Biomedical Research and Therapy 2022, 9(5):5075-5083

Open Access Full Text Article

Review

Post-COVID-19 pulmonary fibrosis: the rationale of mesenchymal stem cell transplantation for lung regeneration

Nhu Ngoc-Quynh Dinh[1,2], Ngoc Bich Vu[2,3], Phuc Van Pham[2,3,4,*]

ABSTRACT

Coronavirus disease 2019 (COVID-19) has caused nearly 15 million deaths worldwide. The rapid development of COVID-19 vaccines and anti-viral drugs significantly decreased the level of mortality related to COVID-19. However, post-COVID-19 pulmonary fibrosis has become a severe problem for some COVID-19 patients. The previous articles present the results of mesenchymal stem cell (MSC) transplantation to treat COVID-19 patients; in this article, we would like to discuss the potential of MSC transplantation to treat and improve post-COVID-19 pulmonary fibrosis. MSCs exhibit immune modulation and anti-inflammation that can control the inflammation caused by coronavirus 2 infection and the cytokine storm that some patients experience during COVID-19. The anti-fibrotic qualities of MSCs have also been demonstrated both *in vitro* and *in vivo*. Based on the current information about the anti-fibrotic effects of MSCs, MSC transplantation can be used to improve post-COVID-19 pulmonary fibrosis.

Key words: Anti-fibrosis, COVID-19, Mesenchymal stem cell, MSC, Post-COVID-19

[1]*EMCAS Hospital, District 10, Ho Chi Minh City, Viet Nam*

[2]*Stem Cell Institute, University of Science Ho Chi Minh City, Viet Nam*

[3]*Vietnam National University Ho Chi Minh City, Viet Nam*

[4]*Laboratory of Stem Cell Research and Application, University of Science Ho Chi Minh City, Viet Nam*

Correspondence

Phuc Van Pham, Stem Cell Institute, University of Science Ho Chi Minh City, Viet Nam

Vietnam National University Ho Chi Minh City, Viet Nam

Laboratory of Stem Cell Research and Application, University of Science Ho Chi Minh City, Viet Nam

Email: phucpham@sci.edu.vn

History

- Received: April 20, 2022
- Accepted: May 15, 2022
- Published: May 31, 2022

DOI : 10.15419/bmrat.v9i5.741

Check for updates

INTRODUCTION

The coronavirus disease 2019 (COVID-19) pandemic is the fifth documented pandemic since the 1918 flu pandemic. The first reported case of COVID-19 was recorded in Wuhan, China, before the virus spread worldwide. Since 2019, there have been nearly 15 million deaths due to COVID-19. Vaccines and anti-viral drugs significantly decreased mortality related to COVID-19 around the world. However, the rate of COVID-19 infections has continuously increased in most countries, even after vaccination. Some reports have shown that although the mortality was reduced, the rates of post-COVID-19 syndrome and post-COVID-19 pulmonary fibrosis (PF) were not significantly reduced.

Post-COVID-19 PF has mainly been observed in patients with comorbidities such as diabetes, hypertension, or coronary disease[1]. In 2020, Wu *et al.* showed that up to 40% of patients who had recovered from COVID-19 may develop ARDS; 20% of them may develop PF[2]. In some other reports, PF is estimated to affect about 1/3 of the patients hospitalized with SARS-CoV-2[3,4].

Therefore, post-COVID-19 PF poses a serious threat to patients who have recovered from COVID-19. Many of the potential treatments reported in the literature can improve post-COVID-19 PF; however, there is no fully proven treatment for this condition. This review aims to comprehensively review PF in post-COVID-19 patient, some current treatments for PF, and some early results using mesenchymal stem cells (MSCs) for post-COVID-19 PF treatment.

SARS-COV-2 AND COVID-19 PATHOPHYSIOLOGY

Virus SARS-CoV-2 profile and viral life cycle

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which is responsible for the COVID-19 pandemic, belongs to the family *Coronaviridae*. The Coronaviridae family has 2 subfamilies, Coronavirinae and Torovirinae. Coronavirinae includes seven coronavirus-caused respiratory diseases in human, which are divided into two groups: normal coronaviruses (HCoV-229E, HCoV-NL63, HCoV-HKU1, HCoV-OC43) and novel coronaviruses (MERS-CoV, SARS-CoV and SARS-CoV-2)[5]. The normal coronaviruses usually causes acute mild upper tract respiratory diseases (except NL63), while the novel coronaviruses can lead to pneumonia and severe acute respiratory syndrome (SARS). Among the three novel coronaviruses, SARS-CoV-2 has had the lowest case fatality rate (CFR)—under 5% — but the highest level of transmissibility.

Coronaviruses (CoVs) have large single-stranded positive-sense RNA genomes—around 30 kilobases, containing a 5'-cap and 3' poly (A) tail structure[6]. CoVs have a lipid bilayer membrane and include four main structural proteins: nucleocapsid (N) protein,

BioMedPress The Open Access Publisher

Cite this article : Dinh N N, Vu N B, Pham P V. **Post-COVID-19 pulmonary fibrosis: the rationale of mesenchymal stem cell transplantation for lung regeneration**. *Biomed. Res. Ther.* 2022; 9(5):5075-5083.

membrane (M) protein, envelope (E) protein and, most importantly, spike (S) protein [6,7]. S protein plays a pivotal role in the initial attachment and penetration of the virus to the host cell by binding to the angiotensin-converting enzyme 2 receptor (ACE-2). The spike protein is composed of two subunits, S1 and S2. S1 mediates the binding of the virus to the host cell receptor, while S2 is responsible for the fusion of virion and cell membrane [7]. Inside the host cell, the virus replicates and performs exocytosis, after which it is ready to invade adjacent cells.

