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Early View

Review

Current Status of Cell-Based Therapies for Respiratory Virus Infections: Applicability to COVID-19

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Title:
Current Status of Cell-Based Therapies for Respiratory Virus Infections: Applicability to COVID-19

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Abstract:

Severe respiratory consequences of the 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 the acute respiratory distress syndrome (ARDS), although not as 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 are a rapidly increasing number of clinical investigations of cell-based therapy approaches for COVID-19. These utilize a range of different cell sources, doses, dosing strategies, and targeted patient populations. To provide a rationale strategy to maximize 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. These are presented along with consideration of current clinical investigations.

Keywords: Coronavirus; COVID-19; Cell therapy; Mesenchymal stem/stromal cells; Acute respiratory distress syndrome.

Introduction

The 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, 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 1-14 days. Most patients present with mild respiratory tract infection, mostly commonly characterized 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 in evolution as the pandemic spreads and depends 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 complications of critical illness are not as yet clear. Current therapeutic approaches include aggressive standard supportive care and treatment of any other co-infections. Anti-viral medications including remdesivir, lopinavir–ritonavir, or lopinavir–ritonavir and interferon beta-1 are under investigation but safety and potential efficacy remains to be determined. Remdesivir and interferon beta-1β appear to have superior antiviral activity to lopinavir and remdesivir *in vitro* for the MERS coronavirus but whether this is the case for SARS-CoV-2 remains to be determined.[2] The 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-IL6 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 utilizing MSC-derived conditioned media (CM) or extracellular vesicles (EVs) 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 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 define the patient population towards 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 labeled MSCs demonstrate the most are cleared within 24-48 hours although there can be longer persistence in injured or inflamed lungs.[4] The clearance mechanisms are still being elucidated but include apoptosis and subsequent effereocytosis 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 (EVs) (**Figure 1**).[8] Direct cell-cell transmission of mitochondria from MSCs to respiratory epithelial and immune cells[9] has also been described.[10] 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 cell surfaces.[11] This includes toll-like receptors (TLRs) that are activated by viral RNA (TLR3) (as in COVID-19) and viral unmethylated CpG-DNA (TLR9), leading to downstream cell signaling pathways resulting in MSC activation.[12] 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[13] while specific inhibitory mRNAs (miRNAs) in EVs are also described as mediating the protective effects of MSCs in pre-clinical models of bacterial or non-infectious acute lung injuries.[14] However, mediators responsible for ameliorating respiratory viral-induced lung injuries remain unclear. In animal models, H9N2 viral infection increases serum and lung chemokines responsible for lung leukocyte infiltration,

including Granulocyte-macrophage colony-stimulating factor (GMCSF), Monocyte chemoattractant protein-1 (MCP-1), Macrophage inflammatory protein-1 alpha (MIP-1α) and others that are markedly reduced by intravenous administration of MSCs.[15] Increased levels of interferon-gamma (IFN-γ), typical of anti-viral 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 immuno modulating effects has been previously demonstrated in a model of Graft *versus* Host Disease (GVHD), where the MSC treated recipients of IFN (–/–) T cell grafts, did not respond to cell therapy, evolving into fatal GVHD. [16] 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, 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*[17]. Umbilical cord-derived MSCs (UC-MSCs) have also been shown to inhibit the cytotoxicity of specific T cells against H1N1 influenza virus *in vitro*, [18] leading perhaps to prolonged infection in recipients. This is in contrast to reports where, for example, in models of CMV infection, MSCs exert differential effects on alloantigen and virus specific T cells that retain the ability to proliferate, produce IFN-*γ*, and to kill CMV-infected cells *in vitro*.[19]

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 anti-viral T cells actions, or whether they are due to overall anti-inflammatory actions have been demonstrated in other models of acute lung injuries.[20] 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 interferon (IFN) stimulated genes (ISG) that

preempt viral infection[21]. MSC ISG expression includes, among others, IFITM (interferon-induced transmembrane family), IFI6, ISG15, SAT1, PMAIP1, p21/CDKN1A and CCL2. Among these antiviral proteins, members of the IFITM family members 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 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 MSC susceptible to infection by a variety of viruses including YFV and ZIKV[21].

