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Long-term outcomes of mesenchymal stem cell therapy in severe COVID-19 patients: 3-year follow-up of a randomized, double-blind, placebo-controlled trial

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Abstract

Background The long-term effects and outcomes of human mesenchymal stem cell (MSC) therapy in patients with severe coronavirus disease 2019 (COVID-19) remain poorly understood. This study aimed to evaluate the extended safety and efficacy of MSC treatment in severe patients with COVID-19 who participated in our earlier randomized, double-blind, placebo-controlled clinical trial, with follow-up conducted over 3 years.

Methods One hundred patients with severe COVID-19 were randomized to receive either an MSC infusion (n = 65, 4×10^7 cells/dose, on days 0, 3, and 6) or a placebo, with both groups receiving the standard of care. At 36 months post-MSC therapy, patients were followed up to long-term safety and efficacy, particularly the effects of MSC therapy on persistent COVID-19 symptoms. Evaluated outcomes included lung imaging results, 6-min walking distance (6-MWD), pulmonary function test results, quality of life scores based on the Short Form-36 (SF-36) health survey, Long COVID symptoms, new-onset comorbidities, tumor marker levels, and rates of COVID-19 reinfection.

Results Three years post-treatment, 46.94% (23/49) of patients in the MSC group and 34.48% (10/29) in the placebo group showed normal findings on computed tomography (CT) images (odds ratio [OR] = 1.68, 95% confidence interval [CI]: 0.65–4.34). The general health (GH) score from the SF-36 was higher in the MSC group (67.0) compared to the placebo group (50.0), with a difference of 12.86 (95% CI: 1.44–24.28). Both groups showed similar results for total lung severity scores (TSS), 6-MWD, pulmonary function tests, and Long COVID symptoms. No significant

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differences between groups were observed in new-onset complications (including tumorigenesis) or tumor marker levels. After adjusting for China's dynamic zero-COVID-19 strategy, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) reinfection rates were 53.06% (26/49) in the MSC group and 67.86% (19/28) in the placebo group (OR = 0.54, 95% CI: 0.20–1.41).

Conclusions These findings support the long-term safety of MSC therapy in patients with severe COVID-19 over 3 years. MSC treatment may offer potential benefits for lung recovery and improved quality of life in patients experiencing Long COVID symptoms.

Trial registration: ClinicalTrials.gov, NCT04288102. Registered 28 February 2020, https://clinicaltrials.gov/study/NCT04288102.

Keywords COVID-19, Mesenchymal stem cell, Cell Therapy, Long-term Follow-up, Long COVID

Background

Since December 2019, coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has continued to impact populations worldwide [1, 2]. As of October 13, 2024, the World Health Organization (WHO) statistics report > 776 million confirmed cases and 7.07 million deaths globally [3]. With faster-spreading variants and increased immune escape, SARS-CoV-2 infections continue to rise [4]. Despite the widespread use of antiviral treatments and vaccines, challenges remain due to viral mutations, antiviral resistance [4, 5], persistence of SARS-CoV-2 in tissues, and reinfections [6, 7]. Patients with severe or critical COVID-19 continue to experience high mortality rates and poor prognoses [8, 9]. Additionally, a significant proportion of patients with COVID-19 experience long-term symptoms across multiple organs and systems even after testing negative for the virus, a condition commonly known as "Long COVID." Symptoms include persistent fatigue, intermittent headaches, shortness of breath, cognitive impairments, loss of smell and taste, and sleep disturbances [10–13]. Thus, there is a need to explore new treatment modalities for these persistent effects.

Mesenchymal stem cells (MSCs), known for their self-renewal, multidirectional differentiation, and immunomodulatory properties, have been extensively studied in animal models and clinical trials for respiratory diseases [14–17]. Over 380 clinical trials of MSC therapy for COVID-19 are currently registered on ClinicalTrials.gov. MSC therapy can reduce inflammatory cytokines and accelerate lung recovery in patients with COVID-19, with good safety observed in short-term follow-ups [18–21]. However, long-term outcomes require further investigation.

During the early phase of the COVID-19 pandemic, we conducted a randomized, double-blind, placebo-controlled clinical trial to assess the safety and efficacy of human umbilical cord MSC (UC-MSC) in treating severe patients with COVID-19 (NCT 04288102). In the MSC group, improvements in lung lesion recovery, symptom relief, and quality of life were observed at 28 d, 1 year, and 2 years post-treatment, with good tolerance [22–24]. This study aimed to evaluate the safety and efficacy of MSC treatment over a period of 3 years, with a secondary focus on its impact on Long COVID. Considering the adjustment in China's dynamic zero-COVID-19 policy in late 2022, we also tracked COVID-19 reinfection rates among enrolled participants to assess the effects of MSC treatment.

Methods

Study design and participants

In our prior randomized, double-blind, placebo-controlled, phase 2 trial conducted from March 6 to March 20, 2020, a total of 101 patients with severe COVID-19 in Wuhan were enrolled and randomized into MSC and placebo groups at a 2:1 ratio. Ultimately, 65 patients received UC-MSC infusion, and 35 received placebo, as one patient in the MSC group withdrew consent prior to infusion. Detailed inclusion and exclusion criteria are available in our previously published article [22]. Both patients and investigators remained blinded in this trial until June 23, 2020, when the 28-d follow-up of the primary outcome was completed [22]. Following the 28-d, 1-year, and 2-year follow-ups [22–24], the current 36-month follow-up study was conducted from March 16, 2023, to May 11, 2023, at the outpatient clinic of the Chinese PLA General Hospital of Central Theater Command in Wuhan, Hubei, China (Fig. 1). Written informed consent was obtained from all participants or their legal representatives prior to screening and the start of any research activities. This study was approved by the Ethics Committee of the Fifth Medical Center of the Chinese PLA General Hospital (Approval No.: 2020–013-D).