COVID-19 pathophysiology

ACE-2 has been determined to be a main functional receptor for SARS-CoV, HCoV-NL63, and SARS-CoV-2. ACE-2 has primarily been expressed in type II pulmonary epithelial cells, so the respiratory system is the easiest method for viruses to infect patients. However, ACE-2 has also been observed in nasal goblet cells, gastrointestinal epithelial cells, pancreatic β cells, and renal podocytes [6,8], leading to digestive issues, myocarditis, kidney diseases and, ultimately, multiple organ dysfunction syndromes. Compared to SARS-CoV, SARS-CoV-2 has a 10–20 times higher affinity for ACE-2 receptors [9], which is why there have been more COVID-19-related myocarditis cases (even among those who are young and have no comorbidities) than in the 2002-2004 SARS outbreak in Hong Kong.

The renin-angiotensin system (RAS) plays an essential role in regulating blood pressure and homeostasis. In the lungs, angiotensin I (Ang I) metabolizes to angiotensin II (Ang II) by the ACE. Ang II acts through angiotensin type I and type II receptors (AT1R and AT2R), leading to the constriction of vascular, water, and sodium retention, stimulation of chronic inflammation, fibrosis, and lung damage. Conversely, the ACE-2 converts Ang II into Ang 1-7, which exerts vasodilation through binding Mas receptor, thus inhibiting inflammation and preventing pulmonary diseases and edema [10]. Therefore, the ACE2/Ang1-7/Mas axis is antagonized to the ACE/AngII/AT1R pathway (classic RAS). When SARS-CoV-2 binds to cells with a high affinity, the bond between the virus and ACE-2 is formed, leading to the down regulation of the ACE-2 axis. This imbalance is partly responsible for the acute and chronic inflammation (due to overactivity of RAS), resulting in medium to severe COVID-19 symptoms and long-term complications.

Many researchers have concluded that there is a correlation between the increasing level of pro-inflammatory cytokines and the outcome of COVID-19 patients; cytokine storm syndrome is the main factor that causes severe and fatal complications in patients [11–14]. Cytokines are small protein molecules secreted through the interaction between cells, including pro-inflammatory and anti-inflammatory cytokines [15]. The virus invades the nasal epithelial cell, replicates, and immigrates to the upper and lower respiratory cells, then to lymphocytes. With COVID-19, cytokines are produced not only through PAMPs (pathogen-associated molecular pattern molecules) and DAMPs (damage-associated molecular pattern molecules) pathways but also during the crosstalk between epithelial cells and immune cells via the trigger of macrophages. In mild to moderate cases of COVID-19, interferon-1 (IFN-1, which includes IFN-α and IFN-ß) is efficiently and productively secreted, functioning as a lung protector. Otherwise, the inflammation is regulated by the balancing between pro-inflammatory (TNF-α, IL-1, IL-6) and anti-inflammatory (IL-10, IL-1) cytokines. This facilitates quicker and more effective virus clearance without any serious complications.

On the other hand, in patients with severe to critical disease, there is not only impaired INF-1 production and activity accelerating DAMPs and PAMPs pathways but also exacerbated inflammation, with an excess of pro-inflammatory cytokines (TNF-α, IL-6), leading to the cytokines auto-amplification and cytokine storm, resulting in ARDS, multi-organ failure or even death [12,14]. Moreover, the imbalance of the ACE and ACE-2 axis that leads to the ACE/AngII/AT1R over-activity will exaggerate the releasing soluble TNF-α, HB-EGF, and IL-6Rα to adjacent cells and activate NK-kB and STAT3 to produce more IL-6 [8]. Serum IL-6 and TNF-α levels can be used to reliably and independently predict poorer prognoses [16]. Therefore, ACE/AngII blockers and anti-IL-6 have recently been considered viable medication for treating disease of medium to critical severity.

Immune response to SARS-CoV-2 and COVID-19

Besides local immune activity (innate cell immunopathology and plasma cytokines signature), understanding the specific immune response is a vital part of controlling the virus invasion, with three major components: antibodies (produced by B cells), $CD4^+$ T cells, and $CD8^+$ T cells. The adaptive immune response works slower than innate immunity; it takes time to proliferate and differentiate naive cells into effector cells. When the

virions, ingested by antigen-presenting cells (APC), are recognized by $CD4^+$ T cells, the T helper cells activate both cytotoxic T cells to destroy infected cells and virus, and B cells produce antibodies. If the adaptive immune response works fast and effectively, eliminating the circulating virions, the disease can be controlled; patients usually have no symptoms. Conversely, T cells and antibodies may not be activated soon enough, in which case the local immune activity can lead to over-activity in cytokine production. In the case of SARS-CoV-2 and COVID-19, the virus can avoid or delay innate immunity related to INF-1 efficiently, leading to the overproduction of pro-inflammatory cytokines[17–20].

POST-COVID -19 SYNDROME/COVID-19 LONG-TERM COMPLICATIONS AND COVID-19 PULMONARY FIBROSIS

Although the accurate pathological mechanism of long-term COVID-19 complications is still a matter of controversy, several mechanisms have been reported to be associated with the following disease sequelae: (1) direct viral tissue damages due to the diverse locations of ACE-2 expression in epithelial cells leads to multiorgan injuries; (2) the high expression of ACE-2 in endothelial cells affects the complexity of blood vessels, which may lead to coagulation and thrombosis; (3) the consequences of cytokine storm syndrome; (4) the imbalance of ACE axis and ACE-2 axis, leading to vascular constriction, lung fibrosis, and chronic inflammation; (5) immune system dysregulation (has been proven by finding auto-reactive T cells in patients with fatal cases of COVID-19, similar to autoimmune disease patients)[8,14,21,22].

Recent research has shown that COVID-19 patients with prolonged symptoms can have SARS-CoV-2 detection blood tests that are either (1) PCR-negative or (2) low-level trace PCR-positive. Patients with trace PCR-positive results may experience long-lasting symptoms, even over 3 months after the initial infection. Remarkably, in long-lasting symptomatic patients who are trace PCR-positive, SARS-CoV-2-specific $CD8^+$ T cells increase in breadth and magnitude, which may be related to the mechanism by which long-term complications occur[19,22–24].

