



Potency of Mesenchymal Stem Cell and Its Secretome in Treating COVID-19

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Abstract

The COVID-19 disease, which is caused by the novel coronavirus, SARS-CoV-2, has affected the world by increasing the mortality rate in 2020. Currently, there is no definite treatment for COVID-19 patients. Several clinical trials have been proposed to overcome this disease and many are still under investigation. In this review, we will be focusing on the potency of mesenchymal stem cells (MSCs) and MSC-derived secretome for treating COVID-19 patients. Fever, cough, headache, dizziness, and fatigue are the common clinical manifestations in COVID-19 patients. In mild and severe cases, cytokines are released hyperactively which causes a cytokine storm leading to acute respiratory distress syndrome (ARDS). In order to maintain the lung microenvironment in COVID-19 patients, MSCs are used as cell-based therapy approaches as they can act as cell managers which accelerate the immune system to prevent the cytokine storm and promote endogenous repair. Besides, MSCs have shown minimal expression of ACE2 or TMPRSS2, and hence, MSCs are free from SARS-CoV-2 infection. Numerous clinical studies have started worldwide and demonstrated that MSCs have great potential for ARDS treatment in COVID-19 patients. Preliminary data have shown that MSCs and MSC-derived secretome appear to be promising in the treatment of COVID-19.

Lay Summary

The COVID-19 disease is an infection disease which affects the world in 2020. Currently, there is no definite treatment for COVID-19 patients. However, several clinical trials have been proposed to overcome this disease and one of them is using mesenchymal stem cells (MSCs) and MSC-derived secretome for treating COVID-19 patients. During the infection, cytokines are released hyper-actively which causes a cytokine storm. MSCs play an important role in maintaining the lung microenvironment in COVID-19 patients. They can act as cell managers which accelerate the immune system to prevent the cytokine storm and promote the endogenous repair. Therefore, it is important to explore the clinical trial in the world for treating the COVID-19 disease using MSCs and MSC-derived secretome.

Keywords COVID-19 · SAR-CoV-2 · Mesenchymal stem cell · Secretome · Exosome · Clinical trial

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Introduction

In early 2020, the world was horrified by a severe acute atypical respiratory syndrome caused by viral respiratory diseases. The World Health Organization (WHO) office in China received the first report in late December 2019 that several patients in Wuhan, Hubei Province, were diagnosed with pneumonia of unknown cause. Later on, the causative agent was identified using sequencing technology as the novel coronavirus [1]. This novel coronavirus was originally called 2019-nCoV and later renamed officially as SARS-CoV-2 by the International Committee on Taxonomy of Viruses. This virus

causes the disease called COVID-19 that has affected not only China but also worldwide.

The estimated mortality rate of the disease is up to 5% with confirmed infected cases increasing daily [2]. Currently, there is no definite treatment for COVID-19; however, clinical trials have been performed using potential antimicrobials, immunoglobulin- or antibody-based therapy, and cell-based therapy [3–6]. The use of stem cell therapy has shown a promising impact in treating various diseases including degenerative and genetic disorders [7]. Therefore, in this review, we will discuss the potency of mesenchymal stem cells (MSCs) and MSC-derived secretome for COVID-19 treatment.

Coronavirus Disease 2019

Coronavirus disease 2019 (COVID-19) is a highly transmissible severe acute respiratory syndrome caused by SARS-CoV-2, an enveloped, single-stranded RNA virus belonging to the family Coronaviridae [8], alongside Middle East respiratory syndrome coronavirus (MERS-CoV) and severe acute respiratory syndrome coronavirus (SARS-CoV), which broke out in 2012 and 2001, respectively. SARS-CoV-2 was reported to be a member of the β group of coronaviruses, Nidovirales order [9]. The homology of SARS-CoV-2 with SARS-CoV is up to 79.6% which was confirmed by full-length viral genome sequences [5, 10–12].

The possibility of animal to human transmission has been proven in previous outbreaks of SARS-CoV and MERS-CoV [13]. Several reports stated that the bat acts as the natural host for SARS-CoV-2 [12, 14], but the direct transmission to humans is unlikely to happen because of the differences in the habitats between them. A further study should be conducted in order to identify the intermediate host which transfers the infection from the bat to humans [15]. The virus spreads from human to human through droplets and aerosol transmission. A close contact with confirmed infected patients could increase the virus transmission, especially when they talk, cough, or sneeze [16, 17]. In combating the COVID-19, WHO and many countries have issued regulations such as social distancing and the use of masks in public areas [18]. Still, an indirect transmission when people touch an object and then touch their faces in particular their nose, mouth, or eyes might allow the virus to enter their body [19]. Moreover, it has been reported that SARS-CoV-2 could also spread through airborne transmission [20].

The clinical manifestations of a person who gets infected with SARS-CoV-2 are remarkably varied and include fever, cough, headache, dizziness, and fatigue. Other symptoms also include diarrhea. Coughing, breathing difficulties, dyspnea, and pneumonia are the respiratory problems that lead to acute respiratory distress syndrome (ARDS) [12, 13]. The development of ARDS in COVID-19 patients could progress rapidly

resulting in high mortality rate [11]. The severity of COVID-19 patients was divided into 5 categories shown in Table 1.

