan, China, is rapidly and continuously spreading globally and can result in serious significant respiratory morbidity and mortality [1]. The responsible agent, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), is an enveloped RNA virus of the *Coronaviridae* virus family. Human-to-human transmission occurs through respiratory droplets or contaminated surfaces [1]. The average incubation period is 5 days, but ranges from 1 to 14 days. Most patients present with mild respiratory tract infection, most commonly characterised by fever (82%) and cough (81%). Severe pneumonia and acute respiratory distress syndrome (ARDS) have been described in 14% of the reported cases, and the overall mortality is around 2% [2]. However, these numbers are evolving as the pandemic spreads, and depend on the country involved.

For COVID-19 patients who develop ARDS requiring intubation and mechanical ventilation, shock and multiple organ failure can also develop, although whether this is a direct consequence of viral infection or due to complications of critical illness is not yet clear. Current therapeutic approaches include aggressive standard supportive care and treatment of any other co-infections. Antiviral medications, including remdesivir, lopinavir–ritonavir, or lopinavir–ritonavir and interferon (IFN)- β 1, are under investigation but safety and potential efficacy remain to be determined. Remdesivir and IFN- β 1 β appear to have superior antiviral activity to lopinavir and remdesivir *in vitro* for the Middle East respiratory syndrome (MERS) coronavirus but whether this is the case for SARS-CoV-2 remains to be determined [2]. The US Food and Drug Administration (FDA) has recently approved use of hydroxychloroquine in COVID-19 patients but the efficacy remains to be determined. Growing information also suggests that virus-induced cytokine storm in the lungs may drive severe pathogenesis and provide potential therapeutic targets, for example anti-interleukin (IL)-6 or anti-IL-1 approaches [3].

More recently, a growing number of clinical investigations of cell-based therapies, primarily involving mesenchymal stem (stromal) cells (MSCs) but also utilising MSC-derived conditioned media or extracellular vesicles and several other cell types, have been initiated in China for COVID-19 respiratory disease. As these encompass a wide range of approaches and targeted patient groups, it is imperative to better understand the rationale of the studies and the potential mechanisms of MSC actions towards respiratory viral infections. Recent pre-clinical data in models of respiratory virus infections and relevant related clinical studies of MSC administration in patients with ARDS can contribute to better definition of the patient population for whom potential MSC-based cell therapy approaches might be considered.

Potential mechanisms of MSC actions in respiratory virus-induced lung injuries

Following systemic administration, the majority of MSCs lodge in the pulmonary vascular bed through as yet unclear interactions with the capillary endothelial cells. Tracking studies using labelled MSCs demonstrate that most are cleared within 24–48 h, although there can be longer persistence in injured or inflamed lungs [4]. The clearance mechanisms are still being elucidated but include apoptosis and subsequent efferocytosis and phagocytosis by resident inflammatory and immune cells, notably macrophages [5]. While lodged in the lungs, the MSCs are able to release a wide variety of soluble mediators including anti-inflammatory cytokines [6], antimicrobial peptides [7], angiogenic growth factors, and extracellular vesicles [8] (figure 1). Direct cell–cell transmission of mitochondria from MSCs to respiratory epithelial and immune cells [10] has also been described [11].

A growing literature demonstrates that the pattern of anti-inflammatory mediators released is specific for the inflammatory lung environment encountered and is mediated through differential activation of damage- and pathogen-associated molecular pathogen receptors expressed on MSC surfaces [12]. This includes Toll-like receptors (TLRs) that are activated by viral RNA (*e.g.* TLR3) (as in COVID-19) and viral unmethylated CpG-DNA (*e.g.* TLR9), leading to downstream cell signalling pathways resulting in MSC activation [13]. MSC-secreted angiopoietin-1 (Ang-1) and keratinocyte growth factor (KGF) contribute to the restoration of alveolar–capillary barriers disrupted as part of ARDS pathogenesis [14], while specific inhibitory microRNAs in extracellular vesicles are also described as mediating the protective effects of MSCs in pre-clinical models of bacterial or non-infectious acute lung injuries [15].

However, mediators responsible for ameliorating respiratory viral-induced lung injuries remain unclear. In animal models, influenza H9N2 viral infection increases serum and lung chemokines responsible for lung leukocyte infiltration, including granulocyte–macrophage colony-stimulating factor (GM-CSF), monocyte chemoattractant protein-1 (MCP-1), macrophage inflammatory protein-1 alpha (MIP-1 α) and others, which are markedly reduced by intravenous administration of MSCs [16]. Increased levels of IFN- γ , typical of antiviral immune responses, alone or together with other pro-inflammatory cytokines, prompt MSC activation including the release of anti-inflammatory mediators. The importance of such IFN “licensing” of immunomodulating effects has been previously demonstrated in a model of graft *versus* host disease (GVHD), where the MSC-treated recipients of IFN- γ ^{-/-} T-cell grafts did not respond to cell therapy,