Outcomes

Outcomes at the 36-month follow-up included: (1) lung imaging results, evaluated by the number of patients

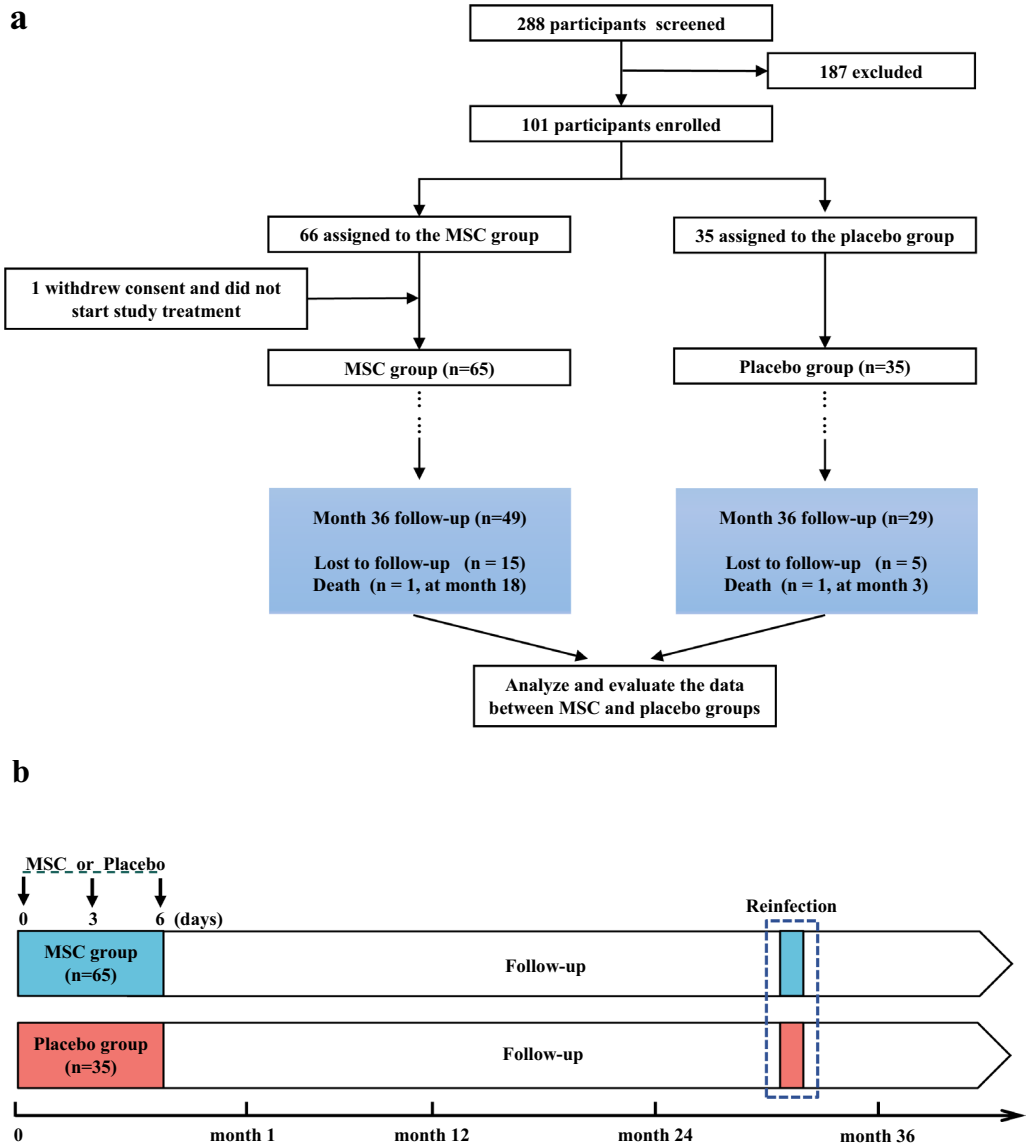

Fig. 1 Overview of the original enrollment and follow-up at month 36. **a** Shows the number of participants in the initial enrollment and at the 36-month follow-up. **b** Shows the time points of previous follow-ups conducted after the transfusion of MSC or placebo

with completely normal lung computed tomography (CT) images and the total severity score (TSS) [25–27]; (2) 6-min walk test results, including the actual value of 6-min walk distance (6-MWD) and the number of patients whose 6-MWD were less than lower limit of the normal range(LLN) [28–30]; (3) pulmonary function tests; (4) quality of life assessment using the Short Form 36 (SF-36) health survey questionnaire [31]; (5) Long COVID symptoms, including chest congestion, breathlessness (modified Medical Research Council scale, mMRC)[32, 33], loss of appetite, sleep difficulties, pain or discomfort, fatigue or muscle weakness, emotional instability, and reduced usual activity); (6) new-onset comorbidities from baseline to 36 months; (7) tumor markers levels; and (8) COVID-19 reinfection survey results.

Procedures

After randomization, patients received three intravenous infusions of UC-MSC (4×10^7 cells/dose) or placebo on days 0, 3, and 6, alongside standard treatment. The UC-MSC was provided by VCANBIO Cell & Gene Engineering Corp (Tianjin, China) as a nearly colorless suspension, containing 4.0×10^7 MSCs in 100 mL/

bag. The placebo, identical in appearance and packaging, consisted of 0.9% sodium chloride and 1% human serum albumin without MSC. Details of cell manufacturing, characterization, and viability are available in our prior study [22].

At the 36-month follow-up, patients underwent a physical examination by experienced physicians and completed questionnaires on SF-36, Long COVID symptoms, and COVID-19 reinfection. Additionally, high-resolution chest CT (HRCT), standardized 6-MWD test, pulmonary function, routine blood and biochemical, and tumor marker tests were conducted. New-onset comorbidities arising within 36 months post-enrollment were thoroughly documented.

As described in our previous 2-year follow-up report [24], three blinded radiologists independently assessed the lung CT images, with discrepancies resolved by consensus. The TSS was determined based on the extent of involvement in each lung lobe (Table 1), with morphological features evaluated by distribution, density, morphology, internal lesion structure, and mediastinal involvement. The 6-MWD test was performed following American Thoracic Society guidelines (ATS) [28], with the calculation method detailed in Table S4. Pulmonary function parameters were tested as previously described [23]. All patients completed a COVID-19 reinfection questionnaire, covering reinfection history, date, symptoms, hospitalizations, treatment, oxygen therapy, complications, and sequelae.