Mechanism of SARS-CoV-2 Invasion

The invasion of the virus into the host cells involves the process of attachment, penetration, biosynthesis, maturation, and release. This mechanism also takes place in the SARS-CoV-2 invasion. There are 4 structural proteins in coronaviruses: spike (S), membrane (M), envelope (E), and nucleocapsid (N) proteins [23, 24]. The spike protein located on the surface of the virus has two subunits: the S1 subunit binds to the host cell receptor in the process of attachment and the S2 subunit functions in the penetration activity, which allows the virus to enter the host cell through membrane fusion or endocytosis [25, 26]. Biosynthesis takes place after the virus released its genetic materials and the RNA enters the nucleus for replication. Once the maturation process is completed, a new virion is released. Similar to SARS-CoV, the angiotensin-converting enzyme 2 (ACE2) receptor was identified as the receptor of SARS-CoV-2 [11, 27]. The ACE2 receptor is reported to be distributed on the surface of endothelial cells and alveolar epithelial type II cells; therefore, SARS-CoV-2 could infect the cell with the ACE2 receptor [21]. Others also reported that the type 2 transmembrane serine 2 protease (TMPRSS2) and dipeptidyl peptidase IV (DPP-4) proteins act as the entry site of the virus [12]. SARS-CoV-2 attacks the lungs because the alveolar type II cells and the capillary endothelial cells express the ACE2 receptor and TMPRSS2 [28].

The infection will occur when the virions are released into the host's body and spread to other cells. The antigen-presenting cells (APCs) will recognize the virus antigen and present it to the natural killer (NK) cell and CD8-positive cytotoxic T cells (CD8⁺ T cells). Both innate and adaptive immunity will be activated and produce pro-inflammatory cytokines and chemokines. However, in some cases, a massive uncontrolled immune response of the activated immune cells, lymphocytes, and macrophages could occur and might cause a cytokine storm. Overwhelming systemic inflammation, hypercytokinemia, and hyperferritinemia associated with multi-organ failure will appear in COVID-19 patients who experience the cytokine storm. Approximately, around 40% of the patients died due to ARDS-caused cytokine storms. In the event of a cytokine storm, pro-inflammatory cytokines such as interleukin (IL)-1, IL-1 β , IL-6, IL-12, IL-18, IL-33, interferon (IFN)- α , IFN- γ , tumor necrosis factor (TNF)- α , and TNF- β along with chemokine (C-X-C motif) ligand (CXCL)10, CXCL8, CXCL9, CC chemokine ligand (CCL)2, and CCL5 increase (Fig. 1) [29–32].

Among all the pro-inflammatory cytokines, IL-6, and anti-inflammatory cytokine, IL-10 are reported to be the main factors in the cytokine storm [33]. During the cytokine storm,

Table 1 Classification of COVID-19 patients [11, 21, 22]

Classification	Symptoms
Asymptomatic	PCR tested positive without any clinical symptoms and signs. Chest imaging is normal
Mild	Symptoms of acute upper respiratory tract infection or digestive symptoms Cannot be detected by imaging procedures
Moderate/common	Mild to high fever Pneumonia with no hyposmia Chest CT with lesion
Severe	Pneumonia with hypoxemia (SpO ₂ <92%) Respiration rate higher than 30/min Pressure of oxygen in arteries is less than 300 mmHg
Critical	Acute respiratory distress syndrome (ARDS) Multiple organ failure May have shock, encephalopathy, myocardial injury, heart failure, coagulation dysfunction, and acute kidney injury

granulocyte colony–stimulating factor (GCSF), IFN-gamma inducible protein (IP)-10, monocyte chemoattractant protein (MCP)-1, macrophage inflammatory proteins (MIP)-1A, IL-2, and IL-7 are released which causes immune cell death and tissue damage resulting in clinical features such as edema, air exchange dysfunction, ARDS, and other secondary infections leading to death (Fig. 2) [31, 35, 36]. Lymphopenia is a common feature in COVID-19 patients with mild infection [37]. The CD4⁺ T cells, CD8⁺ T cells, B cells, and NK cells are

decreased following the declining expression of IFN-γ in CD4⁺ T cells and upregulated NKG2A receptors on NK cells and CD8⁺ T cells [38, 39].

Patient Management

As there is no targeted therapy available until now, clinicians and researchers are mobilized to find the cure for COVID-19.