Many systems are affected by long-term COVID-19, including: respiratory (dyspnea, pulmonary fibrosis, lung function abnormalities, ventilator/oxygen dependence, *etc.*), cardiological (chest pain, arrhythmia, ventricular systolic dysfunction, heart failure, *etc.*), hematological (thrombosis, hypoxia, *etc.*), neurological (fatigue, myalgia, headache, migraine, insomnia, *etc.*), dermatological (urticaria, alopecia, rash, *etc.*), urological (acute kidney injury, renal dysfunctional, *etc.*), digestive (nausea, vomiting, poor appetite, *etc.*) and so on[8]. In this review, we focused on post-COVID-19 lung fibrosis.

Pulmonary fibrosis is also called interstitial lung disease or diffuse parenchymal lung disease. The term "post-COVID19 fibrosis" includes any "fibrotic-like" conditions (reversible diseases) and fibrosis (irreversible diseases) with specific CT imaging features[25]. The high level of transforming growth factor-beta 1 (TGF-β1) is considered to be the main factor for organizing pneumonia (based on recent knowledge of past influenza and SARS pandemics). The mechanism is the production of profibrotic agents such as TGF-β1, PDGF, VEGF, EGF, *etc.*, which leads to the activation of fibroblasts (which stay resident and recruit from the blood) and subsequent differentiation into myofibroblasts, stimulating collagen synthesis[26]. The fibroblasts and myofibroblasts will organize into fibrotic foci. The growth factors targeting tyrosine kinase pathways are released continuously to promote fibrotic foci formation and evolution in a fibrosed lung. Collagen type 3 is a predominant form of collagen during the early stage, while collagen type 1 is revealed during the late stage.

In SARS-CoV-2 infection, the exact mechanism of fibrosis is still unknown; however, it is believed to be multifactorial, primarily a result of the release of cytokines and abnormal coagulopathy (which is the difference between COVID-19-induced ARDS and classic ARDS), as well as the down regulation of the ACE-2 axis (as described above). In addition to these are two iatrogenic factors, namely, oxygenic toxicity and ventilator-induced lung injury. Patients exposed to high doses of oxygen experience enhanced levels of oxygen-derived free radicals, which damage the epithelial cells. Patients with severe disease who are supported by prolonged mechanical ventilation experience extended high pressure inside their stiff lungs; this physical stress also contributes to pulmonary fibrosis[26].

The clinical symptoms of organizing pulmonary fibrosis differ from patient to patient; however, all are very similar to those common of respiratory diseases, including prolonged dry cough, shortness of breath, fatigue, decreased exercise tolerance, or even a lack of symptoms. Risk factors such as age, comorbidities (hypertension, diabetes, cardiovascular disease, cancer, pulmonary tuberculosis, *etc.*), prolonged ICU stay and ventilation period, smoking, and a history of

alcohol abuse play an important role in the progression of severe COVID-19 [27]. Specific paraclinical test features consist of the reduction of lung diffusion capacity of carbon monoxide (DLCO) and the formations associated with lung scarring, which are identified via CT scan, such as traction dilation of bronchi, honeycombing, pleural thickening or bands in the focal area [28]. According to Wu *et al.*, 30% of COVID-19 patients suffer from at least some lung complication at 9 months after hospitalization; a third of them develop fibrotic-like damages without any reduction trends [29]. Another study by Wu *et al.* shows a similar result: 40% of COVID-19 patients develop ARDS and 20% of ARDS cases are severe, with imaging results showing diffuse alveolar damage; about 10% of recovered patients experience lung fibrosis [30]. Long-term research carried out on SARS patients for 15 years has shown that the lesions due to lung damage can be reduced within 12 months after recovery, without any evolution or changes observed 14 years later [31]; post-COVID-19 lesions due to lung damage are now considered to progress in a similar way.

CURRENT STATUS OF POST-COVID-19 PULMONARY FIBROSIS TREATMENT

Currently, there are no fully proven treatment methods for COVID-induced pulmonary fibrosis. Different approaches are being researched, with a focus on anti-inflammatory and anti-fibrotic drugs. Nintedanib and pirfenidone—oral therapeutics commonly used to treat idiopathic pulmonary fibrosis (IPF)—are the most commonly investigated anti-fibrotic medicines. In IPF patients, nintedanib and pirfenidone have been shown to inhibit the reduction of lung function and increase life expectancy [32]. Nintedanib (6-methoxycarbonyl-substituted indolinone) prevents pro-fibrotic signaling pathways through mediators (TGF-β1, PDGF, VEGF, EGF). Pirfenidone (5-methyl-1-phenyl-2-(1H)-pyridone) works via multiple mechanisms by regulating pro-fibrotic and pro-inflammatory agents, inhibiting the activation of collagen synthesis and fibroblast differentiation through TGF-β1 and playing an antioxidant role. Despite their potential, the side effects and drug interactions must be taken into account. Several common reactions, such as diarrhea, fatigue, and poor appetite, are similar to COVID-19 symptoms, which can affect physicians' evaluations of patients. Nintedanib and pirfenidone can also cause damage to the liver, which can be seriously affected by COVID-19 itself [28]. When using anticoagulants, it is necessary to delay Nintedanib, which can increase the risk of bleeding [33].

Besides, several new medicines have been the subject of ongoing clinical trials around the world since 2020. These include two multicenter, randomized, placebo-controlled research projects with Treamid (NCT04527354), Bov Hyaluronidase azoximer (NCT04645368), and LYT-100 (NCT04652518), one multicenter, randomized trial compared to standard therapy with Anluohuaxian (NCT0433426) and many single-center, randomized, placebo-controlled trials with Collagen Polyvinylpyrrolidone, Prednisone, Genistein, Tetrandrine, *etc*. In addition, cell therapy—especially using mesenchymal stem cells—has been reported as a potential treatment method.

MESENCHYMAL STEM CELLS AND THEIR BIOEFFECTS TARGETING PULMONARY FIBROSIS

Preclinical trials and clinical trials for pulmonary fibrosis

To date, there have been more than 20 reports on the use of MSCs for PF in animal models. Most of the studies used MSCs from bone marrow or adipose tissues. Other kinds of MSCs used to treat PF in animal models include umbilical cord MSCs, amniotic membrane MSCs, human embryonic MSCs, and human menstrual blood–derived MSCs. In a 2021 review by Li *et al.* [34], 12 animal studies showed that animals treated with MSCs had significantly improved survival rates compared to the control group ($p < 0.001$) [34]. Moreover, in this meta-analysis review, Li *et al.* (2021) showed that MSC transplantation clearly reduced pulmonary fibrosis scores compared to the control group ($p < 0.01$) [34].