Table 1 Evaluation of chest CT in the MSC and placebo groups at month 36

	MSC group	Placebo group	Difference/OR (95% CI)	*p* value
No. normal CT images [a]				
Month 36	23 /49(46.94)	10 /29(34.48)	1.68(0.65,4.34)[c]	0.2819[e]
Age < 65 y	15 /30(50.00)	8/20 (40.00)	1.50(0.48,4.72)[c]	
Age ≥ 65 y	8/19 (42.11)	2/9 (22.22)	2.55(0.41,15.65)[c]	
BMI ≤ 24	5/16 (31.25)	4/12 (33.33)	0.91(0.18,4.50)[c]	
BMI > 24	16/30 (53.33)	5/13 (38.46)	1.83(0.48,6.90)[c]	
TSS [b]				
Month 36	1.0 (0.0, 9.0)	4.0 (0.0, 10.0)	−1.92(−4.98,1.15) [d]	0.2164[f]
Age < 65 y	0.5 (0.0, 11.0)	4.0 (0.0, 11.5)	−1.87(−6.27,2.54)[d]	
Age ≥ 65 y	2.0 (0.0, 6.0)	5.0 (3.0, 6.0)	−1.64(−5.12,1.84)[d]	
BMI ≤ 24	4.5 (0.0, 9.5)	4.0 (0.0, 7.5)	−0.85(−6.25,4.54)[d]	
BMI > 24	0.0 (0.0, 9.0)	4.0 (0.0, 10.0)	−1.85(−6.41,2.71)[d]	

Data are presented as n/N (%) or median (IQR) unless otherwise specified. The available chest CT values were 49 in the MSC group and 29 in the placebo group at month 36. *P*-values are provided only for descriptive purposes

[a] When all the 5 lobes were normal (score = 0), we counted the number of patients with completely normal lung CT

[b] ***TSS*** = The total severity score, was the sum of scores of 5 lung lobes (scored in proportion according to the extent of lung lesions, and the specific data were score 0 = 0%, score 1 = 1–5%, score 2 = 5–25%, score 3 = 26–50%, score 4 = 51–75%, and score 5 = 76–100%, respectively), ranging from 0 to 25 [25–27]

[c] Calculated by the logistic regression model. OR = odds ratio

[d] Differences are expressed as group t-test and 95% confidence interval (CI)

[e] Group difference assessed by Chi-square test or Fisher's exact test

[f] Group difference assessed by t-test

Statistical analyses

Safety and efficacy outcomes were analyzed using statistical tests, confidence intervals (CIs), and *p*-values, which are applied for descriptive statistics rather than inferential purposes, as no predefined hypotheses were set in this study. Continuous variables were summarized as median values (interquartile range [IQR]), and statistical differences between groups were tested using the two-sample T-Test, which was appropriate as the data satisfied the conditions of independence, normality, and homogeneity of variance. Categorical variables were presented as n/N (%) with 95% CIs calculated using the Clopper–Pearson method. Statistical differences between groups were tested using the chi-square or Fisher's exact tests. Ordinal variables were analyzed using the Cochran–Mantel–Haenszel (CMH) chi-square test, and Odds ratios (ORs) were estimated using logistic regression models. A post-hoc subgroup analysis was conducted, stratified by age (< 65 years vs ≥ 65 years) and body mass index (BMI) (≤ 24 vs 24 kg/m^2). Multiple comparisons were not involved in this study. Except for the SF-36 quality of life scale, no other missing safety or efficacy data were processed. Statistical analyses were performed using SAS software (version 9.4; Gary, NC, USA).

Results

Follow-up and baseline characteristics

During previous follow-ups, a 62-year-old male in the placebo group died of liver cancer at month 3 [23], and a 64-year-old male in the MSC group died of an unknown cause at month 18 [24]. For the present 36-month follow-up, 64 patients in the MSC group and 34 patients in the placebo group were followed. Of these, 20 cases were lost to follow-up (six could not be contacted, and 14 refused follow-up due to lack of time or long distance). A total of 78 patients (49/64 in the MSC group, 29/34 in the placebo group) were assessed, with a median follow-up time of 1106.0 d (IQR: 1102, 1127; Fig. 1). No new deaths occurred during this study.

At baseline, the MSC group was well-matched to the placebo group in terms of age, sex, BMI, the time interval from symptom onset to baseline, complications, concomitant medications, and the proportion of lung lesions observed on chest CT (Table S1) [22]. A post-hoc subgroup analysis stratified by age (< 65 years and ≥ 65 years, respectively) and BMI (≤ 24 and > 24 kg/m^2, respectively)

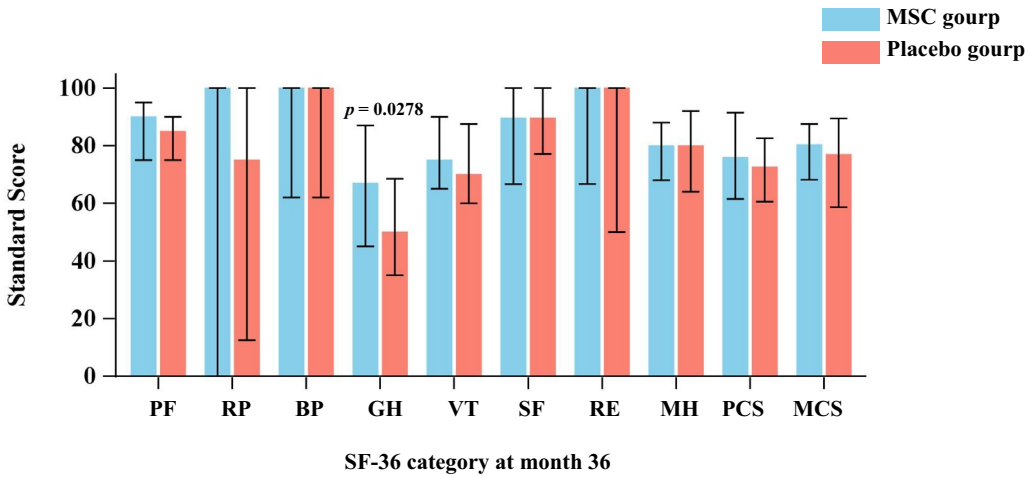

Fig. 2 Ten SF-36 category scores in the MSC and placebo groups at month 36. Data are presented as the median (IQR). At 36-month follow-up, the available SF-36 values were 49 in the MSC group and 28 in the placebo group. I-bars indicate Q1 (first quartile) and Q3 (third quartile), and points indicate the median. The blue bars represent the MSC group, while the red bars represent the placebo group. Group differences were assessed using t-tests. SF-36, 36-Item Short Form Health Survey (range 0–100); PF, Physical Functioning; RP, Role-Physical; BP, Bodily Pain; GH, General Health; VT, Vitality; SF, Social Functioning; RE, Role-Emotional; MH, Mental Health; PCS, Physical Component Summary; MCS, Mental Component Summary

revealed no significant differences between the MSC and placebo groups (Table S1) [24].