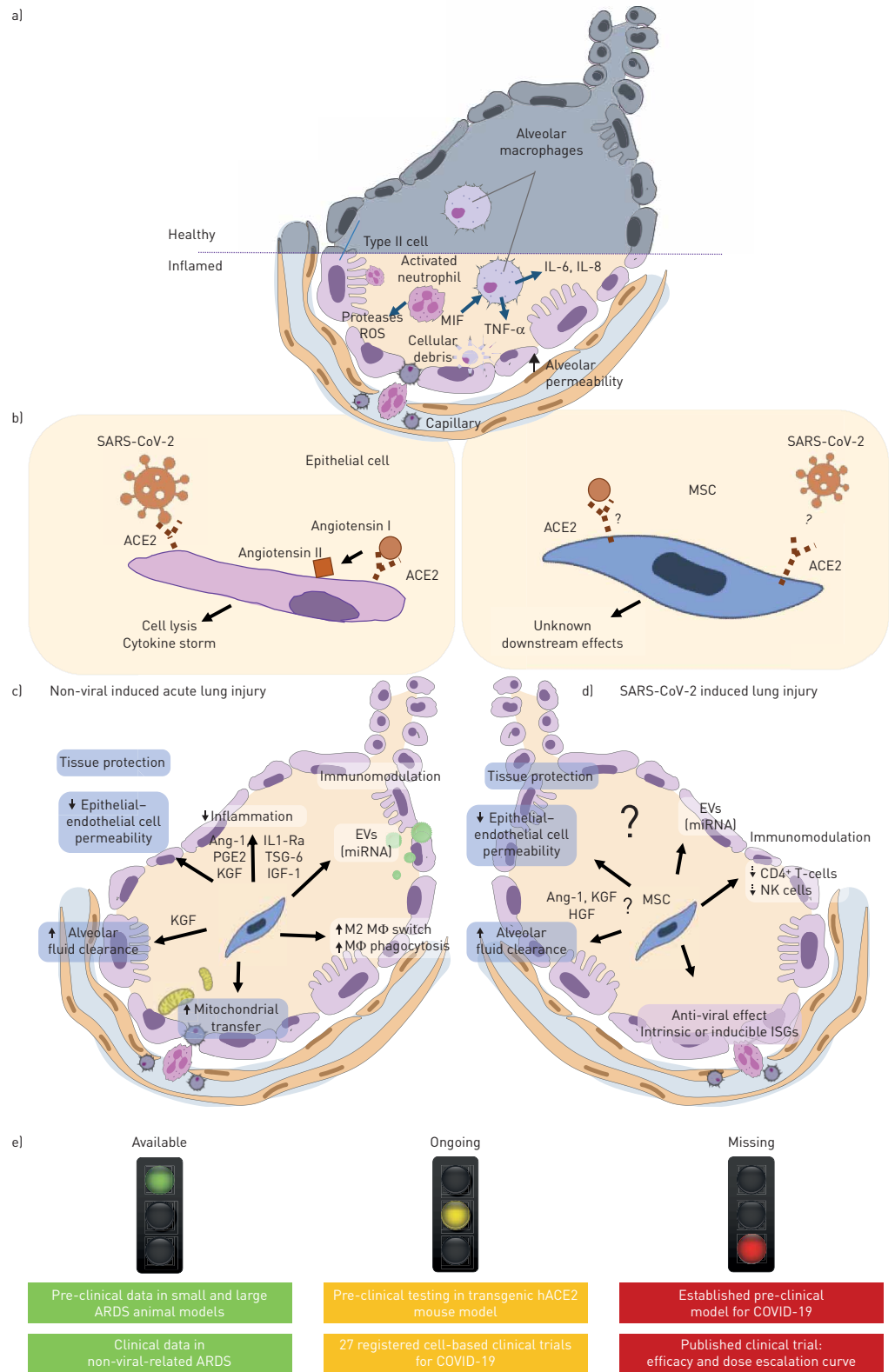


FIGURE 1 Potential therapeutic effects of mesenchymal stem (stromal) cells (MSCs) in respiratory lung injury are mediated by different mechanisms, including but not limited to secreted paracrine factors, extracellular vesicles (EVs) and possibly mitochondrial transfer, promoting tissue protection, immunomodulation and possibly viral resistance. a) Schematic of a healthy alveolus (top) and inflamed/oedematous alveolus (bottom) and mechanisms involved in acute respiratory distress syndrome (ARDS) pathogenesis. b) Schematic of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infecting a lung epithelial cell, with subsequent lysis and cytokine storm (left), and of potential MSC infection by SARS-CoV-2, with unknown downstream

consequences [right]. c) Some of the known mechanisms by which MSCs ameliorate non-viral acute lung injury. Adapted from LAFFEY and MATTHAY [9]. d) Limited information on mechanisms by which MSCs might ameliorate SARS-CoV-2 lung damage, based on limited pre-clinical data in influenza infection models. e) Current state-of-the-art of cell-based therapy in coronavirus disease 2019 (COVID-19), based on pre-clinical and clinical studies. MIF: macrophage migration inhibitory factor; TNF: tumour necrosis factor; IL: interleukin; ROS: reactive oxygen species; ACE2: angiotensin-converting enzyme 2; Ang-1: angiopoietin-1; PGE2: prostaglandin E2; KGF: keratinocyte growth factor; IL1-Ra: IL-1 receptor antagonist; TSG-6: TNF-stimulated gene 6; IGF-1: insulin-like growth factor 1; miRNAs: microRNAs; MΦ: macrophage; M2 MΦ: macrophage type 2; HGF: hepatocyte growth factor; NK cells: natural killer cells; ISGs: interferon-stimulated genes; hACE2: human ACE2.

evolving into fatal GVHD [17]. Unpublished data from COVID-19 patients in Italy suggest that high levels of IFN- γ are found and thus may influence systemically administered MSCs (Massimo Dominici, University Hospital of Modena and Reggio Emilia, Modena, Italy; personal communication). However, “licensed” MSCs can also suppress alloantigen-induced T-cell functions *in vitro*, potentially compromising antiviral responses needed for disease control. For example, MSCs suppress lymphocyte proliferation in response to the activation of influenza-specific T-cells *in vitro* [18]. Umbilical cord-derived MSCs (UC-MSCs) have also been shown to inhibit the cytotoxicity of specific T-cells against H1N1 influenza virus *in vitro* [19], leading perhaps to prolonged infection in recipients. This is in contrast to reports where, for example, in models of cytomegalovirus (CMV) infection, MSCs exert differential effects on alloantigen and virus-specific T-cells that retain the ability to proliferate, produce IFN- γ and kill CMV-infected cells *in vitro* [20].

An important question that remains to be resolved in respiratory virus infections is whether protective MSC actions are directly against viral infection, perhaps by stimulating antiviral T-cell actions, or whether they are due to overall anti-inflammatory actions that have been demonstrated in other models of acute lung injuries [9]. The latter may be particularly relevant for cytokine storm and it is likely that a combination of actions will be responsible.

Effects of respiratory viruses on MSC actions

MSCs are generally resistant to viral infection compared to their differentiated progeny [21]. In part this reflects intrinsic expression of IFN-stimulated genes (ISGs) that pre-empt viral infection [21]. MSC ISG expression includes, among others, IFITM family proteins, IFI6, ISG15, SAT1, PMAIP1, p21/CDKN1A and CCL2. Among these antiviral proteins, members of the IFITM family are unique as they prevent infection before a virus can traverse the lipid bilayer of the cell. These activities limit infection in cultured cells by many viruses, including dengue virus, Ebola virus, influenza A virus and severe acute respiratory syndrome (SARS) coronavirus [22]. Silencing of one of the most highly expressed ISGs in MSCs (p21/CDKN1A) specifically increased their susceptibility to chikungunya virus (CHIKV), whereas knockdown of IFITM3 rendered MSCs susceptible to infection by a variety of viruses, including yellow fever virus and Zika virus [21].

Using RNA sequencing (GEO dataset GSE97987) and validation by quantitative reverse transcription PCR [21], we established a list of ISGs constitutively expressed by human embryonic stem cell-derived MSCs. Bioinformatic analysis of five independent GEO databases for expression changes following different pro-inflammatory cytokine stimulation of intrinsic ISGs in human MSCs (three bone marrow-derived MSCs (BM-MSCs), one UC-MSCs and one adipose tissue-derived MSCs (AD-MSCs), with the respective GEO datasets GSE68610, GSE77814, GSE46019, GSE46019 and GSE18662; figure 2) demonstrated that pro-inflammatory cytokines including IFN- γ induced non-constitutive ISGs including MT1X, MT1G, SERPING1, SAT1, IFNAR2 and CD74, while significantly increasing the expression of constitutive antiviral genes such as IFI6, ISG15, CCL2, SAT1, PMAIP1 and IFITM1.

Hence, in the context of a respiratory viral infection, including COVID-19, MSCs might present two distinct antiviral mechanisms: constitutively elevated levels of MSC-specific ISGs to function as mediators of an antiviral protection, and a secondary response to IFN, leading to ISG induction and broad viral resistance. Conversely, MSCs could present a mix of intrinsic and inducible innate antiviral defences that could lead to therapeutic benefits in COVID-19 patients.

In contrast, some literature demonstrates that human BM-MSCs are permissive to avian influenza A (H5N1) infection, losing viability and immunoregulatory activities [16]. This can occur rapidly following exposure of uninfected MSCs [23], and virus-infected MSCs may thus not be functionally effective at stopping virus replication and lung inflammation [24–27]. BM-MSCs express influenza virus alpha-2,3 and alpha-2,6 sialic acid receptors on their cell surfaces and can support replication of both avian H1N1 and H9N5 influenza strains [24, 25]. Influenza-infected MSCs undergo cell lysis apoptosis within 18 h post exposure, with corresponding production of pro-inflammatory cytokines and chemokines [24] potentially