In a 2013 study, Tzouvelekis *et al.* used adipose-derived stromal vascular fraction (SVF) to treat 14 PF patients [35]. In this study, the researchers used a low dose of SVF (0.5 million cells per kg of body weight per infusion). Although no cases of serious adverse events were reported, treatment efficacy was not significantly improved in patients who received transplants [35].

Averyanov *et al.* (2019) used a high dose of MSCs to treat PF in 20 human patients. This clinical trial showed that the cumulative dose of 2 billion cells is safe and tolerable in PF patients. After 52 weeks of follow-up, the authors confirmed that MSC transplantation with a cumulative high dose is effective for treating rapidly progressing PF [36].

A study by Campo *et al.* (2021) transplanted autologous bone marrow MSCs (BM-MSCs) for 13 patients[37]. The transplantation of BM-MSCs was determined to be safe, and the mean forced vital capacity declined by 8.1% at 3 months[37].

Mechanisms of MSCs for pulmonary fibrosis

Effects of MSCs on COVID-19 treatment, including (1) anti-viral effects, (2) immune modulation, and (3) lung regeration, have been comprehensively discussed in some previous publications[38,39]. Unlike COVID-19 treatment, post-COVID-19 treatment can be effective if the fibrosis process is reversed. Ideally, the treatment for fibrosis should achieve three steps of fibrotic tissue regeneration, namely, the inhibition or reduction in fibrosis progression (via inhibiting inflammation, reducing myofibroblast activity, and preventing further collagen synthesis), the degradation of the accumulated/existing collagen, and the triggering of lung regeneration (**Figure 1**). MSCs can target three therapeutic mechanisms of fibrotic lung regeneration.

MSCs can display immune modulation that significantly reduces inflammation by suppressing T lymphocytes, B lymphocytes, dendritic cells, and NK cells and stimulating regulatory T cells. MSCs also secrete an array of anti-inflammatory cytokines (reviewed in Pham and Vu, 2020[40]). In a model of radiation-induced pulmonary fibrosis in animals, Dong *et al.* (2015) showed that MSCs can reduce fibrosis in PF in animals by stimulating the endogenous secretion of HGF and PGE2[41]. MSCs also secrete HGF that inhibits epithelial-to-mesenchymal cell transition and promotes myofibroblast apoptosis[42]. The role of MSC-derived HGF in protection from bleomycin-induced PF was confirmed by Cahill *et al.* (2016). Cahill *et al.* (2016) compared the effects of MSCs and HGF-knockdown MSCs on bleomycin-induced PF and showed that HGF knockdown MSCs could not protect against fibrosis *in vivo*[43].

The degradation of existing cumulated collagen in fibrotic lungs is an essential step in fibrotic lung regeneration. Transplantation of MSCs can activate the host's macrophages, especially M2 macrophage, to synthesize the MMP-9 degrading the collagen[44]. Indeed, in animal models of PF, UCMSC transplantation reduced collagen deposition (determined by Sirius red staining)[44]. Indeed, overexpression of MMP9 in macrophages can attenuate bleomycin-induced PF[45]. *In vitro*, Khan *et al.* (2017) also showed that MSCs could decrease Col1A1 deposition by increasing the ratio of MMP-1 to TIMP-1[46].

The last step of anti-fibrosis in PF is tissue regeneration. Previous studies have confirmed that MSCs could produce and secrete some growth factors that can activate the local stem cells for tissue homeostasis[47,48]. Chu *et al.* (2020) determined the indirect way that MSC transplantation triggers tissue regeneration in PF through TLR-4[44]. Transplanted MSCs will promote the expression of TLR-4 in the host's alveolar epithelial cells. TLR-4 promotes alveolar progenitor cell renewal and prevents severe pulmonary fibrosis[49].

Xiao *et al.* (2020) reported that MSC-derived exosomes could reverse the progression of LPS-induced lung injury and fibrosis[50]. Exosomes from MSCs transmitted miR-23a-3p and miR-182-5p to the MLE-12 cells and inhibited the NF-kB and Hedgehog pathways by silencing lkbkb and destabilizing IKKbeta[50]. Zhang *et al.* (2021) also demonstrated that exosomes from BMMSCs can reverse the epithelial-mesenchymal transition to alleviate silica-induced pulmonary fibrosis[51]. Indeed, exosomes from BMMSCs caused an increase in epithelial markers (including E-cadherin and cytokeratin 19) and reduced the expression of fibrosis marker proteins (including alpha-SMA). The authors proposed that a mechanism for the anti-fibrotic effects of exosomes could be the attenuation of the Wnt/beta-catenin signaling pathway[51].

AN UPDATE ON USAGE OF MESENCHYMAL STEM CELLS FOR POST-COVID-19

Rahulan *et al.* (2022) recently used MSC transplantation to treat five patients with post-COVID ARDS on ECMO who were referred for lung transplantation[52]. All patients received 5 million cells per kilogram infused over 30 minutes for 3 doses on days 0, 3, and 6. The results showed that all patients tolerated MSCs well without experiencing any observable side effects. Three patients recovered from post-COVID-19 and weaned off ECMO successfully; 1 patient did not improve and expired; 1 patient did not recover and underwent lung transplantation[52].