Lung images

Normal CT images were observed in 46.94% (23/49) patients in the MSC group and 34.48% (10/29) in placebo groups (OR = 1.68, 95% CI: 0.65–4.34), suggesting that MSC transfusion contributed to lung damage recovery (Tables 1 and S2). The TSS was 1.0 (IQR: 0.0, 9.0) in the MSC group and 4.0 (IQR: 0.0, 10.0) in the placebo group, with a difference of −1.92 (95% CI: −4.98–1.15; Table 1). Among the CT images with abnormalities, the most common morphological features were fibrous stripes (46.94% in the MSC group and 65.52% in the placebo group), followed by ground-glass opacities (40.82% in the MSC group and 48.28% in the placebo group), reticular opacities (30.61% in the MSC group and 37.93% in the placebo group), interlobular septal thickening, honeycombing, and mixed ground-glass opacities (Table S3). Representative lung images of 20 patients at the 36-month follow-up are shown in Figure S1.

6-MWD test and pulmonary function

The 6-MWD test was conducted to assess exercise capacity post-MSC treatment. The median 6-MWD in the MSC and placebo groups were 430.00 m (IQR: 390.00, 465.00) and 420.00 m (IQR: 386.00, 465.00) respectively, with a difference of −1.28 (95% CI: −28.58–26.02). The proportion of patients with a 6-MWD below the LLN was 16.33% (8/49) in the MSC group and 21.43% (6/28) in the placebo group (OR = 0.72, 95% CI: 0.22–2.33; Tables S4 and S5). Although there was numerical improvement in the MSC group, no statistical differences in the 6-MWD were observed between the two groups.

To further evaluate long-term lung recovery, pulmonary function tests were performed, including measurements of the diffusing capacity of the lungs for carbon monoxide (DLCO) and forced vital capacity (FVC) (Table S6). Additionally, we assessed the severity of diffusion impairment and categorized hypoventilation based on the results of these tests (Table S7). In the MSC group, 62.22% (28/45) of patients exhibited decreased diffusion function, compared to 79.17% (19/24) in the placebo group, with most cases being mild. No significant differences were observed between the two groups.

Impact of MSC to Long COVID

To evaluate the impact of MSC treatment on Long COVID, we administered the SF-36 and Long COVID-related symptoms questionnaires at the 36-month follow-up. The general health (GH) score in the MSC group (median 67.0 [IQR: 45.0, 87.0]) was considerable higher than that in the placebo group (median 50.0 [IQR: 35.0, 68.5]) with a difference of 12.86 (95% CI: 1.44–24.28, $p = 0.0278$) (Table S8 and Fig. 2), consistent with the findings at the 2-year follow-up [24]. In the subgroup of patients aged ≥ 65 years, the GH score at month 36 was 70.0 (IQR: 45.0, 87.0) in the MSC group compared to 42.5 (IQR: 32.5, 52.5) in the placebo group, showing a difference of 25.47 (95% CI: 9.44–41.49, $p = 0.0031$)

(Table S8 and Figure S2). No significant differences were observed in other aspects of the SF-36 between these two groups.

Among all 77 patients (except for one in the placebo group who did not complete the questionnaire), the most common Long COVID-related symptoms were chest congestion (55/77, 71.43%), followed by breathlessness (52/77, 67.53%), fatigue or muscle weakness (44/77, 57.14%), sleep disorders (41/77, 53.25%), pain or discomfort (32/77, 41.56%), emotional instability (17/77, 22.08%), decreased usual activity (16/77, 20.78%), and loss of appetite (10/77, 12.99%). No significant differences were observed between the two groups, with the detailed data provided in Table 2.

New-onset complications and tumor markers

From baseline to the 36-month follow-up, both groups reported a similar number of new-onset complications, with 34 occurrences in total. These complications affected 22 patients (44.90%) in the MSC group and 21 patients (72.41%) in the placebo group. The most common complication in the MSC group was hypertension (6/49, 12.24%), followed by coronary heart disease (3/49, 6.12%), hyperlipidemia (2/49, 4.08%), and hyperthyroidism (2/29, 4.08%). In the placebo group, the most common complication was hypertension (6/29, 20.69%), followed by hyperlipidemia (3/29, 10.34%), diabetes (2/29, 6.90%), and nephrolithiasis (2/29, 6.90%) (Table 3).

At the 36-month follow-up, a male patient in the MSC group was diagnosed with type 1 papillary renal cell carcinoma and underwent laparoscopic partial nephrectomy. He was still alive at month 36 (March 2023) at 72-years-old. Most tumor markers remained within normal ranges, and no significant differences were observed between the two groups (Table 4).

Reinfection

In late 2022 and early 2023, during the Omicron wave in China, 45 patients were reinfected with SARS-CoV-2: 26 patients (26/49, 53.06%) in the MSC group and 19 patients (19/28, 67.86%) in the placebo group (OR = 0.54, 95% CI: 0.20–1.41). Among these, three patients (3/26, 11.54%) in the MSC group and one patient (1/19, 5.26%) in the placebo group required hospitalization. Most patients received basic symptomatic and supportive treatment, with 65.38% in the MSC group and 84.21% in the placebo group. None of the patients received antiviral therapy, and no severe, critical, or fatal cases were reported (Tables 5 and S9).