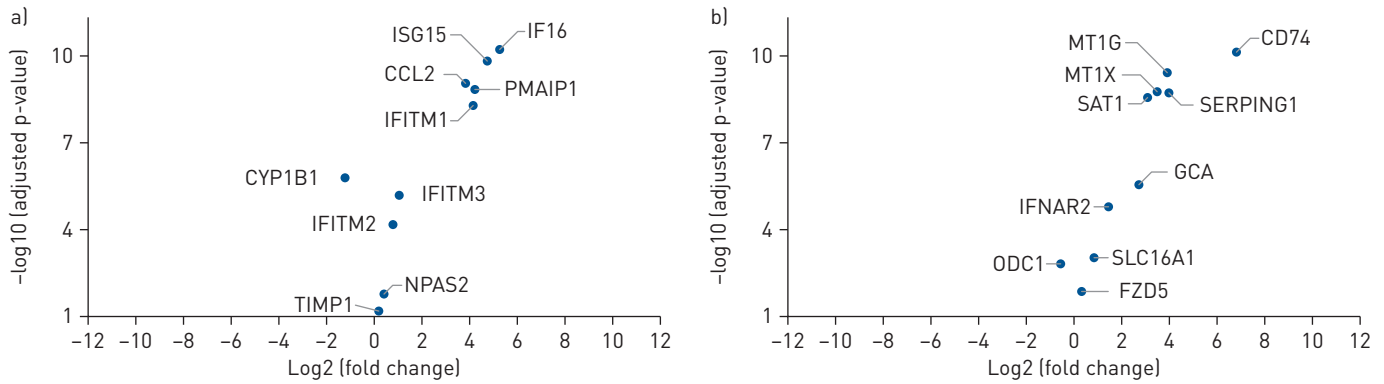


FIGURE 2 Representative volcano plot analysis of gene expression of the interferon-stimulated genes (ISGs) in human bone marrow-derived mesenchymal stem (stromal) cells [BM-MSCs] activated with pro-inflammatory cytokines [interleukin- 1β , tumour necrosis factor- α and interferon (IFN)- γ], versus control, in GEO dataset GSE68610. Gene expression analysis of a) constitutive and b) non-constitutive ISGs. ISG15: ubiquitin-like protein; CCL2: C-C motif chemokine 2; IFI6: IFN- α -inducible protein 6; PMAIP1: phorbol-12-myristate-13-acetate-induced protein 1; IFITM: IFN-induced transmembrane protein; CYP1B1: cytochrome p450 1b1; NPAS2: neuronal PAS domain-containing protein 2; TIMP-1: metalloproteinase inhibitor 1; MT1G/MT1X: metallothionein 1G/X; SERPING1: serpin family G member 1 (plasma protease C1 inhibitor); SAT1: sulfate anion transporter 1; GCA: grancalcin; IFNAR2: IFN- α/β receptor 2; SLC16A1: monocarboxylate transporter 1; ODC1: ornithine decarboxylase 1; FZD-5: Frizzled-5.

subverting their protective immunomodulatory properties [26]. The respiratory syncytial virus can also infect MSCs, modifying immune cell proliferation and activity [27]. As such, depending on the virus type and the level of expression or percentage of MSCs expressing the virus receptor, MSCs may get infected if infused into a patient with an ongoing respiratory virus infection. How this would affect potential beneficial effects remains to be determined.

Do MSCs express ACE2, the functional receptor of SARS-CoV-2?

Angiotensin-converting enzyme 2 (ACE2) has been reported to be the main host cell receptor for SARS-CoV-2 entry, and the virus uses the host cell serine protease TMPRSS2 for S protein priming [28]. ACE2 is highly expressed in respiratory epithelial cells, thus plays a crucial role in the entry of virus into these cells [29]. ACE2 was recently also demonstrated to be an ISG in nasal epithelial cells [30]. Hence, SARS-CoV-2 may exploit IFN-driven upregulation of ACE2, a key tissue-protective mediator during lung injury, to enhance infection. Conversely, ACE2 was reported to protect against non-viral lung injury by degrading the profibrotic peptide angiotensin II [31]. *In vivo* gene silencing of ACE2 enhances bleomycin-induced lung collagen deposition in mice, whereas systemic administration of purified ACE2 inhibits the fibrotic response [32]. Murine BM-MSCs overexpressing the ACE2 gene, following lentiviral vector transduction, offered additional anti-inflammatory and endothelial-protective effects against endotoxin-induced lung injury in mice [33, 34]. However, there may be a downside to this if ACE2 overexpression results in infection and subsequent deleterious effects on the MSCs. As the level of gene expression is a key determinant of SARS-CoV-2 transmissibility [28], it is relevant to assess whether MSCs of any origin constitutively or inducibly express ACE2 or TMPRSS2.

MSCs in respiratory virus-related lung injury: pre-clinical evidence

There is a large body of literature demonstrating efficacy of either systemic or direct intratracheal MSC administration in pre-clinical models of respiratory diseases, including those involving acute lung injury induced by bacteria or bacterial products (endotoxins) or other means [35]. The models include rodents and large animals (pigs and sheep), as well as explanted human lungs. A range of approaches have been utilised for dose, dosing and MSC source, with MSCs from bone marrow, adipose tissue, umbilical cord, cord blood and placenta being investigated. A recent systematic review indicated that BM-MSCs and UC-MSCs were more effective than AD-MSCs in reducing mortality in pre-clinical acute lung injury models [36].

However, there are only a small number of pre-clinical studies investigating effects of MSC administration in pre-clinical models of respiratory virus infections. These have been further limited to influenza viruses, have produced conflicting results, and have not as yet directly addressed coronavirus respiratory infections (table 1). Notably, two earlier studies found MSCs not to be protective against influenza respiratory infections in mice. DARWISH *et al.* [37] assessed the effects of a single systemic administration in immunocompetent mice of either syngeneic murine BM-MSCs or xenogeneic human BM-MSCs on lung injury induced by mouse-adapted H1N1 or swine-origin pandemic H1N1. Two different doses of MSCs (2.5×10^5 or 5×10^5 cells-mouse $^{-1}$) were administered at different time-points after virus administration

TABLE 1 Pre-clinical studies of MSCs in respiratory virus-related lung injury

First author, year [ref.]	Experimental model, route of infection, type of virus	MSC source, passage, number, administration route, timing of treatment	Time of outcome analysis	Adjuvant therapy	Outcome	Mechanisms of action	Control group
DARWISH, 2013 [37]	C57BL/6 mice aged 7–10 weeks, <i>i.n.</i> infection, influenza A/PuertoRico/8/34 (mouse- adapted H1N1) or influenza A/Mexico/410 8/2009 (swine-origin pandemic H1N1)	Mouse BM-MSCs (P6–P9) or human BM- MSCs (P3), 2.5×10^5 or 5×10^5 cells-mouse ⁻¹ , <i>i.v.</i> (tail vein), single dose, days –2, 0, 2 or 5 post infection	Day 7 or when euthanasia criteria were met	Oseltamivir $2.5 \text{ mg} \cdot \text{kg}^{-1}$, oral gavage, once daily for 5 days	Prophylactic and therapeutic syngeneic and xenogeneic administration of MSCs failed to improve survival, failed to affect weight loss, and failed to decrease lung parenchyma inflammation and BALF cell counts	Nonspecified (soluble mediators)	No
GOTTS, 2014 [38]	C57BL/6 mice aged 7–10 weeks, <i>i.n.</i> infection, influenza A/Puerto Rico/8/34 (mouse-adapted H1N1)	Mouse BM-MSCs ($\leq P7$) or human BM-MSCs ($\leq P7$), 5×10^5 cells-mouse ⁻¹ , <i>i.v.</i> (retro-orbital injection) or <i>i.t.</i> (data not shown); two doses: 1) days 5 and 6 post infection, 2) days 2 and 3 post infection (data not shown)	Days 7, 9 or 11	No	Mouse MSCs prevented influenza-induced thrombocytosis and caused a modest reduction in lung viral load on day 7; early (data not shown) and late syngeneic and xenogeneic <i>i.v.</i> administration of MSCs failed to affect weight loss, failed to decrease lung water, failed to decrease BALF inflammation, and failed to improve lung histology; <i>i.t.</i> administration increased severity of model (data not shown)	Nonspecified (soluble mediators)	No
CHAN, 2016 [39]	BALB/c mice aged 6–8 weeks (young) or 8–12 months (old), <i>i.n.</i> infection, influenza A/Hong Kong/486/1997(H5N1)	Human BM-MSCs (passage not mentioned), 5×10^5 cells-mouse ⁻¹ , <i>i.v.</i> , single dose, day 5 post infection	Days 7, 10 or 18	No	In older mice, but not younger mice, allogeneic MSCs increased survival, reduced weight loss, reduced lung histopathological lesions, increased M2 macrophages in BALF, reduced lung pro-inflammatory cytokines and chemokines (MCP-1, MCP-3, MIP-1 α , RANTES, IL-4, IL-17, TNF- α), but did not reduce lung virus titres	Paracrine soluble mediators, partially due to Ang-1 and KGF secretion	NIH 3T3 mouse embryo fibroblasts