Utilizing RNAseq (GSE97987) and validated by qRT-PCR,[21] we established a list of ISGs constitutively expressed by human embryonic stem cell derived-MSCs. Bioinformatic analysis of 5 independent GEO databases for expression changes following different pro-inflammatory cytokine stimulation of intrinsic ISGs in human MSCs (3 BM-MSC, 1 UC-MSC, 1 AD-MSC, with the respective GEO data set of GSE68610, GSE77814, GSE46019, GSE46019 and GSE18662) (**Figure 2**). demonstrated that pro-inflammatory cytokines including IFN-γ induced non-constitutive ISGs includingMT1X, MT1G, SERPING1, SAT1, IFNAR2, 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 defenses 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[15]. 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 hours post -exposure, with corresponding production of pro-inflammatory cytokines and chemokines[24] potentially subverting their protective immunomodulatory properties.[26] The respiratory syncytial virus (RSV) 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 of the SARS-COv-2 entry and the serine protease TMPRSS2 for S protein priming.[28] ACE2 is highly expressed in respiratory epithelial cells thus playing 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 (Ang) 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 over-expressing 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 over-expression 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 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 bacterial or bacterial products (endotoxin) or other means.[35] The models include both rodents as well as large animal (pig, sheep) and explanted human lungs. A range of approaches have been utilized for dose, dosing, and MSC source with MSCs of bone marrow (BM), adipose, umbilical cord, cord blood, and placenta being investigated . A recent systematic review indicated that BM and UC-MSCs were more effective than adipose tissue derived 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 further been 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 and colleagues assessed the effects of a single systemic administration in immunocompetent mice of either syngeneic murine bone marrow-derived MSCs or xenogeneic human bone marrow-derived MSCs on lung injury induced by mouse-adapted H1N1 or swine-origin pandemic H1N1.[37] Two different doses of MSCs (2.5 or 5 x 10^5 cells/mouse) were administered at different time points after virus administration: Day -2, 0, 2 and 5 post-infection (p.i.). Using survival and different measures of lung inflammation as outcome endpoints on day 7 p.i., neither syngeneic or 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, Gotts and colleagues assessed the effect of both systemic and intratracheal administration of human and mouse MSCs (5 x 10^5 cells/mouse), administered in two doses, either earlier (day 2 and 3 p.i.) or later (day 5 and 6 p.i.), in mouse adapted H1N1-induced lung injury in immunocompetent mice.[38] 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 and colleagues found in *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 up-regulated sodium and chloride transporters. Systemic administration of 5 x 10^5 human bone-marrow derived MSCs/mouse on day 5 p.i. in aged (8-12 months) immunocompetent mice infected with Influenza A (H5N1)[39] reduced virus-induced mortality (until day 18 p.i.), weight loss (day 6-10 p.i.), lung edema (day 7 p.i.), BALF CD4+ T and natural killer (NK) cells (day 7 p.i.), lung histopathological lesions (day 18 p.i.), pro-inflammatory cytokines and chemokines (day 7 p.i.) without reducing lung virus titers (day 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 (6-8 weeks) mice, no effects were observed in mortality and body weight loss. Thus, the data suggests 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 and colleagues investigated the impact of low dose (10^5 cells/mouse) of murine bone marrow-derived MSCs in Avian Influenza virus (H9N2)-induced lung injury in young immunocompetent mice .[15] A single intravenous administration led on day 3 p.i. to reduction in mortality, lung edema, histologic injury, bronchoalveolar lavage fluid (BALF) and serum chemokines and cytokines, as well as improving gas-exchange and levels of anti-inflammatory mediators, although not reducing lung virus titration when administered either 30 minutes or 24 hours 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 monokine induced by interferon-γ (MIG), GM-CSF; BALF and serum interleukin 1-α and interferon-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 TNF-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, authors speculated that the beneficial effects might be a specific consequence of different pathogenic features as compared to swine origin H1N1 infection. [15]

Loy and colleagues found that UC-MSCs were more effective than human BM-derived MSCs (BM-MSCs) at restoring impaired alveolar fluid clearance and permeability *in vitro* airway epithelial cell models.[40] These effects were partially mediated through MSC secretion of Ang-1 and hepatocyte growth factor (HGF). The authors subsequently compared administration of UC-MSCs to BM-MSCs (5×10^5 cells/mouse, day 5 p.i.) in experimental lung injury induced by Influenza A (H5N1) infection in female 6-8 week old immunocompetent mice. Despite failure to reduce virus titer and increase survival rate, a single dose of UC-MSCs decreased body weight loss (days 16, 17 p.i.), lung edema (days 10, 14 p.i.), and inflammation in H5N1-induced lung injury (day 7 p.i.).

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MSCs-derived EVs have been demonstrated to have comparable and in some cases more effective than MSCs themselves in ameliorating inflammation and injury in a range of pre-clinical lung injury models.[41, 42] Khatri and colleagues found that systemic administration of EVs isolated from pig bone marrow-derived MSCs was safe and reduced virus shedding in nasal swabs, influenza replication in the lungs, BALF pro-inflammatory cytokines and chemokines, histopathological changes when administered 12 hours after viral inoculation in a mixed swine (H3N2, H1N1) and avian (H9N5, H7N2) influenza-induced pig lung injury model.[43] These findings suggest systemic EV administration as a potential cell-free strategy for use in respiratory virus-induced lung injuires.