On the clinicaltrials.gov website, there are three active clinical trials using mesenchymal stem cells for post-COVID-19 treatment and one active clinical trial using extracellular vesicles from MSCs for post-COVID-19 treatment (**Table 1**). Two clinical trials use allogeneic expanded ADSCs; the clinical trial uses autologous ADSCs. It seems that while umbilical cord-derived MSCs (UCMSCs) are usually used to treat COVID-19 because of the strong immune modulation of UCMSCs, adipose tissue-derived MSCs are

Table 1: Some clinical trials using MSCs for post-COVID-19 treatment

ClinicalTrials.gov Identifier	Title	Phase/Patients	Kind of cells	Methods	Country
NCT04909892	Study of Allogeneic Adipose-Derived Mesenchymal Stem Cells to Treat Post COVID-19 "Long Haul" Pulmonary Compromise	Phase II (60 patients)	Allogeneic culture-expanded adipose-derived mesenchymal stem cells	MSCs will be administered intravenously on Day 0, Day 2, and Day 4 with 2 groups of 2 doses: one vial, ~18.5 million cells per infusion and ~37 million cells per infusion	USA
NCT05126563	Randomized Double-Blind Phase 2 Study of Allogeneic HB-adMSCs for the Treatment of Chronic Post-COVID-19 Syndrome (HBPCOVID02)	Phase II (80 patients)	Allogeneic culture-expanded adipose-derived mesenchymal stem cells	Dose: 200 million Route: Intravenous Regimen: Weeks 0, 2, 6, and 10.	USA
NCT04798066	HBPCOV01:"Intermediate Size Expanded Access Protocol for the Treatment of Post-COVID-19 Syndrome"	Phase I (5 patients)	Autologous adipose derived mesenchymal stem cells	Dose: 200 million HB-adMSCs Route: intravenous infusion only, Regimen: a treatment duration of 14 weeks	USA
NCT05116761	ExoFlo^{TM} Infusion for Post-Acute COVID-19 and Chronic Post-COVID-19 Syndrome	Phase I/II (60 patients)	Bone Marrow Mesenchymal Stem Cell Derived Extracellular Vesicles	Dose: Normal saline 85 mL and ExoFlo 15 mL, which is 10.5 x 10^8 EV Route: Intravenous	USA

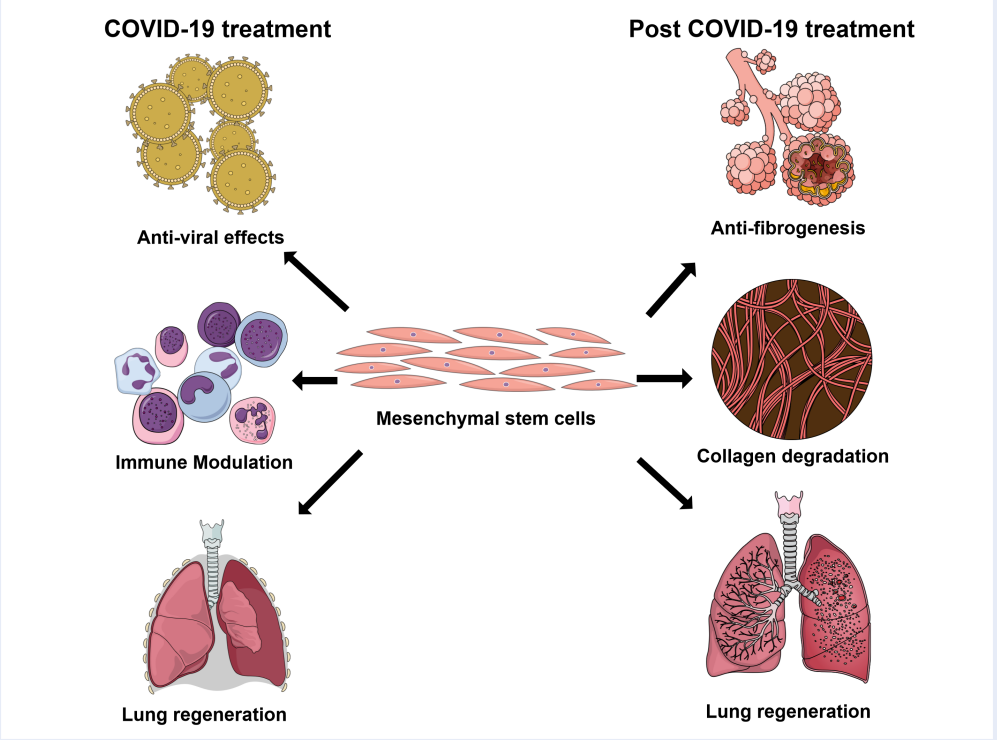

Figure 1: **Mechanisms of MSC transplantation for COVID-19 and post-COVID-19 treatment**. In COVID-19 treatment, MSCs can directly affect the virus, modulate the host's immune system, or trigger lung regeneration. In post-COVID-19 treatment, MSCs can reduce or inhibit fibrogenesis, degrade the existing collagen, and stimulate lung regeneration.

used to treat post-COVID-19. Indeed, in the previous publication[53], ADSCs were considered a better cell source than BM-MSCs to treat hindlimb ischemia in mice[53]. In another study, Liu *et al.* (2020) showed that ADSCs are better than UCMSCs in treating spinal cord injury in animals[54]. These results show that ADSCs are a more suitable cell source than BMSCs or UCMSCs for tissue regeneration, while UCMSCs are suitable for treating anti-inflammation through their immunomodulation capacity[55]. However, more studies have confirmed this observation.

In addition to mesenchymal stem cells used to treat post-COVID-19, extracellular vesicles from MSCs are also being used in a clinical trial (NCT05116761). In this study, EVs from BMMSCs were collected and used with a dose of 10.5 x 10^8 EV per patient.

CONCLUSION

COVID-19 has become the most serious pandemic on record. Some novel therapeutic treatments and vaccines have significantly reduced mortality; however, post-COVID-19, especially post-COVID-19 pulmonary fibrosis, is on the rise as a new condition affecting many patients who have tested negative for SARS-CoV-2. Although it is difficult to conclude based off of the current evidence that this therapy is efficacious for treating post-COVID-19 PF, the first clinical report of MSC transplantation for post-COVID-19 ARDS on ECMO patients referred for lung transplantation and the current results reported on the usage of MSCs for PF suggest that MSC transplantation should be considered a promising therapy for post-COVID-19. More clinical trials should be performed to evaluate this therapy.