Continued

TABLE 1 Continued

First author, year [ref.]	Experimental model, route of infection, type of virus	MSC source, passage, number, administration route, timing of treatment	Time of outcome analysis	Adjuvant therapy	Outcome	Mechanisms of action	Control group
Li, 2016 [16]	C57BL/6 mice aged 6–8 weeks, <i>i.n.</i> infection, avian influenza virus Hong Kong/2108/2003 (H9N2)	Mouse BM-MSCs (P3–P10), 1×10^5 cells-mouse ⁻¹ , <i>i.v.</i> (tail vein), single dose, 30 min or day 1 post infection	Day 3	No	Regardless of time of administration, syngeneic MSCs did not reduce lung virus titration, increased survival rate, decreased lung oedema, decreased histological injury, and improved gas exchange; early and late administration reduced BALF and serum cytokines (IL-6 and TNF- α); early administration reduced BALF chemokine (GM-CSF), reduced BALF and serum chemokines and cytokines (MIG, IL-1 α , IFN- γ), and increased anti-inflammatory cytokine IL-10 in BALF and serum	Nonspecified (soluble mediators)	No
KHATRI, 2018 [40]	White Duroc crossbred pigs aged 8 weeks, <i>i.n.</i> infection, influenza virus swine/TX/98 (H3N2) and swine/MN/08 (H1N1)	Pig BM-MSC extracellular vesicles (P3–P5), 80 $\mu\text{g}\cdot\text{kg}^{-1}$ body weight (produced by 10×10^6 MSCs), <i>i.t.</i> , single dose, 12 h post infection	Days 1 or 3	No	BM-MSC-derived extracellular vesicles decreased virus shedding in nasal swabs, reduced influenza virus replication in the lungs, prevented virus-induced production of pro-inflammatory cytokines (TNF- α , CXCL-10), and reduced histological injury and lung oedema	Nonspecified (extracellular vesicles)	No
Loy, 2019 [41]	BALB/c mice aged 6–8 weeks, <i>i.n.</i> infection, influenza A/Hong Kong/486/1997 (H5N1)	Human UC-MSCs (\leq P7) 5×10^5 cells-mouse ⁻¹ , <i>i.v.</i> , single dose, day 5 post infection	Days 7, 10, 14 or 18	No	UC-MSCs failed to decrease lung virus titration, failed to increase survival rate, reduced body weight loss, decreased lung oedema, and decreased BALF cytokines (IP-10, MCP-1, RANTES, IL-1 β)	Paracrine soluble mediators, partially due to Ang-1 and HGF secretion	NIH 3T3 mouse embryo fibroblasts

MSCs: mesenchymal stem (stromal) cells; *i.n.*: intranasal; *i.v.*: intravenous; *i.t.*: intratracheal; BM: bone marrow-derived; UC: umbilical cord-derived; BALF: bronchoalveolar lavage fluid; MCP: monocyte chemoattractant; MIP: macrophage inflammatory protein; RANTES: regulated upon activation, normal T-cell expressed and presumably secreted; IL: interleukin; TNF: tumour necrosis factor; Ang-1: angiopoietin-1; KGF: keratinocyte growth factor; GM-CSF: granulocyte-macrophage colony-stimulating factor; MIG: monokine induced by interferon- γ ; IFN: interferon; CXCL-10: C-X-C motif chemokine 10; IP-10: interferon γ -induced protein 10; HGF: hepatocyte growth factor.

(days -2, 0, 2 and 5 post infection (p.i.)). Using survival and different measures of lung inflammation as outcome end-points on day 7 p.i., neither syngeneic nor xenogeneic MSC administration, either alone or as an adjuvant therapy with oseltamivir, was effective either when administered prophylactically prior to virus inoculation, or when therapeutically administered. Similarly, GOTTIS *et al.* [38] assessed the effect of both systemic and intratracheal administration of human and mouse MSCs (5×10^5 cells-mouse⁻¹), administered in two doses, either earlier (days 2 and 3 p.i.) or later (days 5 and 6 p.i.), in mouse-adapted H1N1-induced lung injury in immunocompetent mice. However, MSCs did not improve influenza-mediated lung injury, regardless of administration route.

In contrast, more recent studies have demonstrated protective effects of systemic MSC administration in rodent and pig models of influenza respiratory infections. CHAN *et al.* [39] found, through *in vitro* assays, that MSCs improved the dysregulated alveolar fluid clearance and protein permeability induced by H5N1 and H7N9 influenza viruses, in part by releasing soluble mediators that upregulated sodium and chloride transporters. Systemic administration of 5×10^5 human BM-MSCs per mouse on day 5 p.i. in immunocompetent mice aged 8–12 months infected with influenza A (H5N1) reduced virus-induced mortality (until day 18 p.i.), weight loss (days 6–10 p.i.), lung oedema (day 7 p.i.), bronchoalveolar lavage fluid (BALF) CD4⁺ T-cells and natural killer (NK) cells (day 7 p.i.), lung histopathological lesions (day 18 p.i.), and pro-inflammatory cytokines and chemokines (day 7 p.i.), without reducing lung virus titres (days 7 and 10 p.i.). They further found that Ang-1 and KGF released by MSCs were important, but not enough to attenuate the effects of viral infection on alveolar fluid clearance and permeability. However, in young mice (aged 6–8 weeks), no effects were observed in mortality and body weight loss. Thus, the data suggest that systemic MSC administration may provide benefit in older patients who are at higher risk for severe pulmonary illness caused by H5N1. Why this was less effective in younger mice is not clear at present.

LI *et al.* [16] investigated the impact of a low dose (10^5 cells-mouse⁻¹) of murine BM-MSCs in avian influenza virus (H9N2)-induced lung injury in young immunocompetent mice. A single intravenous administration led, on day 3 p.i., to reduction in mortality, lung oedema and histological injury, as well as in BALF and serum chemokines and cytokines, and to improved gas exchange and levels of anti-inflammatory mediators, although it did not reduce lung virus titration when administered either 30 min or 24 h after infection induction. Differences between early and later administration of MSCs were only observed in some BALF and serum inflammatory mediators. Only early administration reduced BALF levels of GM-CSF, reduced BALF and serum monokine induced by IFN- γ (MIG), IL-1 α and IFN- γ , and increased levels of BALF and serum IL-10. Both early and later administration led to reduction of BALF and serum IL-6 and tumour necrosis factor- α . This might reflect that early administration seems more geared towards prevention of cell infection and inflammation, rather than dealing with more clinically relevant sequelae of epithelial infection. Avian influenza virus infection can trigger a very intense pro-inflammatory response compared to other influenza viruses; thus, LI *et al.* [16] speculated that the beneficial effects might be a specific consequence of different pathogenic features, compared to swine-origin H1N1 infection.