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 primate and in inbred strains of mice following intranasal infection, but these models failed to show clinical signs of pulmonary disease as seen in human.[44] hACE2 transgenic mice infected with SARS-CoV-2 demonstrated virus replication in lung and interstitial pneumonia with lymphocyte

and monocytes 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 differing features of each approach including the host (age), MSCs (source, number, route of administration), and different 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, EVs might be an option for further studies. Clearly, further pre-clinical studies must be done evaluating 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 just published single center open-label pilot investigation from the YouAn Hospital in Beijing administered BM-derived MSCs to seven patients with COVID-19 pneumonia with differing degrees of severity including one patient with critically severe disease requiring ICU care.[47] The MSCs were given as a single intravenous administration at a dose of 10^6 cells/kg body weight in 100 ml saline at various times after initial symptomatic presentation. The MSCs were assessed by RNAseq for expression of ACE2 or TMPRSS2 prior to administration and each was found to be minimally expressed (1/12,500 cells and 7/12,500 cells, respectively) although the RNAseq results were not validated for gene (qRT-PCR) or protein expression.

The seven patients were categorized 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 endpoints were assessed. No infusional toxicities, allergic reactions, secondary infections, or severe attributable adverse events were observed and patients, including the one categorized 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 6 patients or for the three placebo patients. Comparably, analyses of viral titers, 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.

As such, more detailed information as to inclusion and exclusion criteria, timing of MSC administration relative to onset of disease, co-morbidities, 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 IV administrations of menstrual blood-derived cells (10^6 cells/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, 3 are described as receiving 3 separate infusions during early stage infection, 6 patients received 3 infusions at late stage infection and 8 patients received 4 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 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. 3 patients in the MSC-treated group died (82.4% survival) whereas 24 in the control died (45.5% survival). However, no details 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, were provided. 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, co-morbidities, 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 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 on the NIH clinicaltrials.gov and the Chinese Clinical Trial Registry (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 enrollement (Table 2). There are three main interventions: MSCs (n=17, 781patients), MSC derivatives (CM, EVs, n=4, 176 patients), or other cell sources (n=6, 330 patients). General common features of the investigations include a) systemic administration, jointly or followed by the recommended conventional supportive treatments for severe or critical SARS-CoV-2 infection;[49] b) age range 18-80 years old

with no gender restrictions; c) follow up for at least three months; and d) clinical samples collected will be throat secretions and/or blood. For the MSC investigations, 9/17 will utilize UC-MSCs, 1/17 will utilize menstrual blood-origin MSCs, and 6/17 do not disclose the MSC tissue source. Notably, no apparent MSCs of bone marrow origin are being utilized despite the majority of pre-clinical investigations for non-viral induced acute lung injuries having utilized BM-MSCs. There was little clarification of use of cryopreserved versus continuously cultured cells [5]. Only 6/16 disclosed the intended cell injection dose, among which only 4 were correlated with the patient body weight. The intravenous dosing range varies between 0.4 and $42x10^6$ cells/kg. In comparison, the highest dose of MSCs used in the published literature for clinical trials in non-viral ARDS was $10x10^6$ cells/kg (START trial).[50] The dosing strategy ranged between a single and 5 doses with an average frequency of every 2 days.

Four of the trials will utilize either MSC-derived CM or EVs. Two of these propose aerosol inhalation of MSC-derived EVs, one from adipose-derived MSCs, for which there is no pre-clinical supporting data. Six investigations will utilize other cells including umbilical cord blood derived mononuclear cells (UC-BMC); cytotoxic T cells (CTL); dendritic cells (DC); natural killer cells (NK); cord blood stem cells(CB-SC), or cytokine-induced killer cells (CIK), of which only the latter investigation describes dosing and frequency of injections. As best as we can ascertain, here is 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 health care providers and researchers during an infectious disease outbreak, including clinical trials, are aimed towards finding rapid and effective responses for the treatments of infected patients. These actions need to occur under appropriate ethical guidelines. The WHO has guidelines which 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 etiologies, 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 recognized endpoints, including but not limited to overall mortality, length of ICU and hospital stay and ventilator-free days. We also strongly advocate for utilizing 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 COVID19 spread and the increasing numbers of cell-based therapy investigations, a central coordinating center 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. 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 be made as well for severely infected patients with currently recognized risk factors such as increased age, diabetes and/or cardiovascular disease at potentially higher risk for clinical deterioration. In this instances, 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 indictors 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 supports 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 utilize genetically modified MSCs is unclear at present. This in part reflects the current lack of understanding as to which action, ie., 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, for example an IL-6 or IL-1 receptor antagonist that the MSCs could be engineered to produce, also will have proven efficacy. This may change rapidly as current treatment investigations involving these and other agents evolve. Further, as genetically modified cell products require a more a more lengthy regulatory approval, this may not be a strategy of choice to face the current outbreak.