The results of the post-hoc subgroup analyses are presented in Tables 1–2, S5–S6, S8–S9, and Figure S2. All results were derived using descriptive statistical analyses.

Discussion

To the best of our knowledge, this study is the first and longest prospective investigation to assess the long-term effects of MSC therapy in patients with severe COVID-19. Based on our previous follow-ups, MSC therapy has the potential to improve lung damage, enhance activity endurance, and increase quality of life, all with good tolerance in patients with severe COVID-19 [22–24]. Herein, the MSC group demonstrated accelerated recovery of lung damage and a higher GH score on the SF-36 compared to the placebo group over 3-years follow-up. Additionally, the incidence of new-onset complications, including tumorigenesis, was similar between the two groups. Collectively, these findings suggest that MSC therapy offers long-term safety and potential therapeutic benefits. Moreover, this study provides important data on reinfection incidence among the enrolled patients with COVID-19 at the 36-month follow-up.

The results reported by another research indicate that a quarter of patients continue to exhibit abnormal lung imaging even 12 months after infection with SARS-CoV-2 [34]. According to our previous follow-up data, 92.31, 88.37, 71.25, and 68.35% of all enrolled patients still had abnormal chest CT images at months 6, 12, 18, and 24, respectively [23, 24]. In this study, we extended the follow-up to examine changes in chest CT images after 36 months and found that 45 individuals (57.69%) had not recovered from lung injury. Notably, 46.94% of patients in the MSC group exhibited normal CT images, whereas 34.48% of patients in the placebo group showed recovery from lung damage at this time point (Tables 1 and S2). The 6-MWD and pulmonary function tests are practical tools for the assessment of the cardiac-pulmonary reserve function of patients with COVID-19 [27], with another previous study suggesting a prolonged decline in pulmonary function even after 2 years of infection [35]. In our present study, while the MSC group showed numerical improvements in the 6-MWD at 36 months post-infection, the differences between the groups were not statistically significant (Table S5). Over 3 years, MSC-treated patients generally exhibited superior lung CT normalization (Table S2) and better 6-MWD outcomes (Table S4) compared to the placebo group at most follow-up points. Lung CT normalization occurred notably earlier in the MSC group (month 3) compared to the placebo group (month 18) (Table S2). Thus, MSC therapy may have a long-term effect on expediting the rehabilitation of exercise capacity and inducing physiological improvements in patients with severe COVID-19.

Some individuals infected with SARS-CoV-2 experience long-term effects, broadly defined as signs and symptoms that persist beyond the acute phase of

Table 2 Long COVID-related symptoms in the MSC and placebo groups at month 36

	MSC group	Placebo group	OR (95% CI) [a]	*p* value [b]	All groups [c]
Chest congestion					
Month 36	35/49 (71.43)	20/28 (71.43)	1.00(0.36,2.79)	1.0000	55/77 (71.43)
Age < 65 y	20/30 (66.67)	12/20 (60.00)	1.33(0.41,4.31)	0.6304	32/50 (64.00)
Age ≥ 65 y	15/19 (78.95)	8/8 (100.00)	NA	0.2855	23/27 (85.19)
BMI ≤ 24	11/16 (68.75)	8/12 (66.67)	1.10(0.22,5.45)	1.0000	19/28 (67.86)
BMI > 24	21/30 (70.00)	9/12 (75.00)	0.78(0.17,3.56)	1.0000	30/42 (71.43)
Breathlessness (mMRC grade ≥ 1) [d]					
Month 36	33/49 (67.35)	19/28 (67.86)	0.98(0.36,2.64)	0.9633	52/77 (67.53)
Age < 65 y	16/30 (53.33)	12/20 (60.00)	0.76(0.24,2.40)	0.6418	28/50 (56.00)
Age ≥ 65 y	17/19 (89.47)	7/8 (87.50)	1.21(0.09,15.66)	1.0000	24/27 (88.89)
BMI ≤ 24	9/16 (56.25)	6/12 (50.00)	1.29(0.29,5.77)	0.7428	15/28 (53.57)
BMI > 24	22/30 (73.33)	10/12 (83.33)	0.55(0.10,3.07)	0.6956	32/42 (76.19)
Fatigue or muscle weakness					
Month 36	26 /49 (53.06)	18/28 (64.29)	0.63(0.24,1.63)	0.3384	44/77 (57.14)
Age < 65 y	14/30 (46.67)	11/20 (55.00)	0.72(0.23,2.23)	0.5637	25/50 (50.00)
Age ≥ 65 y	12/19 (63.16)	7/8 (87.50)	0.24(0.02,2.43)	0.3645	19/27 (70.37)
BMI ≤ 24	7/16 (43.75)	6/12 (50.00)	0.78(0.17,3.49)	0.7428	13/28 (46.43)
BMI > 24	17/30 (56.67)	10/12 (83.33)	0.26(0.05,1.40)	0.1580	27/42 (64.29)
Sleep difficulties					
Month 36	23 /49 (46.94)	18/28 (64.29)	0.49(0.19,1.28)	0.1422	41/77 (53.25)
Age < 65 y	17/30 (56.67)	11/20 (55.00)	1.07(0.34,3.34)	0.9074	28/50 (56.00)
Age ≥ 65 y	6/19 (31.58)	7/8 (87.50)	0.07(0.01,0.66)	0.0128	13/27 (48.15)
BMI ≤ 24	7/16 (43.75)	6/12 (50.00)	0.78(0.17,3.49)	0.7428	13/28 (46.43)
BMI > 24	15/30 (50.00)	8/12 (66.67)	0.50(0.12,2.02)	0.3269	23/42 (54.76)
Pain or discomfort					
Month 36	21 /49 (42.86)	11/28 (39.29)	1.16(0.45,2.99)	0.7597	32/77 (41.56)
Age < 65 y	12/30 (40.00)	8/20 (40.00)	1.00(0.32,3.17)	1.0000	20/50 (40.00)
Age ≥ 65 y	9/19 (47.37)	3/8 (37.50)	1.50(0.28,8.14)	0.6957	12/27 (44.44)
BMI ≤ 24	8/16 (50.00)	3/12 (25.00)	3.00(0.59,15.36)	0.2530	11/28 (39.29)
BMI > 24	13/30 (43.33)	6/12 (50.00)	0.76(0.20,2.93)	0.6950	19/42 (45.24)
Emotional instability					
Month 36	7 /49 (14.29)	10/28 (35.71)	0.30(0.10,0.91)	0.0292	17/77 (22.08)
Age < 65 y	5/30 (16.67)	7/20 (35.00)	0.37(0.10,1.40)	0.1825	12/50 (24.00)
Age ≥ 65 y	2/19 (10.53)	3/8 (37.50)	0.20(0.03,1.52)	0.1358	5/27 (18.52)
BMI ≤ 24	0/16 (0.00)	3/12 (25.00)	NA	0.0672	3/28 (10.71)
BMI > 24	7/30 (23.33)	7/12 (58.33)	0.22(0.05,0.90)	0.0666	14/42 (33.33)
Decreased usual activity					
Month 36	8 /49 (16.33)	8/28 (28.57)	0.49(0.16,1.49)	0.2027	16/77 (20.78)
Age < 65 y	5/30 (16.67)	5/20 (25.00)	0.60(0.15,2.42)	0.4940	10/50 (20.00)
Age ≥ 65 y	3/19 (15.79)	3/8 (37.50)	0.31(0.05,2.07)	0.3191	6/27 (22.22)
BMI ≤ 24	1/16 (6.25)	2/12 (16.67)	0.33(0.03,4.19)	0.5604	3/28 (10.71)
BMI > 24	7/30 (23.33)	5/12 (41.67)	0.43(0.10,1.77)	0.2740	12/42 (28.57)
Loss of appetite					
Month 36	7 /49 (14.29)	3/28 (10.71)	1.39(0.33,5.86)	0.7390	10/77 (12.99)
Age < 65 y	5/30 (16.67)	1/20 (5.00)	3.80(0.41,35.28)	0.3811	6/50 (12.00)
Age ≥ 65 y	2/19 (10.53)	2/8 (25.00)	0.35(0.04,3.09)	0.5583	4/27 (14.81)
BMI ≤ 24	2/16 (12.50)	1/12 (8.33)	1.57(0.13,19.67)	1.0000	3/28 (10.71)
BMI > 24	5/30 (16.67)	2/12 (16.67)	1.00(0.17,6.03)	1.0000	7/42 (16.67)