ABBREVIATIONS

ACE-2: Angiotensin-converting Enzyme, **APC**: antigen-presenting cells, **COVID-19**: Coronavirus disease 2019, **MSC**: mesenchymal stem cell, **PF**: pulmonary fibrosis, **SARS-CoV-2**: Severe acute respiratory syndrome coronavirus 2, **SVF**: stromal vascular fraction

ACKNOWLEDGMENTS

None

AUTHOR'S CONTRIBUTIONS

All authors equally contributed to this work. All authors read and approved the final manuscript.

FUNDING

None.

AVAILABILITY OF DATA AND MATERIALS

Not applicable.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

CONSENT FOR PUBLICATION

Not applicable.

COMPETING INTERESTS

The authors declare that they have no competing interests.

REFERENCES

1. Spagnolo P, Balestro E, Aliberti S, Cocconcelli E, Biondini D, Casa GD. Pulmonary fibrosis secondary to COVID-19: a call to arms? The Lancet Respiratory Medicine. 2020;8(8):750–2. PMID: 32422177. Available from: 10.1016/S2213-2600(20)30222-8.
2. Wu C, Chen X, Cai Y, Zhou X, Xu S, Huang H, et al. Wu C, Chen X, Cai Y, Zhou X, Xu S, Huang H, Zhang L, Zhou X, Du C, Zhang Y, Song J. Risk factors associated with acute respiratory distress syndrome and death in patients with coronavirus disease 2019 pneumonia in Wuhan, China. JAMA internal medicine. 2020;180(7):934–943. Available from: 10.1001/jamainternmed.2020.0994.
3. Tale S, Ghosh S, Meitei SP, Kolli M, Garbhapu AK, Pudi S. Post-COVID-19 pneumonia pulmonary fibrosis. QJM: An International Journal of Medicine. 2020;113(11):837–8. PMID: 32814978. Available from: 10.1093/qjmed/hcaa255.
4. Vasarmidi E, Tsitoura E, Spandidos DA, Tzanakis N, Antoniou KM. Pulmonary fibrosis in the aftermath of the COVID-19 era (Review). Experimental and Therapeutic Medicine. 2020;20(3):2557–60. PMID: 32765748. Available from: 10.3892/etm.2020.8980.
5. Ludwig S, Zarbock A. Coronaviruses and SARS-CoV-2: A Brief Overview. Anesthesia and Analgesia. 2020;131(1):93–6. PMID: 32243297. Available from: 10.1213/ANE.0000000000004845.
6. Wang Y, Grunewald M, Perlman S. Coronaviruses: An Updated Overview of Their Replication and Pathogenesis. Methods in Molecular Biology (Clifton, NJ). 2020;2203:1–29. PMID: 32833200. Available from: 10.1007/978-1-0716-0900-2_1.
7. Parasher A. COVID-19: current understanding of its Pathophysiology, Clinical presentation and Treatment. Postgraduate Medical Journal. 2021;97(1147):312–20. PMID: 32978337. Available from: 10.1136/postgradmedj-2020-138577.
8. Desai AD, Lavelle M, Boursiquot BC, Wan EY. Long-term complications of COVID-19. American Journal of Physiology: Cell Physiology. 2022;322(1):1–11. PMID: 34817268. Available from: 10.1152/ajpcell.00375.2021.
9. Shirbhate E, Pandey J, Patel VK, Kamal M, Jawaid T, Gorain B. Understanding the role of ACE-2 receptor in pathogenesis of COVID-19 disease: a potential approach for therapeutic intervention. Pharmacological Reports. 2021;73(6):1539–50. PMID: 34176080. Available from: 10.1007/s43440-021-00303-6.
10. Paul M, Mehr AP, Kreutz R. Physiology of local renin-angiotensin systems. Physiological Reviews. 2006;86(3):747–803. PMID: 16816138. Available from: 10.1152/physrev.00036.2005.
11. Yang L, Xie X, Tu Z, Fu J, Xu D, Zhou Y. The signal pathways and treatment of cytokine storm in COVID-19. Signal transduction and targeted therapy. 2021;6(1):1–20. PMID: 34234112. Available from: 10.1038/s41392-021-00679-0.
12. Tang Y, Liu J, Zhang D, Xu Z, Ji J, Wen C. Cytokine Storm in COVID-19: The Current Evidence and Treatment Strategies. Front Immunol. 2020;11:1708. PMID: 32754163. Available from: 10.3389/fimmu.2020.01708.
13. Henderson LA, Canna SW, Schulert GS, Volpi S, Lee PY, Kernan KF, et al. On the Alert for Cytokine Storm: immunopathology in COVID-19. Arthritis & Rheumatology (Hoboken, NJ). 2020;72(7):1059–63. PMID: 32293098. Available from: 10.1002/art.41285.
14. Mehta P, McAuley DF, Brown M, Sanchez E, Tattersall RS, Manson JJ, et al. COVID-19: consider cytokine storm syndromes and immunosuppression. Lancet. 2020;395(10229):1033–4. PMID: 32192578. Available from: 10.1016/S0140-6736(20)30628-0.
15. Zhang JM, An J. Cytokines, inflammation, and pain. International Anesthesiology Clinics. 2007;45(2):27–37. PMID: 17426506. Available from: 10.1097/AIA.0b013e318034194e.
16. Valle DMD, Kim-Schulze S, Huang HH, Beckmann ND, Nirenberg S, Wang B, et al. An inflammatory cytokine signature predicts COVID-19 severity and survival . Nat Med. 2020;26(10):1636–1643. PMID: 32839624. Available from: 10.1038/s41591-020-1051-9.
17. Sette A, Crotty S. Adaptive immunity to SARS-CoV-2 and COVID-19. Cell. 2021;184(4):861–80. PMID: 33497610. Available from: 10.1016/j.cell.2021.01.007.
18. Bastard P, Rosen LB, Zhang Q, Michailidis E, Hoffmann HH, Zhang Y, et al. Autoantibodies against type I IFNs in patients with life-threatening COVID-19. Science. 2020;370(6515). PMID: 32972996. Available from: 10.1126/science.abd4585.
19. Blanco-Melo D, Nilsson-Payant BE, Liu WC, Uhl S, Hoagland D, MØller R. Imbalanced Host Response to SARS-CoV-2 Drives Development of COVID-19. Cell. 2020;181(5). PMID: 32416070. Available from: 10.1016/j.cell.2020.04.026.
20. Arunachalam PS, Wimmers F, Mok CK, Perera RA, Scott M, Hagan T. Systems biological assessment of immunity to mild versus severe COVID-19 infection in humans. Science. 2020;369(6508):1210–20. PMID: 32788292. Available from: 10.1126/science.abc6261.
21. Vabret N, Britton GJ, Gruber C, Hegde S, Kim J, Kuksin M, et al. Immunology of COVID-19: Current State of the Science. Immunity. 2020;52(6):910–941. PMID: 32505227. Available from: 10.1016/j.immuni.2020.05.002.
22. Carfì A, Bernabei R, Landi F, Group GACPACS. Persistent Symptoms in Patients After Acute COVID-19. Journal of the American Medical Association. 2020;324(6):603–5. PMID: 32644129. Available from: 10.1001/jama.2020.12603.
23. Ngai JC, Ko FW, Ng SS, To KW, Tong M, Hui DS. The long-term impact of severe acute respiratory syndrome on pulmonary function, exercise capacity and health status. Respirology (Carlton, Vic). 2010;15(3):543–50. PMID: 20337995. Available from: 10.1111/j.1440-1843.2010.01720.x.
24. Shang J, Ye G, Shi K, Wan Y, Luo C, Aihara H, et al. Structural basis of receptor recognition by SARS-CoV-2. Nature. 2020;581(7807):221–224. PMID: 32225175. Available from: 10.1038/s41586-020-2179-y.
25. Nabahati M, Ebrahimpour S, Tabari RK, Mehraeen R. Post-COVID-19 pulmonary fibrosis and its predictive factors: a prospective study. Egyptian Journal of Radiology and Nuclear Medicine. 2021;52(1):1–7. Available from: 10.1186/s43055-021-00632-9.
26. Udwadia ZF, Koul PA, Richeldi L. Post-COVID lung fibrosis: the tsunami that will follow the earthquake. Lung India : Official Organ of Indian Chest Society. 2021;38(7):41–7. PMID:

33686978. Available from: 10.4103/lungindia.lungindia_818_20.
27. Rai DK, Sharma P, Kumar R. Post covid 19 pulmonary fibrosis. Is it real threat? Indian journal of tuberculosis. 2021;68(3):330–333. Available from: 10.1016/j.ijtb.2020.11.003.
28. Bazdyrev E, Rusina P, Panova M, Novikov F, Grishagin I, Nebolsin V. Lung Fibrosis after COVID-19: Treatment Prospects. Pharmaceuticals (Basel). 2021;14(8):807. PMID: 34451904. Available from: 10.3390/ph14080807.
29. Wu X, Liu X, Zhou Y, Yu H, Li R, Zhan Q, et al. 3-month, 6-month, 9-month, and 12-month respiratory outcomes in patients following COVID-19-related hospitalisation: a prospective study. The Lancet: Respiratory Medicine. 2021;9(7):747–54. PMID: 33964245. Available from: 10.1016/S2213-2600(21)00174-0.
30. Wu C, Chen X, Cai Y, Xia J, Zhou X, Xu S. Risk Factors Associated With Acute Respiratory Distress Syndrome and Death in Patients With Coronavirus Disease 2019 Pneumonia in Wuhan, China. JAMA Internal Medicine. 2020;180(7):934–43. PMID: 32167524. Available from: 10.1001/jamainternmed.2020.0994.
31. Zhang P, Li J, Liu H, Han N, Ju J, Kou Y. Long-term bone and lung consequences associated with hospital-acquired severe acute respiratory syndrome: a 15-year follow-up from a prospective cohort study. Bone Research. 2020;8(1):8. PMID: 32128276. Available from: 10.1038/s41413-020-0084-5.
32. George PM, Wells AU, Jenkins RG. Pulmonary fibrosis and COVID-19: the potential role for antifibrotic therapy. The Lancet Respiratory Medicine. 2020;8(8):807–15. PMID: 32422178. Available from: 10.1016/S2213-2600(20)30225-3.
33. Tang N, Bai H, Chen X, Gong J, Li D, Sun Z. Anticoagulant treatment is associated with decreased mortality in severe coronavirus disease 2019 patients with coagulopathy. Journal of Thrombosis and Haemostasis. 2020;18(5):1094–9. PMID: 32220112. Available from: 10.1111/jth.14817.
34. Li DY, Li RF, Sun DX, Pu DD, Zhang YH. Mesenchymal stem cell therapy in pulmonary fibrosis: a meta-analysis of preclinical studies. Stem Cell Res Ther. 2021;12(1):461. Available from: 10.1186/s13287-021-02496-2.
35. Tzouvelekis A, Paspaliaris V, Koliakos G, Ntolios P, Bouros E, Oikonomou A, et al. A prospective, non-randomized, no placebo-controlled, phase Ib clinical trial to study the safety of the adipose derived stromal cells-stromal vascular fraction in idiopathic pulmonary fibrosis. J Transl Med. 2013;11:171. PMID: 23855653. Available from: 10.1186/1479-5876-11-171.
36. Averyanov A, Koroleva I, Konoplyannikov M, Revkova V, Lesnyak V, Kalsin V. First-in-human high-cumulative-dose stem cell therapy in idiopathic pulmonary fibrosis with rapid lung function decline. Stem Cells Translational Medicine. 2020;9(1):6–16. PMID: 31613055. Available from: 10.1002/sctm.19-0037.
37. Campo A, González-Ruiz JM, Andreu E, Alcaide AB, Ocón MM, De-Torres J, et al. Endobronchial autologous bone marrow-mesenchymal stromal cells in idiopathic pulmonary fibrosis: a phase I trial. ERJ Open Res. 2021;7(2):00773–2020. PMID: 34195252. Available from: 10.1183/23120541.00773-2020.
38. Vu NB, Pham PV. Umbilical cord-derived mesenchymal stem cell transplantation for COVID-19 patients: long-term benefits for lung regeneration. Biomedical Research and Therapy. 2022;9(2):4950–2. Available from: 10.15419/bmrat.v9i2.725.
39. Vu NB, Pham PV. Umbilical Cord Tissue-derived Mesenchymal Stem Cells Should be Considered as Adjuvant Therapy for COVID-19 Treatment: An Opinion from Pooled Clinical Evidence. Biomedical Research and Therapy. 2021;8(9):4583–95. Available from: 10.15419/bmrat.v8i9.694.
40. Pham PV, Vu NB. Off-the-shelf mesenchymal stem cells from human umbilical cord tissue can significantly improve symptoms in COVID-19 patients: an analysis of evidential relations. World Journal of Stem Cells. 2020;12(8):721–30. PMID: 32952854. Available from: 10.4252/wjsc.v12.i8.721.
41. Dong LH, Jiang YY, Liu YJ, Cui S, Xia CC, Qu C, et al. The anti-fibrotic effects of mesenchymal stem cells on irradiated lungs via stimulating endogenous secretion of HGF and PGE2. Sci Rep. 2015;5:8713. PMID: 25736907. Available from: 10.1038/srep08713.