Challenges and Perspectives

The global pandemic of COVID19 repiratory 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 utilized to support rationally designed and conducted clinical investigations. However, more pre-clinical data is 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 take 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. We urge all of these to uphold the highest standards for rationale and approrpaitely designed investigations. Only in these ways can a rationale 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 COVID19. The ISCT, ISSCR, and a number of other professional and scientific organizations have taken leadership positions in this area.[54],[55],[56]. The potential for abuse is high given the desperate circumstances of the COVID-19 pandemic and unauthorized use of unproven therapies is a clear danger.

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CONFLICT OF INTERESTS

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Table 1. Preclinical studies: MSCs in respiratory virus-related lung injury

Reference	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 et al., 2013	C57BL/6 mice 7-10 weeks IN infection Influenza A/PuertoRico/ 8/34 (mouse-adapted H1N1) or Influenza A/Mexico/4108/2009 (swine-origin pandemic H1N1)	Mouse BM -MSCs (P6-P9) Human BM – MSCs (P3) 2.5 x 10^5 cells 5 x 10^5 cells IV (tail vein) Single dose Day -2, 0, 2, 5 post infection	Day 7 or when euthanasia criteria was met	Oseltamivir 2.5 mg/kg Oral gavage Once daily 5 days	Prophylatic 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.	Non-specified (soluble mediators)	No
Gotts et al., 2014	C57BL/6 mice 7-10 weeks IN infection Influenza A/Puerto Rico/8/34 (mouse-adapted H1N1)	Mouse BM–MSCs (P7 or less) Human BM–MSCs (P7 or less) 5 x 10^5 cells IV (Retro-orbital injection)	Days 7, 9 or 11	No	Mouse MSCs: Prevented influenza-induced thrombocytosis, Caused a modest reduction in lung viral load on *day 7*, Early (data not shown) and late syngeneic and	Non-specified (soluble mediators)	No

		IT (Data not shown) Two doses Either: Day 5 and 6 post infection, or Day 2 and 3 post infection (data not shown)			xenogeneic intravenous administration of MSCs: Failed to affect weight loss. Failed to decrease lung water. Failed to decrease BALF inflammation, and Failed to improve lung histology Intratracheal administration increased the severity of model (data not shown).		
Chan, 2016	BALB/c mice 6-8 weeks - young 8-12 months - old IN infection with Influenza A /Hong Kong/486/1997(H5N1)	Human BM–MSCs (Passage not mentioned) 5 x 10^5 cells IV Single dose Day 5 post infection	Days 7, 10 or 18	No	In aged mice, but not young mice, allogeneic MSCs: Increased survival, Reduced weight loss, Reduced lung histopathological lesions, Increased M2 macrophages in BALF, Reduced lung proinflammatory cytokines and chemokines (MCP-1, MCP-3, MIP-1 α, RANTES, IL-4, IL-17, TNF-α), and Reduced lung virus titers	Paracrine soluble mediators, partially due to Ang1 and KGF secretion	NIH 3T3 mouse embryo fibroblasts

Li et al., 2016	C57BL/6 mice 6-8 weeks IN infection with Avian Influenza virus Hong Kong/2108/2003 (H9N2)	Mouse BM-MSCs (P3-P10) 1 x 10^5 cells IV (tail vein) Single dose 30 minutes, or Day 1 post-infection	Day 3	No	Regardless of time administration, syngeneic MSCs: Did not reduce lung virus titration, Increased survival rate, Decreased lung edema, Decreased histologic injury, Improved gas exchange, Reduced BALF chemokines and cytokines – early administration: GM-CSF, MIG content, IL-1α, IFN-γ, IL-6, TNF-α, Reduced serum chemokines and cytokines – early administration: MIG content, IL-1α, IFN-γ, IL-6, TNF- α, Early administration increased anti-inflammatory cytokine IL-10 in BALF and serum.	Non-specified (soluble mediators)	No
Khatri et al., 2016	White-Duroc crossbred pigs 8 weeks	Pig BM-MSCs EVs (P3-P5)	Days 1 or 3	No	BM-MSC-derived EVs: Decreased virus shedding in nasal swabs, Reduced influenza virus replication in the lungs,	Non-specified (extracellular vesicles)	No