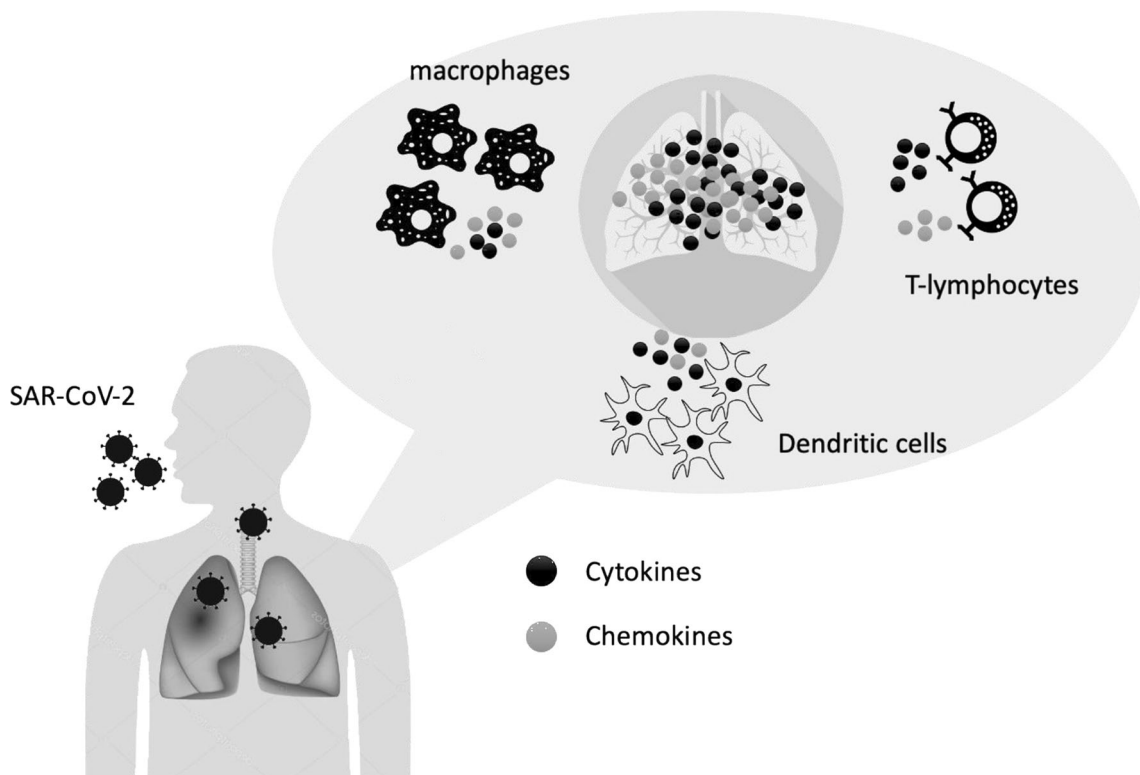


Fig. 1 The development of “cytokine storm” after SARS-CoV-2 infection due to the hyperactivation of immune cells