Data are n/N (%). The available values were 49 in the MSC group and 28 in the placebo group

P-values are provided only for descriptive purposes

Table 2 (continued)

[a] Calculated by the logistic regression model. OR = odds ratio. NA, not applicable

[b] Group difference assessed by Chi-square test or Fisher's exact test

[c] The total numbers of people who had Long-COVID-related symptoms in the two groups

[d] Breathlessness (mMRC) = The mMRC scale was used to measure the decreased ability to perform daily activities caused by shortness of breath, and patients with mMRC grade ≥ 1 were considered to have shortness of breath

Table 3 New-onset comorbidities from baseline to month 36

	MSC group (N = 49) n/N (%)	Placebo group (N = 29) n/N (%)
Cardio-cerebrovascular diseases		
Hypertension	6/49 (12.24)	6/29 (20.69)
Hyperlipidemia	2/49 (4.08)	3/29 (10.34)
Coronary heart disease	3/49 (6.12)	1/29 (3.45)
Angina pectoris	0/49 (0.00)	1/29 (3.45)
Other heart disease	0/49 (0.00)	1/29 (3.45)
Atrial fibrillation	0/49 (0.00)	1/29 (3.45)
Lacunar infarction	1/49 (2.04)	0/29 (0.00)
Metabolic disease		
Diabetes	0/49 (0.00)	2/29 (6.90)
Hypoglycemia	1/49 (2.04)	0/29 (0.00)
Hyperuricemia	1/49 (2.04)	0/29 (0.00)
Hyperthyroidism	2/49 (4.08)	0/29 (0.00)
Hypothyroidism	0/49 (0.00)	1/29 (3.45)
Respiratory disease		
Influenza A	1/49 (2.04)	0/29 (0.00)
Lung nodule	0/49 (0.00)	1/29 (3.45)
Hydrothorax	1/49 (2.04)	0/29 (0.00)
Urinary system		
Renal tumor	1/49 (2.04)	0/29 (0.00)
Chronic glomerulonephritis	1/49 (2.04)	0/29 (0.00)
Nephrolithiasis	0/49 (0.00)	2/29 (6.90)
Digestive system		
Cholelithiasis	1/49 (2.04)	1/29 (3.45)
Gastritis	0/49 (0.00)	1/29 (3.45)
Gastric polyposis	0/49 (0.00)	1/29 (3.45)
Others		
Rheumatism	1/49 (2.04)	1/29 (3.45)
Breast nodule	0/49 (0.00)	1/29 (3.45)
Lumbar disc herniation	0/49 (0.00)	1/29 (3.45)
Urticaria	1/49 (2.04)	0/29 (0.00)
Tinnitus	1/49 (2.04)	0/29 (0.00)

Data are n/N (%). The available values were 49 in the MSC group and 29 in the placebo group at month 36. During the previous follow-up, a 62-year-old male in the placebo group developed liver cancer at month 3 [23]

infection, commonly referred to as Long COVID. Long COVID can last for months or even years, and may include symptoms such as fatigue, lethargy, pharyngeal discomfort, cough, chest pain, sleep disturbances, memory loss, and decreased exercise capacity [10–12, 36]. Herein, 20.78% of patients reported a decline in daily activities at the 36-month follow-up (Table 2), and 14 patients (eight from the MSC group and six from the placebo group) could not return to work. Therefore, COVID-19 continues to have long-term effects on enrolled patients. Notably, higher GH scores on the SF-36 were observed in the MSC group compared to