	IN infection with Swine/TX/98 Influenza virus - H3N2 and Swine/MN/08 (H1N1)	80 μg/kg body weight (produced by 10×10^6 MSCs) IT Single dose 12 hours post-infection			Prevent virus-induced production of pro-inflammatory cytokines (TNF-α, CXCL-10), Reduced histologic injury and lung edema.		
Loy et al., 2019	BALB/c mice 6-8 weeks IN infection with Influenza A /Hong Kong/486/1997 (H5N1)	Human UC–MSCs (P7 or less) 5×10^5 cells IV 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 edema, and Decreased BAL cytokines (IP-10, MCP-1, RANTES, IL-1β.	Paracrine soluble mediators, partially due to Ang1 and HGF secretion	NIH 3T3 mouse embryo fibroblasts

Preclinical studies showing the effects of MSCs in virus-mediated lung injury. ALI- acute lung injury; Ang1 – angiopoietin 1; BM-MSCs – bone marrow-derived mesenchymal stem (stromal) cells; CXCL-10 - C-X-C motif chemokine; EV- extracellular vesicles; GM-CSF - granulocyte-macrophage colony-stimulating fator; IL-Interleukin; IN – Intranasal; IT – intratracheal; IP-10 - interferon γ–induced protein 10; IV – Intravenous; MCP – monocyte chemoattractant; MIG- monokine induced by interferon-γ ; MIP – macrophage inflammatory protein; RANTES - Regulated upon Activation, Normal T Cell Expressed and Presumably Secreted; TNF – Tumor necrosis factor; UC-MSCs - umbilical cord-derived MSCs

Table 2: Cell-based clinical trials (MSC, MSC derivatives and other cells).

Clinical trials based on MSCs						
	Date of registration/ Execution time	**Study phase/ Recruitment status**	**ID/ URL**	**Title**	**Cell type**	**Number of total particpants/ Intervention or treatment**
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	60 Experimental group: Conventional treatment followed by iv infusion UCB-MSCs 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 and UCB-MSCs	60 Experimental group: High dose group: High-dose NK cells (>$5x10^9$) and MSCs (>$5x10^9$), iv infusion once, every 2 days for a total of 5 times Conventional dose group: Conventional dose NK cells (>$3x10^9$) and MSCs (>$3x10^9$), iv infusion once every 2 days for a total of 3 times Preventive dose group: Preventive dose NK cells (>$3x10^9$) and MSCs (>$3x10^9$), iv 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: 1. Conventional treatment followed by iv infusion of MenSCs. Control group A: Conventional treatment Experimental group B: 1: Artificial liver therapy+conventional treatment 2: Artificial liver therapy followed by iv infusion of MenSCs +conventional treatment Control group A: 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 3.3x107 cells/50ml/bag, 3 bags each time. And UC-MSCs will be infused iv on the 1st, 3rd, 5th, and 7th days after enrollment, 1 time 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.
6	27-01-2020/	1 / Recruiting	NCT04252118/ https://clinicaltrials.gov/	Mesenchymal Stem Cell Treatment for Pneumonia Patients	MSCs	20

	21-01-2020 to 00-12 2021		show/NCT04252118	Infected With 2019 Novel Coronavirus		Experimental group: 3.0x10^7 MSCs iv at day 0, day 3, day 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 (0.5x10^6 UC-MSCs/kg body weight iv at day 1, day 3, day 5, day 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: Convention 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: Iv 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
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-Whartons Jellys MSCs	40 Experimental group: Iv injection of Wharton's Jelly MSCs (1×10^6/kg), cell suspension volume: 40 ml Control group: Iv 40ml 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: MSCs 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 (（body weight≥70kg, $4.0x10^7$ cells per time; body weight<70kg, $3.0x10^7$ cells per time) iv at day 0, day 3, day 6). Control group: Saline containing 1% human serum albumin（solution of MSCs）3 times of placebo（iv at day 0, day 3, day 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: Participants will receive conventional treatment plus 4 times of $0.5x10^6$ UC-MSCs /kg body weight suspended in 100 mL saline containing 1% human albumin iv, at day 1, day 3, day 5, day 7. Control group: Participants will receive conventional treatment plus 4 times of placebo (100 mL saline containing 1% human albumin) iv at day 1, day 3, day 5, day 7.
Clinical trials based on MSCs-derivatives						
	Date of registration/ Execution time	**Study phase/ Recruitment status**	**ID/ URL**	**Title**	**Cell type**	**Number of total participants/ Intervention or treatment**
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
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-MSCs Exo	30 Experimental group: 5 times aerosol inhalation of MSCs-derived exosomes ($2.0x10^8$ nano vesicles/3 ml at day 1, day 2, day 3, day 4, day 5). Control group: None specified
3	2020-02-26 / 28-02-2020 to 31-05-2020	0 / Not Recruiting	ChiCTR2000030261/ http://www.chictr.org.cn/showprojen.aspx?proj=49963	A study for the key technology of mesenchymal stem cells exosomes atomization in the treatment of novel coronavirus pneumonia (COVID-19)	MSCs Exo	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	HUMSCs and Exosomes Treating Patients with Lung Injury following Novel Coronavirus Pneumonia (COVID-19)	UC-MSCs and Exo	90 Experimental group: Group 1: HUMSCs iv, $5×10^7$ cells / time, once / week, twice/course Group 2: HUMSCs iv infusion, $5×10^7$ cells / time, 1 time / week, 2 times / course, a total of 2 courses; Exosomes: iv