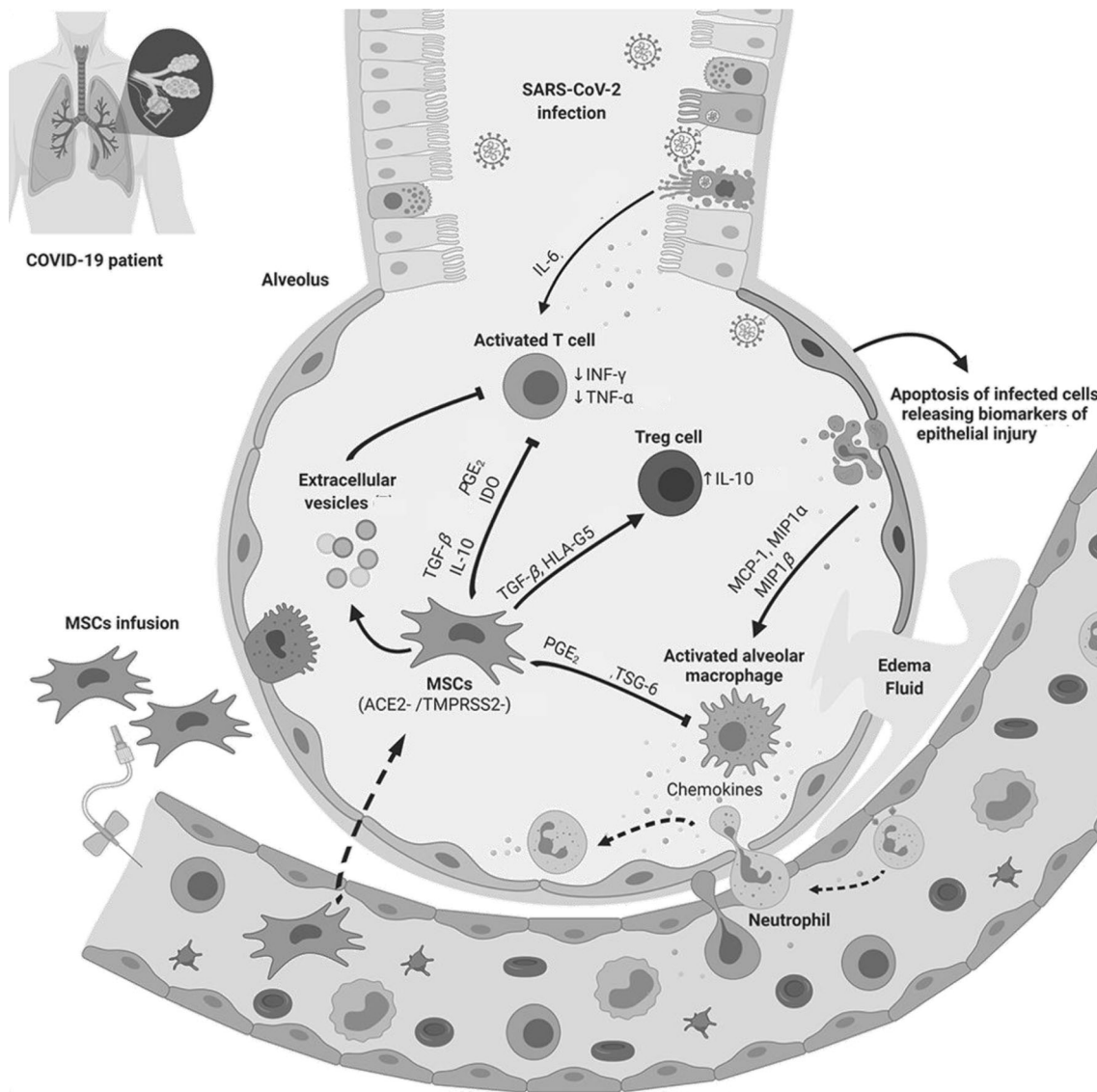


Fig. 2 MSC mechanism of action against COVID-19 [34]

Clinical management of COVID-19 patients are currently only to control the infection progress and acts as supportive care. Oxygen and ventilator are used for treating COVID-19 patients when they need it [40]. Antimicrobial drugs such as lopinavir-ritonavir, remdesivir, ribavirin, favipiravir, hydroxychloroquine, corticosteroids, and azithromycin are also used for COVID-19 patients [41–43].

Several studies have shown that bone marrow, lymph nodes, thymus, spleen, and immune cells are negative for ACE2 suggesting the use of immunoglobulin for treating the patients [5, 44]. Antibody-based therapies using immunoglobulin, IL-6[®] monoclonal antibody, and convalescent plasma have also been tested in several clinical trials [45, 46]. Although the International Society for Stem Cell Research (ISSCR) has not approved stem cell-based approaches, many researchers have introduced MSCs for treating severe COVID-19 patients [4, 5].

MSC Mechanism in COVID-19

Cell-based therapy using stem cells, especially MSCs, also known as mesenchymal stromal cells and medicinal signaling cells, has become a promising tool in treating COVID-19 patients [47, 48]. MSCs are a promising candidate to treat COVID-19 because of the overreaction of the immune response. Hypothetically, MSCs act as the cell manager that re-activates the immune system to prevent the cytokine storm and promotes the endogenous repair [49, 50].

Long before COVID-19, MSCs have been used to treat immune and inflammatory diseases based on their potency as an immunomodulator [51]. The immunomodulatory activities include the proliferation inhibition of immune cells such as T cells, B cells, dendritic cells, and NK cells. The production of IL-10 is inhibited when the monocyte polarized into the anti-inflammatory M2 macrophages, leading to decreasing

production of TNF- α and IL-12 [5, 52]. MSCs also secrete anti-inflammatory molecules that reduce inflammation during injury [53].

After intravenous injection, the MSCs will be trapped in the lung. The secretion of a variety of soluble mediators such as growth factors, antimicrobial peptides, and extracellular vesicles by the MSCs will restore the pulmonary microenvironment resulting in the restoration of alveolar-capillary barriers, protection of alveolar epithelial cells, and interception of pulmonary fibrosis to cure dysfunction of the lung and COVID-19 pneumonia [54–56].

Different anti-inflammatory mediators are released into the lung microenvironment through different activation receptors. Toll-like receptors (TLRs) are activated by viral unmethylated CpG-DNA (TLR9) and viral RNA (TLR3), leading to a subsequent cellular signaling pathway. Growth factors such as keratinocyte growth factor (KGF) and angiopoietin-1 (Ang-1) promote the restoration of disrupted alveolar-capillary barriers [21, 22, 54]. In addition, MSCs also produce LL37, an antibiotic protein that neutralizes the SARS-CoV-2 [57]. A variety of paracrine factors that are secreted by MSCs interact with the immune cells in order to improve the condition of COVID-19 patients

Some findings show that MSCs are resistant to viral infection compared to other differentiated cells. This is due to the presence of IFN-stimulated genes (ISG) which could block the virus from entering the cells. In addition, MSCs also express indoleamine 2,3-dioxygenase (IDO) that leads to the decrease of viral production [58]. Additionally, MSCs also promoted the regeneration of alveolar epithelial type II cells and prevented apoptosis through growth factors KGF, VEGF, and HGF [59].

The benefits of using MSCs in cell-based therapy are the easy way to obtain and free of ethical issues compared to embryonic stem cells [60]. In the MSC-based treatment of COVID-19 patients, MSCs were implicated to be free from SARS-CoV-2 infection because they minimally expressed ACE2 or TMPRSS2 receptors. This was proven by 10 \times RNA sequencing which shows that only 1 per 12,500 cells and 7 per 12,500 cells are expressed, respectively [22, 54]. Numerous clinical studies of MSC treatment for COVID-19 patients have begun worldwide and some publications have shown that MSCs are safe and have great potential for treating ARDS in COVID-19 patients. In one of the COVID-19 clinical trials, after the infusion of MSCs, the levels of C-reactive protein (CRP) in patients decreased while the number of peripheral lymphocytes increased [22, 61].