Table 4 Tumor markers at month 36

Tumor Marker	MSC group (N = 49) Abnormal n/N (%)	Placebo group (N = 29) Abnormal n/N (%)	*p* value
Total-Prostate specific antigen *	4/30 (13.33)	2/15 (13.33)	1.0000
Carcinoembryonic antigen	0 /48(0.00)	0/28 (0.00)	1.0000
Neuron-specific enolase	2 /48(4.17)	3/28 (10.71)	0.3512
Free-Prostate specific antigen *	4/30 (13.33)	2/15 (13.33)	1.0000
Carbohydrate antigen 125	0/48 (0.00)	0/28 (0.00)	1.0000
Carbohydrate antigen 15–3	0 /48(0.00)	0/28 (0.00)	1.0000
Alpha fetoprotein	0/48 (0.00)	0/28 (0.00)	1.0000
Free-β-HCG	0/48 (0.00)	0/28 (0.00)	1.0000
Carbohydrate antigen 19–9	0 /48(0.00)	0/28 (0.00)	1.0000
Carbohydrate antigen 24–2	0 /48(0.00)	0/28 (0.00)	1.0000
Cytokeratin 19 fragment (CYFRA21-1)	2/48 (4.17)	2/28 (7.14)	0.6225
Squamous cell carcinoma antigen	2/48 (4.17)	1/28 (3.57)	1.0000

* Only in male patients. At the 36-month follow-up, 30 male patients in the MSC group and 15 male patients in the placebo group were tested

Group difference assessed by Fisher's exact test

These *p* values are provided for descriptive purposes only

the placebo group, suggesting potential benefits of MSC therapy in improving quality of life and mitigating Long COVID symptoms. This is consistent with findings from another clinical trial investigating MSC treatment for long-COVID [37]. The hypothesized mechanisms underlying Long COVID pathogenesis include immune dysregulation, autoimmunity, dysfunctional neurological signaling, clotting and endothelial abnormality, and microbiota disruption [10–12]. These effects may be linked to the immunomodulatory properties of MSCs [38], which, at sites of inflammation, can restore immune homeostasis by influencing both innate and adaptive immune cells, thereby inhibiting the cascade of immune responses.

SARS-CoV-2 infection impacts various tissues and organs, and studies have reported that some patients may develop conditions such as hypertension, diabetes, coronary heart disease, and myocarditis among others [39, 40]. Our clinical trial found that MSC therapy did not result in a higher incidence of new-onset complications compared to the placebo group over 36-months follow-up. Additionally, tumorigenesis and tumor marker levels were similar between the two groups. This study provides the longest follow-up data on the safety of MSC therapy in patients with severe COVID-19. While several studies have demonstrated the short-term safety of MSC transplantation in patients with COVID-19, our research offers valuable insights into its extended safety profile. Moving forward, we plan to continue monitoring this cohort for any new-onset complications, including tumorigenesis.

Since China modified its dynamic zero-COVID-19 strategy in December 2022, the number of infections has risen rapidly. At the 3-year follow-up, 53.06% and 67.86% of patients in the MSC and placebo groups, respectively, experienced reinfection with SARS-CoV-2. Notably, these reinfections were associated with milder clinical symptoms compared to the initial infections, which is consistent with findings from other studies [39, 41]. This could be attributed to the humoral and cellular immunity induced by prior infections and vaccinations [42]. Although the proportion of reinfections was numerically lower in the MSC group, the difference between the two groups was not statistically significant. The effect of MSC treatment on the rate and severity of reinfection remain unclear and warrants further investigation.

This study had several limitations. First, the participants were drawn from the early phases of the pandemic, meaning the findings may not fully represent the characteristics of COVID-19 in later stages. Second, reliance on self-reported health outcomes in the follow-up data introduces the possibility of information bias. Third, the extended follow-up period presents the challenge of maintaining participant engagement, which can result in a loss of follow-up. This loss may reduce statistical power, potentially leading to an overestimation of treatment safety or efficacy.

Table 5 Reinfection of SARS-CoV-2 from month 24 to 36

	MSC group	Placebo group	OR/Difference (95% CI)	*p* value
Reinfection				
Number of people, n/N (%)	26/49 (53.06)	19/28 (67.86)	0.54(0.20,1.41) [a]	0.2050[c]
Time from reinfection onset to the 3-year follow-up time points, days, Median (IQR)	102.5 (92.0, 112.0)	106.0 (91.0, 110.0)	1.06(−13.18,15.31) [b]	0.8809[d]
Symptoms, n/N (%)				
Pyrexia	15/26 (57.69)	10/19 (52.63)	1.23(0.37,4.03) [a]	0.7358[c]
Maximum temperature of fever, ℃, Median (IQR)	38.20 (37.80, 39.00)	38.20 (38.00, 38.50)	0.01(−0.52,0.55) [b]	0.9592[d]
Fever duration, days, Median (IQR)	2.0 (1.0, 3.0)	2.5 (1.0, 3.0)	−0.43(−1.39,0.52) [b]	0.3578[d]
Runny nose	10/25 (40.00)	9/19 (47.37)	0.74(0.22,2.47) [a]	0.6250[c]
Cough	18/26 (69.23)	14/19 (73.68)	0.80(0.22,3.00) [a]	0.7448[c]
Expectoration	12/26 (46.15)	8/19 (42.11)	1.18(0.36,3.89) [a]	0.7872[c]
Sore throat	13/26 (50.00)	11/19 (57.89)	0.73(0.22,2.39) [a]	0.6001[c]
Headache	11/26 (42.31)	7/19 (36.84)	1.26(0.37,4.23) [a]	0.7116[c]
Muscle pain	10/26 (38.46)	8/19 (42.11)	0.86(0.26,2.87) [a]	0.8053[c]
Chill	6/26 (23.08)	3/19 (15.79)	1.60(0.35,7.42) [a]	0.7123[c]
Fatigue or weakness	16/26 (61.54)	10/19 (52.63)	1.44(0.43,4.77) [a]	0.5502[c]
Joint pain	11/26 (42.31)	6/19 (31.58)	1.59(0.46,5.50) [a]	0.4634[c]
Chest congestion	8/26 (30.77)	9/19 (47.37)	0.49(0.14,1.68) [a]	0.2566[c]
Breathless	10/26 (38.46)	9/19 (47.37)	0.69(0.21,2.30) [a]	0.5502[c]
Nausea	0/26 (0.00)	2/19 (10.53)	NA	0.1727[c]
Vomiting	0/26 (0.00)	4/19 (21.05)	NA	0.0260[c]
Diarrhea	2/26 (7.69)	5/19 (26.32)	0.23(0.04,1.37) [a]	0.1144[c]
Voice hoarseness	8/26 (30.77)	5/19 (26.32)	1.24(0.33,4.65) [a]	0.7448[c]
Anosmia	5/26 (19.23)	5/19 (26.32)	0.67(0.16,2.74) [a]	0.7203[c]
Ageusia	4/26 (15.38)	7/19 (36.84)	0.31(0.08,1.28) [a]	0.1601[c]
Conjunctivitis	0/26 (0.00)	1/19 (5.26)	NA	0.4222[c]
Treatment and Medication, n/N(%)[e]				
Hospitalized	3/26 (11.54)	1/19 (5.26)	2.35(0.22,24.51) [a]	0.6270[c]
Bed rest	15/26 (57.69)	8/19 (42.11)	1.87(0.57,6.21) [a]	0.3015[c]
Symptomatic and supportive treatment	17/26 (65.38)	16/19 (84.21)	0.35(0.08,1.55) [a]	0.1584[c]
Supplemental oxygen therapy (Nasal catheter or mask)	3/26 (11.54)	4/18 (22.22)	0.46(0.09,2.35) [a]	0.4190[c]
Antiviral therapy	0/26 (0.00)	0/19 (0.00)	NA	1.0000[c]
Antibiotic drug treatment	8/26 (30.77)	6/19 (31.58)	0.96(0.27,3.45) [a]	0.9538[c]
Traditional Chinese medicine (TCM) therapy	3/26 (11.54)	1/19 (5.26)	2.35(0.22,24.51) [a]	0.6270[c]
Sequelae of reinfection, n/N (%)	5/26 (19.23)	3/19 (15.79)	1.27(0.26,6.12) [a]	1.0000[c]
Anosmia or ageusia	1/26 (3.85)	0/19 (0.00)	NA	NA
Respiratory-related sequelae	4/26 (15.38)	2/19 (10.53)	NA	NA
Others	1/26 (3.85)	1/19 (5.26)	NA	NA