						administration, 180 mg / time, 1 time / day, 7 days / course, 2 courses in total Control group: Same amount of placebo (stem cell solvent)
Clinical trials based on other cell types						
	Date of registration/ Execution time	**Study phase/ Recruitment status**	**ID/ URL**	**Title**	**Cell type**	**Number of participants/ Intervention or treatment**
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)	UCBMC	60 Experimental group: Conventional treatment followed by iv of 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	UCBMC	30 Experimental group: Conventional treatment combined with UCBMC 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: Patients will receive approximately $5x10^6$ LV-DC vaccine or $1x10^8$ CTLs as a single infusion via subcutaneous fluids / Iv injection and may receive additional infusions. Control group: None specified
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 ($0.1\text{-}2x10^7$ NK cells/kg 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-SC	20 Experimental group: Combination Product: Stem Cell Educator-Treated Mononuclear Cells Apheresis SCE therapy circulates a patient's blood through a blood cell separator, briefly cocultures the patient's immune cells with adherent CB-SC in vitro, and returns the "educated" autologous immune cells to the patient's circulation. Control group: Conventional treatment of patients with SARS-CoV-2. Patients will receive the regular treatments by 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	90 Experimental group: CIK group: UCB CIK cells (1.6×10^8/kg) were injected twice every other day. NK group: UCB NK cells (1.6×10^8/kg) were injected twice every other day. Control group: Conventional therapy

CB-MSCs, Umbilical cord blood derived-mesenchymal stem cells; UCB-NK, Umbilical cord blood derived-natural killer; MenSCs, mesenchymal stem cells derived from menstrual uid; UC-MSCs, umbilical cord derived mesenchymal stem cells; UC-MSCs CM, umbilical cord derived mesenchymal stem cells-conditioned médium; AT-MSCs Exo, Adipose tissue xosomes; MSCs Exo, mesenchymal stem cells exosomes; UCBMC, umbilical cord blood derived mononuclear cells; CTL, cytotoxic T cells; LV, lentivirus; DC, dendritic cells; NK, atural killer cells; CB-SC, cord blood stem cells; CIK, Cytokine-induced killer cells; Iv, intravenous.