MSC-Derived Secretome for COVID-19

Other than orchestrating the cells to maintain and repair the injured tissue, MSCs also secrete multiple critical factors such

as hormones and cytokines for tissue regeneration called secretome. During *in vitro* culture, MSCs secrete a complex mixture of soluble protein including exosomes, extracellular vesicles (EVs), cytokines, chemokines, and growth factors. This complex mixture is called the conditioned medium (CM). The CM emerges as a promising tool in cell-free therapy. Secretome shows immunomodulatory, anti-inflammatory, pro-angiogenic, and anti-protease properties similar to MSCs. Trophic factors such as tumor growth factor (TGF)- β , hepatocyte growth factor (HGF), leukemia inhibitory factor (LIF), vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), brain-derived neurotrophic factor (BDNF), and nerve growth factor (NGF) are also secreted by MSCs [52, 62].

As an immunomodulator, the PGE2, TGF- β , and HGF play roles in inhibition of dendritic cell maturation. PGE2 induces the elevation of IL-10, an anti-inflammatory factor, which inhibits the activation of CD4⁺ T cells and upregulates the Treg population [63]. Other growth factors such as VEGF and TGF- β are important to promote angiogenesis by activating the PI3K/Akt and MAPK pathways. The HGF and KGF are used for alveolar epithelial cell protection from apoptosis. The IGF-1 and IL-6 also elevated the secreted frizzled-related protein 2 (SFRP2) as an anti-apoptotic mediator [64].

As reported in a previous study, intravenous injection of secretome is distributed to the lungs and remains stable in the body [65]. Moreover, using secretome has its advantages compared to MSCs themselves especially in COVID-19 emergency because it has already been prepared before and could be used as ready-to-use products. The MSC-derived secretome has an advantage in storage conditions such as stability and lower cost compared to MSCs [66]. The MSC-derived secretome can activate the endogenous stem cells and progenitor cells, regulate the inflammatory response, stimulate angiogenesis and remodeling of the extracellular matrix, suppress apoptosis, mediate chemoattraction, and reduce fibrosis [4]. The MSC-derived secretome approach also avoids the risk of teratoma formation [67].

MSC-Based Clinical Trials for COVID-19

The first clinical trial using MSCs for treating COVID-19 in China evaluates the efficacy and safety of MSCs. Clinical trials registered through <http://www.clinicaltrials.gov> showed different approaches in treating COVID-19. Until July 2020, using the terms “COVID-19” and “mesenchymal stem cells”, we found 42 clinical trials of MSCs for COVID-19, most of them are in the recruiting of participant phase and several clinical trials that have been FDA-approved are ongoing at present. Although most of the clinical trials were conducted in China, other countries such as Brazil, Mexico, Pakistan, Spain, Belarus, Jordan, Iran, Colombia, Germany, Ukraine,