Data are presented as n/N (%) or median (IQR) unless otherwise specified. The available values for SARS-CoV-2 reinfection were 26 in the MSC group and 19 in the placebo group at month 36, and all reinfections occurred once. *P*-values are provided only for descriptive purposes

[a] Calculated by the logistic regression model. OR = odds ratio

[b] Differences are expressed as group t-test and 95% confidence interval (CI)

[c] Group difference assessed by Chi-square test or Fisher's exact test

[d] Group difference assessed by t-test

[e] No one of the two groups required prone ventilation, non-invasive ventilation (NIV), invasive mechanical ventilation (IMV) or tracheal cannula

No one of the two groups received immunotherapy, vasoactive drug, anticoagulant drug, extracorporeal membrane oxygenation(ECMO), renal replacement therapy, plasma therapy, hemopurification or salvage therapy

Conclusions

This study represents the longest follow-up of MSC therapy in individuals with severe COVID-19 to date. The results demonstrate the sustained safety of MSC therapy over 36-months follow-up. Additionally, the findings suggest that MSC therapy holds promise as a potential treatment for individuals with severe COVID-19, aiding in recovery from lung damage and enhancing the quality of life for patients with Long COVID. These results establish the foundation for continued clinical trials exploring MSC therapy as an intervention for both acute SARS-CoV-2 infection and Long COVID.

Abbreviations

COVID-19	Coronavirus Disease 2019
SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus 2
MSC	Mesenchymal Stem Cell
UC-MSC	Umbilical Cord Mesenchymal Stem Cells
6-MWD	6-Min Walking Distance
ORs	Odds Ratios
Cis	Confidence Intervals
IQR	Interquartile Range
WHO	World Health Organization
TSS	Total Severity Score
LLN	The Lower Limit of Normal range
HRCT	High-Resolution CT
CMH chi-square test	The Cochran–Mantel–Haenszel (CMH) chi-square test
BMI	Body Mass Index
DLCO	The diffusing capacity of the lungs for carbon monoxide
FVC	Forced Vital Capacity
SF-36	36-Item Short Form Health Survey
PF	Physical Functioning
RP	Role-Physical
BP	Bodily Pain
GH	General Health
VT	Vitality
SF	Social Functioning
RE	Role-Emotional
MH	Mental Health
PCS	Physical Component Summary
MCS	Mental Component Summary

Supplementary Information

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Additional file 1

Additional file 2

Additional file 3

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Author contributions

FSW and Lei Shi contributed to the conception and design of the study. BZ, Le Song, MQY, ZRW, ZYZ, QM, NZ, JLH, YGL, LH, ZX, XY, and JLF were responsible for the acquisition of data. MQY, ZRW, ZYZ, and YYL were responsible for the analysis and interpretation of data. FSW, Lei Shi, BZ, MQY, ZYZ, YGL, and WFX verified the underlying data. ZYZ, MS, Le Song, WQY, CZ, JWS, YZ, and TYD were responsible for laboratory testing and assay development. JMC, JHD, and JZZ performed image analysis. MQY, ZRW, and QM drafted the manuscript. FSW, Lei Shi, and BZ critically revised the manuscript. All authors revised and approved the final version of the manuscript for submission.

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Availability of data and materials

Participant data are available from the lead contact, Lei Shi (shilei302@126.com) under reasonable request.

Declarations

Ethics approval and consent to participate

(1) Title of the approved project: "Treatment with human umbilical cord-derived mesenchymal stem cells for severe coronavirus disease 2019 (COVID-19)." (2) Name of the institutional approval committee or unit: the Ethics Committee of the Fifth Medical Center, Chinese PLA General Hospital. (3) Approval number: 2020–013-D. (4) Date of approval: February 24, 2020.

Informed consent

Written informed consent was obtained from all candidates or their legal representatives before the screening process and initiation of any research.

Consent for publication

Not applicable.

Competing interests

WQY, TYD, and YZ are current employees of Wuhan Optics Valley Zhongyuan Pharmaceutical Co., Ltd. All authors declare no competing interests.

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