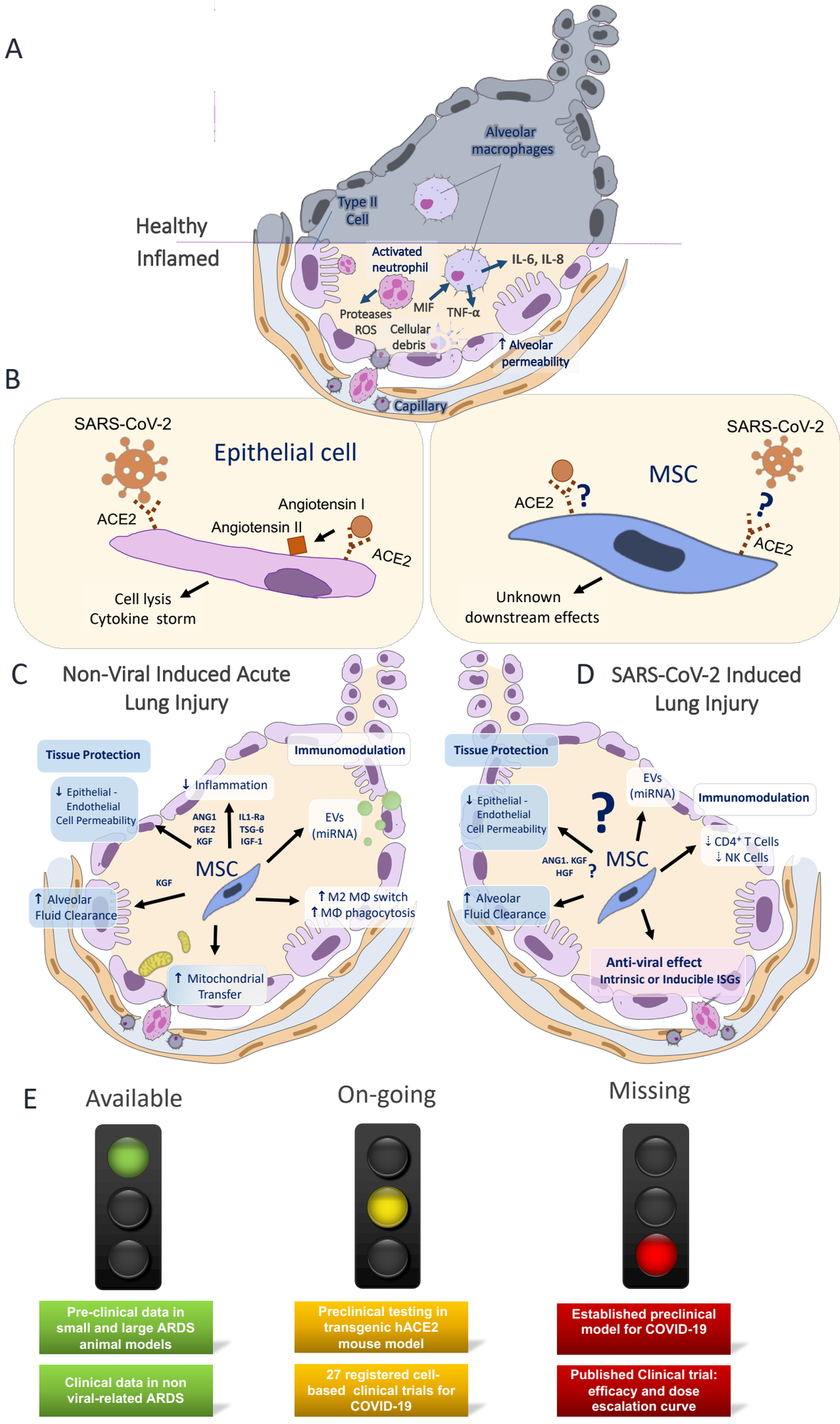

Figure 1. Potential therapeutic effects of MSCs in respiratory lung injury are mediated by different mechanisms including but not limited to secreted paracrine factors, extracellular vesicles (EVs), and possibly mitochondrial transfer, promoting tissue protection, immunomodulation, and possibly viral resistance. A) Schematic of a healthy alveolus (top) and inflamed/edematous alveolus and mechanisms involved in ARDS pathogenesis; B) Schematic of SARS-CoV-2 infecting lung epithelial cells with subsequent lysis and cytokine storm (middle left) and of potential MSC infection with unknown downstream consequences (middle right); C) Some of the known mechanisms by which MSCs ameliorate non-viral acute lung injury (lower left); 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 (lower right). Figure adapted from Laffey and Matthay.[1] E) Current state-of-the-art of cell-based therapy in COVID-19, based on pre-clinical and clinical studies. Angiopoietin-1 (Ang1); keratinocyte growth factor (KGF); Hepatocyte growth factor (HGF); Macrophage-type 2 (M2 MΦ); Macrophage migration inhibitory Factor (MIF); Tumor Necrosis Factor-stimulated Gene-6 (TSG-6); Reactive oxygen species (ROS); Tumor Necrosis Factor alpha (TNF-a); Prostaglandin E2 (PGE2); Angiotensin converting enzyme 2 (ACE2); Interferon (IFN) stimulated genes (ISGs).