LOY *et al.* [41] found that UC-MSCs were more effective than human BM-MSCs at restoring impaired alveolar fluid clearance and permeability, in *in vitro* airway epithelial cell models. These effects were partially mediated through MSC secretion of Ang-1 and hepatocyte growth factor. The authors subsequently compared administration of UC-MSCs to BM-MSCs (5×10^5 cells-mouse⁻¹, day 5 p.i.) in experimental lung injury induced by influenza A (H5N1) infection in female immunocompetent mice aged 6–8 weeks. Despite failure to reduce virus titre and increase survival rate, a single dose of UC-MSCs decreased body weight loss (days 16 and 17 p.i.), lung oedema (days 10 and 14 p.i.) and inflammation (day 7 p.i.) in H5N1-induced lung injury.

MSC-derived extracellular vesicles have been demonstrated to have comparable results, and in some cases are more effective than MSCs themselves in ameliorating inflammation and injury in a range of pre-clinical lung injury models [42, 43]. KHATRI *et al.* [40] found that systemic administration of extracellular vesicles isolated from pig BM-MSCs was safe and reduced virus shedding in nasal swabs, influenza replication in the lungs, BALF pro-inflammatory cytokines and chemokines, and histopathological changes, when administered 12 h after viral inoculation in a mixed swine (H3N2, H1N1) and avian (H9N5, H7N2) influenza-induced pig lung injury model. These findings suggest systemic extracellular vesicle administration as a potential cell-free strategy for use in respiratory virus-induced lung injuries.

There are as yet no pre-clinical data investigating effects of MSC administration in models of coronavirus respiratory infection, mostly due to the lack of an established animal model. SARS-CoV-2 replication was observed in several non-human primates and in inbred strains of mice following intranasal infection, but these models failed to show clinical signs of pulmonary disease as seen in humans [44]. Mice transgenic

for human ACE2, infected with SARS-CoV-2, demonstrated virus replication in the lungs, as well as interstitial pneumonia with lymphocyte and monocyte infiltration into the alveolar interstitium and accumulation of macrophages in alveolar spaces [45]. While this model requires further evaluation, it might facilitate the testing of therapeutics including cell-based therapies for COVID-19. Recently, a non-human primate model for SARS-CoV-2 was able to reflect the same clinical signs, viral replication and pathology observed in humans, with comparable levels of mortality, and might be a valuable model for further evaluation [46].

Overall, it remains unclear whether the varying results reflect the differing features of each approach, including differences in the host (age), in the MSCs (source, number, route of administration), and in the virus-specific inflammatory patterns. Infection of the administered MSCs by the viruses, particularly the avian influenza viruses, might explain the lack of effectiveness observed in some *in vivo* studies, but MSC infection after their administration *in vivo* has not yet been investigated. In order to bypass the impact of viruses on MSCs, extracellular vesicles might be an option for further studies. Clearly, further pre-clinical studies must be done to evaluate the infectiveness of MSCs by coronaviruses and the impact of MSCs in coronavirus-induced lung injury models.

Clinical investigations of MSC administration in patients with coronavirus or other respiratory virus-induced lung injury

Despite suggestive recent evidence of potential efficacy of MSC administration in pre-clinical models of influenza respiratory viral lung infections, there are limited published clinical data available. A recently published single-centre open-label pilot investigation from the YouAn Hospital in Beijing (China) administered BM-MSCs to seven patients with COVID-19 pneumonia with differing degrees of severity including one patient with critically severe disease requiring care in the intensive care unit (ICU) [47]. The MSCs were given as a single intravenous administration at a dose of 10^6 cells·kg⁻¹ body weight in 100 mL saline at various times after initial symptomatic presentation. The MSCs were assessed by RNA sequencing for expression of ACE2 or TMPRSS2 prior to administration and each was found to be minimally expressed (in one out of 12500 cells and seven out of 12500 cells, respectively), although the RNA sequencing results were not validated for gene (quantitative reverse transcription PCR) or protein expression.

The seven patients were categorised as critically severe (n=1), severe (n=4) and common type (n=2). Three additional patients classified as severe received placebo (vehicle) administration for comparison. Patients were followed for 14 days after MSC or placebo administration and a range of safety and efficacy end-points were assessed. No infusional toxicities, allergic reactions, secondary infections or severe attributable adverse events were observed, and patients, including the one categorised as critically severe, apparently demonstrated clinical improvements within 2–4 days after MSC administration. However, while detailed information is provided for the critically severe patient, there is a lack of corresponding information for the other six patients or for the three placebo patients. Analyses of viral titres, circulating pro- and anti-inflammatory mediators, and lymphocyte numbers and populations were presented in detail for the critically severe patient and to a lesser degree for the other patients.

More detailed information as to inclusion and exclusion criteria, timing of MSC administration relative to onset of disease, comorbidities, clinical course of each patient, and evaluation of inflammatory mediators and cell populations for both treated and placebo patients are needed to better determine potential MSC efficacy and mechanisms of action. Importantly, there is no discussion as to whether the approach be further investigated in only critically severe and/or severe patients or for the broader range of clinical presentations of COVID-19 respiratory infection.

A second recently published study evaluated MSC administration in patients with H7N9 influenza virus respiratory infections during the 2013–2014 outbreak in China [48]. In this study, 17 critically ill patients with H7N9-induced ARDS received multiple intravenous administrations of menstrual blood-derived cells (10^6 cells per infusion in Plasmalyte) obtained from a single healthy donor and outcomes were compared to 44 comparably critically ill patients receiving standard antiviral and supportive therapies. Of the treated patients, three are described as receiving three separate infusions during early-stage infection, six patients received three infusions at late-stage infection and eight patients received four infusions at late-stage infection. However, no information is provided concerning the timing between infusions or whether the control patients received vehicle infusions. The MSC-treated and control patients were otherwise fairly well matched for comorbidities and degrees of multi-organ failure and for use of other supportive therapies, except for a higher incidence of shock in the MSC-treated group ($p < 0.03$). No apparent infusional toxicities or serious adverse events were noted. Three patients in the MSC-treated group died (82.4% survival), whereas 24 in the control died (45.5% survival). However, no details were provided on the deaths, including cause and timing related to either infusion or to overall clinical course, or on other

standard assessments including ventilator-free days, ICU stay or hospital stay. Complete blood count and measures of renal, liver, cardiac, and coagulation functions were comparable, except for a higher circulating pro-calcitonin level in the control group, perhaps suggestive of secondary or co-bacterial infections, although no information on other infections was provided. The authors concluded that MSC administration is a viable approach for H7N9-induced ARDS and that this could be potentially applicable to use in COVID-19 patients.

These two studies, while suggestive, highlight a number of issues with respect to potential use of MSCs in coronavirus and other viral respiratory infections. These include but are not limited to source of MSCs, dose and dosing strategies, including the number and timing of administrations. These studies also highlight issues with conduct of clinical trials for respiratory diseases, including those in critically ill patients. Full information about inclusion and exclusion criteria, clinical course, comorbidities, co-infections and laboratory evaluations, including investigative mechanistic evaluations, must be provided in a comprehensive manner.