Table 2 Summary of clinical trials registered at clinicaltrials.gov

Principal investigator, year	Clinical trial number	Title	Number of sample (patient)	Intervention	Dosage	Route of administration	Booster	Study location
Lei Shi & Fu-Sheng Wang, 2020	NCT04252118	Mesenchymal Stem Cell Treatment for Pneumonia Patients Infected With 2019 Novel Coronavirus	20 patients: - treated group - control group	MSC	3.0×10^7 cells	Intravenous	3 times: - Day 1 - Day 4 - Day 7	China
XingHuan Wang & ZhiYong Peng, 2020	NCT04269525	Umbilical Cord (UC)-Derived Mesenchymal Stem Cells (MSCs) Treatment for the 2019-novel Coronavirus (nCOV) Pneumonia	16 patients	UC-MSC	1.0×10^3 MSC in 150 mL	Intravenous	4 times: - Day 1 - Day 3 - Day 5 - Day 7	China
Yang Jin, 2020	NCT04273646	Study of Human Umbilical Cord Mesenchymal Stem Cells in the Treatment of Novel Coronavirus Severe Pneumonia	48 patients: - treated group - placebo group	UC-MSC	0.5×10^6 /kgBW in NaCl + 1% albumin	Intravenous	4 times: - Day 1 - Day 3 - Day 5 - Day 7	China
Jie-ming Qu, 2020	NCT04276987	A Pilot Clinical Study on Inhalation of Mesenchymal Stem Cells Exosomes Treating Severe Novel Coronavirus Pneumonia	30 patients	MSC-derived exosome	2.0×10^8 nano vesicles/3 ml	Aerosol inhalation	5 times: - Day 1 - Day 2 - Day 3 - Day 4 - Day 5	China
Fu-Sheng Wang, 2020	NCT04288102	Treatment With Human Umbilical Cord-derived Mesenchymal Stem Cells for Severe Corona Virus Disease 2019(COVID-19)	100 patients: - treated group - placebo group	UC-MSC	4.0×10^7 in NaCl containing 1% human serum albumin	Intravenous	3 times: - Day 1 - Day 4 - Day 7	China
Yang Jin, 2020	NCT04273646	Study of Human Umbilical Cord Mesenchymal Stem Cells in the Treatment of Novel Coronavirus Severe Pneumonia	48 patients: - treated group - placebo group	UC-MSC	0.5×10^6 UC-MSCs/kg-BW	Intravenous	4 times: - Day 1 - Day 3 - Day 5 - Day 7	China
Martin Iglesias & Carls A Aguilar-Salinas, 2020	NCT04416139	Mesenchymal Stem Cell for Acute Respiratory Distress Syndrome Due for COVID-19 (COVID-19)	10 patients: - treated group - control group	MSC	1.0×10^6 cells/kgBW	Intravenous	-	Mexico
Xanab Akram, 2020	NCT04444271	Mesenchymal Stem Cell Infusion for COVID-19 Infection	10 patients: - treated group - control group	MSC	2.0×10^6 cells/kgBW	Intravenous	2 times: - Day 1 - Day 7	Pakistan
Guillermo Sánchez-Vanegas & Carlos Escobar-Soto, 2020	NCT04429763	Safety and Efficacy of Mesenchymal Stem Cells in the Management of Severe COVID-19 Pneumonia (CELMA)	30 patients: - treated group - control group	MSC	1.0×10^6 cells/kgBW	Intravenous	-	-
Ana Cardesa Gil, 2020	NCT04366323	Clinical Trial to Assess the Safety and Efficacy of Intravenous Administration of Allogeneic Adult Mesenchymal Stem Cells of Expanded Adipose Tissue in Patients With Severe Pneumonia Due to COVID-19	26 patients	AD-MSC	8.0×10^7 cells	Intravenous	2 times: - Day 1 - Day 7	Spain
Jesus Perez, 2020	NCT04456361	Use of Mesenchymal Stem Cells in Acute Respiratory Distress	9 patients	UC-MSC	1.0×10^8 cells	Intravenous	-	Mexico

Table 2 (continued)

Principal investigator, year	Clinical trial number	Title	Number of sample (patient)	Intervention	Dosage	Route of administration	Booster	Study location
Florentino de Araujo Cardoso Filho & Luciana Ferrara, 2020	NCT04315987	Syndrome Caused by COVID-19 NestaCell® Mesenchymal Stem Cell to Treat Patients With Severe COVID-19 Pneumonia (HOPE)	90 patients: - treated group - control group	MSC - NestCell	2.0×10^7 cells	Intravenous	4 times: - Day 1 - Day 3 - Day 5 - Day 7	Brazil
Candy Eller, 2020	NCT04428801	Autologous Adipose-derived Stem Cells (AdMSCs) for COVID-19	200 patients: - treated group - control group	AD-MSC	2.0×10^8 cells	Intravenous	3 times: - Day 1 - Day 4 - Day 7	-
Adeeb M AlZoubi & Ahmad Y AlGhadi, 2020	NCT04313322	Treatment of COVID-19 Patients Using Wharton's Jelly-Mesenchymal Stem Cells	5 patients	WJ-MSC	1.0×10^6 cells/kgBW	Intravenous	3 times: - Day 1 - Day 2 - Day 3	Jordan
Ismail H Dilogo & Tri Kurniawati, 2020	NCT04457609	Administration of Allogenic UC-MSCs as Adjuvant Therapy for Critically-Ill COVID-19 Patients	40 patients: - treated group - control group	UC-MSC	1.0×10^6 cells/kgBW in 100 mL NaCl	Intravenous	-	Indonesia
Qingsong Ye & Chenliang Zhou, 2020	NCT04336254	Safety and Efficacy Study of Allogeneic Human Dental Pulp Mesenchymal Stem Cells to Treat Severe COVID-19 Patients	20 patients: - treated group - control group	DP-MSC	3.0×10^7 cells in NaCl	Intravenous	3 times: - Day 1 - Day 4 - Day 7	China
Thanh Cheng, 2020	NCT04349631	A Clinical Trial to Determine the Safety and Efficacy of Hope Biosciences Autologous Mesenchymal Stem Cell Therapy (HB-adMSCs) to Provide Protection Against COVID-19	5 patients	AD-MSC	-	Intravenous	-	USA
Yan Liu & Yue Zhu, 2020	NCT04339660	Clinical Research of Human Mesenchymal Stem Cells in the Treatment of COVID-19 Pneumonia	30 patients: - treated group - control group	UC-MSC	1.0×10^6 cells/kgBW in 100 mL NaCl	Intravenous	-	China
Shiyue Li & Ming Liu, 2020	NCT04346368	Bone Marrow-Derived Mesenchymal Stem Cell Treatment for Severe Patients With Coronavirus Disease 2019 (COVID-19)	20 patients: - treated group - control group	BM-MSC	1.0×10^6 cells/kgBW	Intravenous	-	China
Thanh Cheng & Joseph Varon, 2020	NCT04348435	A Randomized, Double-Blind, Placebo-Controlled Clinical Trial to Determine the Safety and Efficacy of Hope Biosciences Allogeneic Mesenchymal Stem Cell Therapy (HB-adMSCs) to Provide Protection Against COVID-19	100 patients: - treated 1 group - treated 2 group - treated 3 group - placebo group	AD-MSC	- 0.5×10^8 cells - 1.0×10^8 cells - 2.0×10^8 cells	Intravenous	5 times: - Week 0 - Week 2 - Week 6 - Week 10 - Week 14	USA
Ryan Welter	NCT04352803	Adipose Mesenchymal Cells for Abatement of SARS-CoV-2 Respiratory Compromise in COVID-19 Disease	20 patients: - treated group - control group	AD-MSC	5.0×10^6 cell/kgBW	Intravenous	-	-