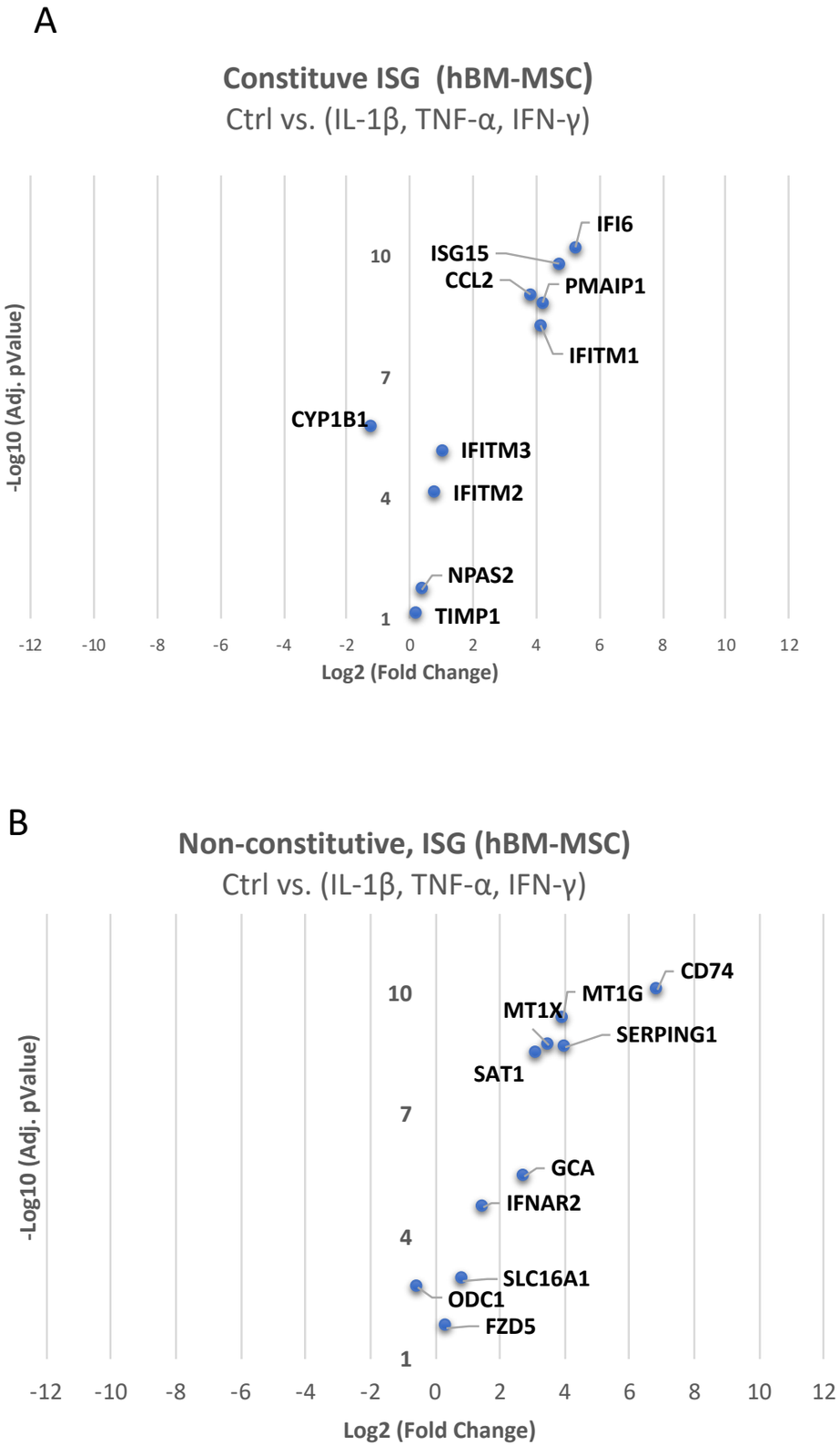

Figure 2. Representative volcano plots analysis of gene expression of the interferon (IFN) stimulated genes (ISGs) in human BM-MSC activated with proinflammatory cytokines (IL1β, TNF-α and IFN-γ), GSE68610 . A. Gene expression analysis of constitutive and **B.** non-constitutive ISGs. Interferon-induced transmembrane protein (IFITIM); metalloproteinase inhibitor 1 (TIMP-1); Plasminogen activator inhibitor 1(SERPINE1); Interferon alpha-inducible protein 6 (IFI6); Ubiquitin-like protein (ISG15); sulfate anion transporter 1 (SAT1); Cytochrome p450 1b1 (CYP1B1); Neuronal pas domain-containing protein 2 (NPAS2); Phorbol-12-myristate-13-acetate-induced protein 1 (PMAIP1); c-c motif chemokine 2 (CCL2); Frizzled-5 (FZD-5); Ornithine decarboxylase 1 (ODC1); Monocarboxylate transporter 1 (SLC16A1); Interferon alpha/beta receptor 2 (IFNAR2); Plasma Protease C1 Inhibitor (SERPING1); Metallothionein 1G/X (MT1G/ MT1X).

1. Laffey JG, Matthay MA. Cell-based therapy for acute respiratory distress syndrome: Biology and potential therapeutic value. *Am J Respir Crit Care Med*. 2017;196(3):266-273. doi:10.1164/rccm.201701-0107CP