Assessment of the ongoing cell-based clinical trials registered during the COVID-19 outbreak

At the time of this review, the number of cell-based clinical investigations to explore the therapeutic potential of cell treatment for SARS-CoV-2 infected patients registered since late January 2020 on the US National Institutes of Health (NIH) ClinicalTrials.gov database and the Chinese Clinical Trial Registry (www.chictr.org.cn), also accessible from the World Health Organization International Clinical Trial Registry Platform (WHO-ICTRP), has reached 27 entries with a total of approximately 1287 patients considered for enrolment (table 2). There are three main interventions: MSCs (n=17; 781 patients), MSC derivatives (conditioned media or extracellular vesicles; n=4; 176 patients), or other cell sources (n=6; 330 patients). General common features of the investigations include: 1) systemic administration, jointly with or followed by the recommended conventional supportive treatments for severe or critical SARS-CoV-2 infection [49]; 2) age range 18–80 years with no sex restrictions; 3) follow-up for at least 3 months; and 4) clinical samples collected will be throat secretions and/or blood. For the MSC investigations, 10 out of 17 will utilise UC-MSCs, one out of 17 will utilise menstrual blood-origin MSCs, and six out of 17 do not disclose the MSC tissue source. Notably, no apparent MSCs of bone marrow origin are being utilised, despite the majority of pre-clinical investigations for non-viral-induced acute lung injuries having utilised BM-MSCs. There is little clarification of use of cryopreserved *versus* continuously cultured cells [5]. Only six out of 16 disclose the intended cell injection dose, among which only four are correlated with the patient body weight. The intravenous dosing range varies between 0.4 and 42×10^6 cells·kg⁻¹. In comparison, the highest dose of MSCs used in the published literature for clinical trials in non-viral ARDS was 10×10^6 cells·kg⁻¹ (START trial) [50]. The dosing strategy ranges between a single dose and five doses, with an average frequency of every 2 days.

Four of the trials will utilise either MSC-derived conditioned media or extracellular vesicles. Two of these propose aerosol inhalation of MSC-derived extracellular vesicles, one from AD-MSCs, for which there is no pre-clinical supporting data. Six investigations will utilise other cells, including umbilical cord blood-derived mononuclear cells, cytotoxic T-cells, dendritic cells, NK cells, cord blood stem cells and cytokine-induced killer cells, of which only the latter investigation describes dosing and frequency of injections. As best as we can ascertain, there are no apparent pre-clinical data to support the rationale for any of these approaches.

Ethical issues for considering cell-based approaches for respiratory virus infections

Activities of healthcare providers and researchers during an infectious disease outbreak, including clinical trials, are aimed towards finding rapid and effective responses for the treatment of infected patients. These actions need to occur under appropriate ethical guidelines. The World Health Organization has guidelines that embed ethical approaches and considerations within the integrated global alert and response system for epidemics and other public health emergencies [51]. These are all applicable to cell-based clinical investigations and include assurance that these are scientifically valid and that potential risks are reasonable in relation to anticipated benefits. With respect to safety of MSC administration in critically ill patients, including those with ARDS resulting from other aetiologies, no significant issues have been described in published articles to date [51]. With respect to efficacy, there is equipoise at present, as only one as yet unpublished exploratory trial of MSC administration has demonstrated beneficial outcomes [52].

It is also imperative that the clinical investigations be conducted in a transparent manner according to established precedents for clinical investigations of potential new therapies for critical illnesses [53]. This includes recognised end-points, including but not limited to overall mortality, length of ICU and hospital

TABLE 2 Cell-based clinical trials using MSCs, MSC derivatives and other cells

	Registration date and execution date range DD-MM-YYYY	Study phase and recruitment status	ID and URL	Title	Cell type	Total participants n	Intervention or treatment
Clinical trials based on MSCs							
1	14-02-2020; 20-02-2020 to 20-02-2021	0 Not recruiting	ChiCTR2000029816; http://www.chictr.org.cn/showproj.aspx?proj=49389	Clinical study for cord blood mesenchymal stem cells in the treatment of acute novel coronavirus pneumonia (COVID-19)	UCB-MSCs; UCB-NK cells	60	Experimental groups: conventional treatment followed by <i>i.v.</i> infusion of 1) UCB-MSCs and 2) UCB-MSCs combined with UCB-NK cells Control group: conventional treatment
2	14-02-2020; 20-02-2020 to 20-02-2021	0 Not recruiting	ChiCTR2000029817; http://www.chictr.org.cn/showproj.aspx?proj=49384	Clinical study of cord blood NK cells combined with cord blood mesenchymal stem cells in the treatment of acute novel coronavirus pneumonia (COVID-19)	NK cells and UCB-MSCs	60	Experimental (high-dose) group: high-dose NK cells ($>5 \times 10^9$) and MSCs ($>5 \times 10^9$), <i>i.v.</i> infusion once every 2 days for a total of 5 times Conventional dose group: conventional dose NK cells ($>3 \times 10^9$) and MSCs ($>3 \times 10^9$), <i>i.v.</i> infusion once every 2 days for a total of 3 times Preventive dose group: preventive dose NK cells ($>3 \times 10^9$) and MSCs ($>3 \times 10^9$), <i>i.v.</i> infusion once every week for a total of 1 time
3	07-02-2020; 15-01-2020 to 31-12-2022	0 Recruiting	ChiCTR2000029606; http://www.chictr.org.cn/showproj.aspx?proj=49146	Clinical study for human menstrual blood-derived stem cells in the treatment of acute novel coronavirus pneumonia (COVID-19)	MenSCs	63	Experimental group A: Conventional treatment followed by <i>i.v.</i> infusion of MenSCs Control group A: conventional treatment Experimental group B: 1: Artificial liver therapy + conventional treatment 2: Artificial liver therapy followed by <i>i.v.</i> infusion of MenSCs + conventional treatment Control group B: conventional treatment
4	07-02-2020; 06-02-2020 to 30-09-2020	2 Recruiting	NCT04269525; https://clinicaltrials.gov/show/NCT04269525	Umbilical cord (UC)-derived mesenchymal stem cells (MSCs) treatment for the 2019-novel coronavirus (nCOV) pneumonia	UC-MSCs	10	Experimental group: UC-MSCs 3.3×10^7 cells per 50 mL per bag, 3 bags each time; UC-MSCs will be infused <i>i.v.</i> on the 1st, 3rd, 5th and 7th days after enrolment, once each day Control group: none specified
5	05-02-2020; 31-01-2020 to 31-12-2020	0 Recruiting	ChiCTR2000029580; http://www.chictr.org.cn/showproj.aspx?proj=49088	A prospective, single-blind, randomized controlled trial for ruxolitinib combined with mesenchymal stem cell infusion in the treatment of patients with severe 2019-nCoV pneumonia (novel coronavirus pneumonia, NCP)	MSCs	70	Experimental group: ruxolitinib combined with MSCs Control group: routine treatment