Table 2 (continued)

Principal investigator, year	Clinical trial number	Title	Number of sample (patient)	Intervention	Dosage	Route of administration	Booster	Study location
Camillo Ricordi, 2020	NCT04355728	Use of UC-MSCs for COVID-19 Patients	24 patients: treated group - placebo group	UC-MSC	1.0×10^8 cell	Intravenous	2 times: - Day 1 - Day 4	USA
Masoumeh Nouri & Hoda Madani, 2020	NCT04366063	Mesenchymal Stem Cell Therapy for SARS-CoV-2-related Acute Respiratory Distress Syndrome	60 patients: - treated 1 group - treated 2 group - control group	- MSC - MSC & EV	1.0×10^8 cell	Intravenous	MSC - 2 time-s: - Day 1 - Day 3 EV - 2 time-s: - Day 5 - Day 7	Iran
Vincent Liao, 2020	NCT04452097	Use of hUC-MSC Product (BX-U001) for the Treatment of COVID-19 With ARDS	9 patients: - treated 1 group - treated 2 group - treated 3 group	UC-MSC	- 0.5×10^6 cells/kgBW - 1.0×10^6 cells/kgBW - 1.5×10^6 cells/kgBW	Intravenous	-	-
Jianming Tan Tan, 2020	NCT04371601	Safety and Effectiveness of Mesenchymal Stem Cells in the Treatment of Pneumonia of Coronavirus Disease 2019	60 patients: - treated group - control group	UC-MSC	1.0×10^6 /kgBW	Intravenous	4 times: - Day 1 - Day 4 - Day 8 - Day 12	China
Alfredo Hernandez-Ruiz & Santiago Saldarriaga-Gomez, 2020	NCT04390152	Safety and Efficacy of Intravenous Wharton's Jelly Derived Mesenchymal Stem Cells in Acute Respiratory Distress Syndrome Due to COVID-19	40 patients: - treated group - control group	WJ-MSC	5.0×10^7 cells	Intravenous	2 times	Colombia
Annetine C Gelijns, 2020	NCT04371393	MSCs in COVID-19 ARDS	300 patients: - treated group - placebo group	MSC - Remestemcell-L	2.0×10^6 cells/kgBW	Intravenous	2 times: - Day 1 - Day 5	USA
Katherine Ruppert & Sherry Diers, 2020	NCT04362189	Efficacy and Safety Study of Allogeneic HB-adMSCs for the Treatment of COVID-19	100 patients: - treated group - placebo group	AD-MSC	1.0×10^8 cell	Intravenous	4 times: - Day 1 - Day 4 - Day 8 - Day 11	USA
Lennie Sender, 2020	NCT04397796	Study of the Safety of Therapeutic Tx With Immunomodulatory MSC in Adults With COVID-19 Infection Requiring Mechanical Ventilation	45 patients: - treated group - placebo group	BM-MSC	-	Intravenous	-	-
Peter Rosenberger, 2020	NCT04377334	Mesenchymal Stem Cells (MSCs) in Inflammation-Resolution Programs of Coronavirus Disease 2019 (COVID-19) Induced Acute Respiratory Distress Syndrome (ARDS)	40 patients: - treated group - control group	BM-MSC	-	Intravenous	-	Germany

Table 2 (continued)