42. Crestani B, Marchand-Adam S, Quesnel C, Plantier L, Borensztajn K, Marchal J. Hepatocyte growth factor and lung fibrosis. Proceedings of the American Thoracic Society. 2012;9(3):158–63. PMID: 22802291. Available from: 10.1513/pats.201202-018AW.
43. Cahill EF, Kennelly H, Carty F, Mahon BP, English K. Hepatocyte growth factor is required for mesenchymal stromal cell protection against bleomycin-induced pulmonary fibrosis. Stem Cells Translational Medicine. 2016;5(10):1307–18. PMID: 27388243. Available from: 10.5966/sctm.2015-0337.
44. Chu KA, Yeh CC, Kuo FH, Lin WR, Hsu CW, Chen TH, et al. Comparison of reversal of rat pulmonary fibrosis of nintedanib, pirfenidone, and human umbilical mesenchymal stem cells from Wharton's jelly. Stem Cell Research & Therapy. 2020;11(1):513. PMID: 33256831. Available from: 10.1186/s13287-020-02012-y.
45. Cabrera S, Gaxiola M, Arreola JL, Ramírez R, Jara P, D'Armiento J, et al. Overexpression of MMP9 in macrophages attenuates pulmonary fibrosis induced by bleomycin. The International Journal of Biochemistry & Cell Biology. 2007;39(12):2324–38. PMID: 17702637. Available from: 10.1016/j.biocel.2007.06.022.
46. Liang J, Zhang Y, Xie T, Liu N, Chen H, Geng Y. Hyaluronan and TLR4 promote surfactant-protein-C-positive alveolar progenitor cell renewal and prevent severe pulmonary fibrosis in mice. Nature Medicine. 2016;22(11):1285–93. PMID: 27694932. Available from: 10.1038/nm.4192.
47. Hofer HR, Tuan RS. Secreted trophic factors of mesenchymal stem cells support neurovascular and musculoskeletal therapies. Stem Cell Res Ther. 2016;7:131. PMID: 27612948. Available from: 10.1186/s13287-016-0394-0.
48. Hou J, Peng X, Wang J, Zhang H, Xia J, Ge Q, et al. Mesenchymal stem cells promote endothelial progenitor cell proliferation by secreting insulin-like growth factor-1. Molecular Medicine Reports. 2017;16(2):1502–1508. PMID: 28627605. Available from: 10.3892/mmr.2017.6741.
49. Khan P, Gazdhar A, Savic S, Lardinois D, Roth M, Tamm M. 118 Lung-derived mesenchymal stem cells exert anti-fibrotic effects in vitro. Chest. 2017;151(5):15. Available from: 10.1016/j.chest.2017.04.016.
50. Xiao K, He W, Guan W, Hou F, Yan P, Xu J, et al. Mesenchymal stem cells reverse EMT process through blocking the activation of NF-κB and Hedgehog pathways in LPS-induced acute lung injury. Cell Death & Disease. 2020;11(10):863. PMID: 33060560. Available from: 10.1038/s41419-020-03034-3.
51. Zhang E, Geng X, Shan S, Li P, Li S, Li W. Exosomes derived from bone marrow mesenchymal stem cells reverse epithelial-mesenchymal transition potentially via attenuating Wnt/β-catenin signaling to alleviate silica-induced pulmonary fibrosis. Toxicology Mechanisms and Methods. 2021;31(9):655–66. PMID: 34225584. Available from: 10.1080/15376516.2021.1950250.
52. Rahulan V, Shah U, Kumar S, Ravipati S, Dutta P, Attawar S. Mesenchymal Stem Cell Therapy for Patients with Post COVID ARDS on ECMO Referred for Lung Transplantation - Single Center Experience from India. The Journal of Heart and Lung Transplantation. 2022;41(S4):S429–S430. Available from: 10.1016/j.healun.2022.01.1084.
53. Vu NB, Phi LT, Dao TT, Le HT, Ta VT, Pham PV. Adipose derived stem cell transplantation is better than bone marrow mesenchymal stem cell transplantation in treating hindlimb ischemia in mice. Biomedical Research and Therapy. 2016;3(9):1–13. Available from: 10.7603/s40730-016-0046-0.
54. Liu AM, Chen BL, Yu LT, Liu T, Shi LL, Yu PP. Human adipose tissue- and umbilical cord-derived stem cells: which is a better alternative to treat spinal cord injury? Neural Regeneration Research. 2020;15(12):2306–17. PMID: 32594054. Available from: 10.4103/1673-5374.284997.
55. Pham PV, Bich NV, Phan NK. Umbilical cord-derived stem cells (MODULATISTTM) show strong immunomodulation capacity compared to adipose tissue-derived or bone marrow-derived mesenchymal stem cells. Biomedical Research and Therapy. 2016;3(6):1–10. Available from: 10.7603/s40730-016-0029-1.