Continued

TABLE 2 Continued

	Registration date and execution date range DD-MM-YYYY	Study phase and recruitment status	ID and URL	Title	Cell type	Total participants n	Intervention or treatment
6	27-01-2020; 21-01-2020 to 31-12-2021	1 Recruiting	NCT04252118; https://clinicaltrials.gov/show/NCT04252118	Mesenchymal stem cell treatment for pneumonia patients infected with 2019 novel coronavirus	MSCs	20	Experimental group: 3.0×10^7 MSCs <i>i.v.</i> at days 0, 3 and 6 Control group: none specified
7	14-02-2020; 16-02-2020 to 15-02-2022	NA Not recruiting	NCT04273646; https://clinicaltrials.gov/ct2/show/NCT04273646	Study of human umbilical cord mesenchymal stem cells in the treatment of novel coronavirus severe pneumonia	UC-MSCs	48	Experimental group: 4 times of UC-MSCs, 0.5×10^6 UC-MSCs·kg ⁻¹ body weight <i>i.v.</i> at days 1, 3, 5 and 7 Control group: none specified
8	28-02-2020; 19-02-2020 to 20-02-2021	1 Recruiting	ChiCTR2000030300; http://www.chictr.org.cn/showprojen.aspx?proj=50022	Umbilical cord mesenchymal stem cells (hucMSCs) in the treatment of high risk novel coronavirus pneumonia (COVID-19) patients	UC-MSCs	9	Experimental group: MSCs Control group: none specified
9	26-02-2020; 14-02-2020 to 31-05-2020	NA Not recruiting	ChiCTR2000030224; http://www.chictr.org.cn/showprojen.aspx?proj=49968	Clinical study of mesenchymal stem cells in treating severe novel coronavirus pneumonia (COVID-19)	MSCs	32	Experimental group 1: critical group, intervention, injecting MSCs Experimental group 2: severe group, intervention, injecting MSCs Control group 3: control of the critical group, intervention, injecting normal saline Control group 4: control of the severe group, intervention, injecting normal saline
10	24-02-2020; 17-02-2020 to 17-04-2020	0 Not recruiting	ChiCTR2000030173; http://www.chictr.org.cn/showprojen.aspx?proj=49229	Key techniques of umbilical cord mesenchymal stem cells for the treatment of novel coronavirus pneumonia (COVID-19) and clinical application demonstration	UC- MSCs	60	Experimental group: UC-MSCs Control group: Conventional treatment
11	24-02-2020; 24-02-2020 to 31-05-2020	2 Not recruiting	ChiCTR2000030138; http://www.chictr.org.cn/showproj.aspx?proj=50004	Clinical trial for human mesenchymal stem cells in the treatment of severe novel coronavirus pneumonia (COVID-19)	UC-MSCs	60	Experimental group: <i>i.v.</i> injection of UC-MSCs Control group: routine treatment + placebo
12	23-02-2020; 01-02-2020 to 31-08-2020	NA Recruiting	ChiCTR2000030116; http://www.chictr.org.cn/showproj.aspx?proj=49901	Safety and effectiveness of human umbilical cord mesenchymal stem cells in the treatment of acute respiratory distress syndrome of severe novel coronavirus pneumonia (COVID-19)	UC-MSCs	16	Experimental group: different stem cell doses Control group: none specified

Continued

TABLE 2 Continued

	Registration date and execution date range DD-MM-YYYY	Study phase and recruitment status	ID and URL	Title	Cell type	Total participants n	Intervention or treatment
13	22-02-2020; 01-03-2020 to 31-12-2021	0 Not recruiting	ChiCTR2000030088; http://www.chictr.org.cn/showproj.aspx?proj=49902	Umbilical cord Wharton's jelly derived mesenchymal stem cells in the treatment of severe novel coronavirus pneumonia (COVID-19)	UC-Wharton's jelly MSCs	40	Experimental group: <i>i.v.</i> injection of Wharton's jelly MSCs (1×10^6 MSCs·kg ⁻¹), cell suspension volume 40 mL Control group: <i>i.v.</i> 40 mL saline
14	20-02-2020; 06-02-2020 to 05-02-2022	NA Recruiting	ChiCTR2000030020; http://www.chictr.org.cn/showproj.aspx?proj=49812	The clinical application and basic research related to mesenchymal stem cells to treat novel coronavirus pneumonia (COVID-19)	MSCs	20	Experimental group: case series, MSC therapy Control group: none specified
15	18-02-2020; 30-01-2020 to 31-03-2020	1-2 Recruiting	ChiCTR2000029990; http://www.chictr.org.cn/showproj.aspx?proj=49674	Clinical trials of mesenchymal stem cells for the treatment of pneumonitis caused by novel coronavirus pneumonia (COVID-19)	MSCs	120	Experimental group: MSCs Control group: saline
16	28-02-2020; 28-02-2020 to 31-12-2021	1-2 Not recruiting	NCT04288102; https://clinicaltrials.gov/ct2/show/NCT04288102	Treatment with mesenchymal stem cells for severe corona virus disease 2019 (COVID-19)	MSCs	45	Experimental group: 3 times of MSCs; if body weight ≥ 70 kg, 4.0×10^7 cells each time; if body weight < 70 kg, 3.0×10^7 cells each time; <i>i.v.</i> at days 0, 3 and 6 Control group: saline containing 1% human serum albumin (solution used for MSCs), 3 times of placebo, <i>i.v.</i> at days 0, 3 and 6
17	24-02-2020; 24-02-2020 to 01-02-2021	NA Recruiting	NCT04293692; https://clinicaltrials.gov/show/NCT04293692	Therapy for pneumonia patients infected by 2019 novel coronavirus	UC-MSCs	48	Experimental group: conventional treatment plus 4 times of 0.5×10^6 UC-MSCs·kg ⁻¹ body weight suspended in 100 mL saline containing 1% human albumin, <i>i.v.</i> at days 1, 3, 5 and 7 Control group: conventional treatment plus 4 times of placebo (100 mL saline containing 1% human albumin), <i>i.v.</i> at days 1, 3, 5 and 7
Clinical trials based on MSC derivatives							
1	04-02-2020; 05-02-2020 to 30-04-2021	0 Not recruiting	ChiCTR2000029569; http://www.chictr.org.cn/showproj.aspx?proj=49062	Safety and efficacy of umbilical cord blood mononuclear cells conditioned medium in the treatment of severe and critically novel coronavirus pneumonia (COVID-19): a randomized controlled trial	UC-MSCs CM	30	Experimental group: conventional treatment combined with UC-MSCs CM Control group: conventional treatment

Continued

TABLE 2 Continued

	Registration date and execution date range DD-MM-YYYY	Study phase and recruitment status	ID and URL	Title	Cell type	Total participants n	Intervention or treatment
2	19-02-2020; 15-02-2020 to 31-07-2020	1 Not recruiting	NCT04276987; https://clinicaltrials.gov/ct2/show/NCT04276987	A pilot clinical study on inhalation of mesenchymal stem cells exosomes treating severe novel coronavirus pneumonia	AT-MSC exosomes	30	Experimental group: 5 times aerosol inhalation of MSC-derived exosomes, 2.0×10^8 nano vesicles per 3 mL at days 1, 2, 3, 4 and 5 Control group: none specified
3	26-02-2020; 28-02-2020 to 31-05-2020	0 Not recruiting	ChiCTR2000030261; http://www.chictr.org.cn/showproj.aspx?proj=49963	A study for the key technology of mesenchymal stem cells exosomes atomization in the treatment of novel coronavirus pneumonia (COVID-19)	MSC exosomes	26	Experimental group: aerosol inhalation of exosomes Control group: blank
4	03-03-2020; 31-01-2020 to 31-01-2021	NA Not recruiting	ChiCTR2000030484; http://www.chictr.org.cn/showproj.aspx?proj=50263	UC-MSCs and exosomes treating patients with lung injury following novel coronavirus pneumonia (COVID-19)	UC-MSCs and exosomes	90	Experimental groups: Group 1: UC-MSCs <i>i.v.</i> 5×10^7 cells each time, once a week, twice per course Group 2: UC-MSCs <i>i.v.</i> 5×10^7 cells each time, once a week, twice per course, a total of 2 courses; exosomes <i>i.v.</i> administration, 180 mg each time, once a day, 7 days per course, 2 courses in total Control group: same amount of placebo (stem cell solvent)
Clinical trials based on other cell types							
1	14-02-2020; 20-02-2020 to 20-02-2021	0 Not recruiting	ChiCTR2000029812; http://www.chictr.org.cn/showproj.aspx?proj=49374	Clinical study for umbilical cord blood mononuclear cells in the treatment of acute novel coronavirus pneumonia (COVID-19)	UCBMCs	60	Experimental group: conventional treatment followed by <i>i.v.</i> UCBMC preparations Control group: conventional treatment
2	05-02-2020; 05-02-2020 to 30-04-2021	0 Recruiting	ChiCTR2000029572; http://www.chictr.org.cn/showproj.aspx?proj=41760	Safety and efficacy of umbilical cord blood mononuclear cells in the treatment of severe and critically novel coronavirus pneumonia (COVID-19): a randomized controlled clinical trial	UCBMCs	30	Experimental group: conventional treatment combined with UCBMCs Control group: conventional treatment
3	17-02-2020; 24-02-2020 to 31-12-2024	1-2 Recruiting	NCT04276896; https://clinicaltrials.gov/show/NCT04276896	Function and safety study of SARS-CoV-2 synthetic minigene vaccines	Autologous LV-DC vaccine or antigen-specific cytotoxic T-cells	100	Experimental group: 5×10^6 LV-DC vaccine or 1×10^8 cytotoxic T-cells as a single infusion <i>via</i> subcutaneous fluids or <i>i.v.</i> injection; may receive additional infusions Control group: none specified