Principal investigator, year	Clinical trial number	Title	Number of sample (patient)	Intervention	Dosage	Route of administration	Booster	Study location
Peter Nemtinov, 2020	NCT04461925	Treatment of Coronavirus COVID-19 Pneumonia (Pathogen SARS-CoV-2) With Cryopreserved Allogeneic P ₀ MMSCs and UC-MMSCs	30 patients: - treated group - control group	UC-MSc	$1.0 \times 10^6/\text{kgBW}$	Intravenous	3 times: - Day 1 - Day 4 - Day 7	Ukraine
Liwei cheng, 2020	NCT04302519	Novel Coronavirus Induced Severe Pneumonia Treated by Dental Pulp Mesenchymal Stem Cells	24 patients	DP-MSc	$1.0 \times 10^6/\text{kgBW}$	Intravenous	3 times: - Day 1 - Day 3 - Day 7	China
Ruth Coll & Joaquin Delgado, 2020	NCT04390139	Efficacy and Safety Evaluation of Mesenchymal Stem Cells for the Treatment of Patients With Respiratory Distress Due to COVID-19 (COVIDMES)	30 patients: - treated group - placebo group	WJ-MSc	$1.0 \times 10^6/\text{kgBW}$	Intravenous	2 times: - Day 1 - Day 3	Spain
Gokhan T Adas & Erdal Karaoz, 2020	NCT04392778	Clinical Use of Stem Cells for the Treatment of COVID-19	30 patients: - treated group - placebo group - control group	UC-MSc	$3.0 \times 10^6/\text{kgBW}$	Intravenous	3 times: - Day 1 - Day 4 - Day 7	Turkey
Barbara Juna & Mireia Arcas, 2020	NCT04348461	BattLe Against COVID-19 Using Mesenchymal Stromal Cells	100 patients: - treated group - control group	AD-MSc	$1.5 \times 10^6/\text{kgBW}$	-	2 times	-
Oscar Simonsson & Karl-Henrik Grinnemo, 2020	NCT04447833	Mesenchymal Stromal Cell Therapy For The Treatment Of Acute Respiratory Distress Syndrome (ARDS-MSc-205)	9 patients: - treated 1 group - treated 2 group	BM-MSc	- $1.0 \times 10^6/\text{kgBW}$ - $2.0 \times 10^6/\text{kgBW}$	-	-	Sweden
Danny F McAuley & Cecilia O’Kane, 2020	NCT03042143	Repair of Acute Respiratory Distress Syndrome by Stromal Cell Administration (REALIST) (COVID-19) (REALIST)	75 patients: - treated group - placebo group	UC-MSc	-	-	-	UK
David Ingbar, 2020	NCT04466098	Multiple Dosing of Mesenchymal Stromal Cells in Patients With ARDS (COVID-19)	30 patients: - treated group - placebo group	MSc	3.0×10^8 in DMSO + dextran 40 + 5% human serum albumin	-	3 times: - Day 1 - Day 3 - Day 5	USA
Brian Miller, 2020	NCT04445220	A Study of Cell Therapy in COVID-19 Subjects With Acute Kidney Injury Who Are Receiving Renal Replacement Therapy	24 patients: - treated 1 group - treated 2 group - placebo group	MSc	- 2.5×10^8 cells + SHAM device - 7.5×10^8 cells + SHAM device - SHAM device	-	-	-

AD-MSc adipose-derived mesenchymal stem cell, BM-MSc bone marrow–derived mesenchymal stem cell, DP-MSc dental pulp–derived mesenchymal stem cell, UC-MSc umbilical cord–derived mesenchymal stem cell, WJ-MSc Wharton jelly–derived mesenchymal stem cell

Turkey, Sweden, the USA, the UK, Denmark, and Indonesia are also executing the clinical trials (Table 2).

The study involves the different sources of MSCs, routes of administration, and also different approaches using cells or their secreted products. Based on the information of cell number use for clinical trial, the range of injected cells is between 0.5×10^6 and 1×10^7 cells/kg. Some studies proposed a single injection and others mention boosted therapy with an interval of 2–5 times. Intravenous, intratracheal, intraperitoneal, and intranasal injection methods are used for the route of administration of the MSCs or MSC-derived secretome. The most common source of MSCs that are used in the study is the allogeneic umbilical cord (UC)/Wharton's jelly because of its non-invasive procedure to obtain and indicated more effective than other sources.

Similar to other clinical trials, a MSC study for COVID-19 involves a control group and a standard treatment for the patient. Other models also used a placebo, which means the standard treatment combination with normal saline as the intervention. The present data reveal that during short-term therapy, MSCs succeed in managing severe and critically severe COVID-19 patient condition and were reported to be safe and has shown efficacy. A study conducted by Leng et al. reported that 7 enrolled patients show positive outcomes 14 days after being injected with MSCs. Positive outcomes are shown by increasing oxygen saturation up to 95% at rest. Analysis of immune cells revealed that there is an increment of Tregs and dendritic cells with the disappearance of T and NK cells. Comparison between the control group and the MSC-treated group displayed that peripheral lymphocytes and levels of IL-10, IP-10, and VEGF rise while C-reactive protein and TNF- α decreased. A case report on a 65-year-old woman by Liang et al. showed no adverse event and patient improvement 4 days post-injection of 5.0×10^7 cells/administration UC-MSCs. However, in the long-term evaluation, a comparison between doses and routes of administration is needed to provide the best outcome for the patient [21, 68]. It is also important that the MSCs and MSC-derived secretome are produced in a good manufacturing practices (GMP) compliance facility to ensure the quality of the cell and eliminate the batch-to-batch variation [34].

Conclusion

MSCs and MSC-derived secretome displayed to be promising treatment candidates for COVID-19. Preliminary data from current clinical trials report that MSCs are safe and have efficacy. Nevertheless, much bigger data are needed for understanding the mechanism of MSCs and MSC-derived secretome for treating COVID-19 patients.

Declarations

Conflict of Interest The authors declare no competing interests.

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