Continued

TABLE 2 Continued

	Registration date and execution date range DD-MM-YYYY	Study phase and recruitment status	ID and URL	Title	Cell type	Total participants n	Intervention or treatment
4	13-02-2020; 20-02-2020 to 30-12-2020	1 Recruiting	NCT04280224; https://clinicaltrials.gov/show/NCT04280224	NK cells treatment for novel coronavirus pneumonia	NK cells	30	Experimental group: conventional treatment plus twice a week of NK cells ($0.1-2 \times 10^7$ NK cells·kg ⁻¹ body weight) Control group: conventional treatment
5	06-03-2020; 10-04-2020 to 10-11-2020	2 Not recruiting	NCT04299152; https://clinicaltrials.gov/ct2/show/NCT04299152	Clinical application of stem cell educator therapy for the treatment of viral inflammation caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)	CB-SCs	20	Experimental group: combination product: stem cell educator-treated mononuclear cells apheresis therapy circulates a patient's blood through a blood cell separator, briefly cocultures the patient's immune cells with adherent CB-SCs <i>in vitro</i> , and returns the "educated" autologous immune cells to the patient's circulation Control group: conventional treatment: regular treatments, only addressing their symptoms, such as reducing fever and cough
6	28-02-2020; 01-03-2020 to 17-02-2021	0 Not recruiting	ChiCTR2000030088; http://www.chictr.org.cn/showproj.aspx?proj=49779	Clinical trial for umbilical cord blood CIK and NK cells in the treatment of mild and general patients infected with novel coronavirus pneumonia (COVID-19)	CIK and NK cells	90	Experimental groups: CIK group: UCB CIK cells (1.6×10^8 cells·kg ⁻¹) injected twice every other day NK group: UCB NK cells (1.6×10^8 cells·kg ⁻¹) injected twice every other day Control group: conventional therapy

MSCs: mesenchymal stem (stromal) cells; *i.v.*: intravenous; UCB: umbilical cord blood; NK: natural killer; MenSCs: mesenchymal stem cells derived from menstrual fluid; UC: umbilical cord; CM: conditioned medium; AT: adipose tissue; UCBMCs: umbilical cord blood-derived mononuclear cells; LV-DC: lentivirus dendritic cell; CB-SCs: cord blood stem cells; CIK: cytokine-induced killer.

stay and ventilator-free days. We also strongly advocate for utilising clinical samples, obtained as part of appropriate clinical care or for monitoring adverse events following cell administration, to obtain mechanistic information including but not limited to analyses of circulating pro- and anti-inflammatory mediators and inflammatory cell populations. Given the rapidity of the COVID-19 spread and the increasing numbers of cell-based therapy investigations, a central coordinating centre to expedite congruent trial design and appropriate data dissemination would be of significant benefit.

A further significant issue is which COVID-19 patient population to target and when to initiate MSC administration. Critically ill patients with ARDS requiring supportive measures including intubation and mechanical ventilation are a logical population to target. Appreciating that there are other potential therapeutic approaches being considered in this population, it would seem reasonable to target these patients as soon as possible following intubation and mechanical ventilation. Arguments can also be made for targeting severely infected patients with currently recognised risk factors such as increased age, diabetes and/or cardiovascular disease, who are at potentially higher risk for clinical deterioration. In this instance, emerging data on clinical and laboratory indications, such as level of oxygenation, changes in oxygenation and elevations in indicators of systemic cytokine storm, may be criteria for initiating MSC administration. As these indicators are evolving, we advocate a broad-minded approach for considering when to consider MSC use. Whether moderate or mild disease patients should be part of clinical investigation remains less clear. The available pre-clinical data and the safety data from clinical trials in non-viral ARDS best support potential use. As it is too early to tell whether there will be any downstream respiratory effects in COVID-19 ARDS survivors, this seems a less urgent population to target. Similarly, targeting recovering mild or moderately affected patients to counter as yet unknown downstream effects on the lungs also seems to be a less urgent consideration. Overall, to date there has been a limited understanding of the pathogenesis of COVID-19, which currently hinders the development of an optimal study design.

Whether to utilise genetically modified MSCs is unclear at present. This in part reflects the current lack of understanding as to which action, *i.e.* secreted mediator constitutively or inducibly produced by MSCs, may be most effective in SARS-CoV-2 respiratory infections. This also reflects the lack of clear evidence at present as to whether any other potentially therapeutic agent will have proven efficacy, for example an IL-6 or IL-1 receptor antagonist that the MSCs could be engineered to produce. This may change rapidly as current treatment investigations evolve, involving these and other agents. Furthermore, as genetically modified cell products require more lengthy regulatory approval, this may not be a strategy of choice to face the current outbreak.

Challenges and perspectives

The global pandemic of COVID-19 respiratory infection has prompted urgent need for novel therapies. Clinical and basic science investigators need to take the lead by promoting and adhering to rigorously designed investigations logically based on available pre-clinical data. The limited available data regarding MSC administration in pre-clinical respiratory disease injury models and current understanding of potential mechanisms of MSC actions in lung injury models can be cautiously and carefully utilised to support rationally designed and conducted clinical investigations. However, more pre-clinical data are necessary, particularly in models of coronavirus-induced lung injuries. While compassionate use of unproven MSC-based therapies may be contemplated in different circumstances, we urge that, whenever possible, this takes place in the larger context of a clinical investigation. Since the original writing of this review, a number of academic and industry-sponsored trials of MSC-based investigations have been initiated globally in addition to the ones in China reviewed here. We urge all of these to uphold the highest standards for rational and appropriately designed investigations. Only in these ways can a rational evidence-based platform for potential therapeutic use of cell-based therapies be developed.

We must also take strong stance against the stem cell clinic industry, which has already begun to offer unproven therapies for COVID-19. The International Society for Cell and Gene Therapy, the International Society for Stem Cell Research and a number of other professional and scientific organisations have taken leadership positions in this area [54–56]. The potential for abuse is high, given the desperate circumstances of the COVID-19 pandemic, and unauthorised use of unproven therapies is a clear danger.

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the submitted work; and is a research scientist for Cells for Cells, a University spin-off developing therapies for osteoarthritis, pulpitis and cardiac failure, and Regenero, a consortium for Chilean regenerative medicine (public and private funding), for skin ulcer and Lupus. F.F. Cruz has nothing to disclose. F.E. Figueroa has a patent WO/2019/051623 pending and is a board member of Cells for Cells, as the director of the programme of translational cell therapy at Universidad de los Andes, the academic institution that originated the Consorcio Corfo Regenero and the Cells for Cells biotechnological spin-off. P.R.M. Rocco has nothing to disclose. D.J. Weiss reports grants from NIH, Cystic Fibrosis Foundation and US Department of Defense, outside the submitted work.

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