ORIGINAL ARTICLE

A Randomized Trial of Mesenchymal Stromal Cells for Moderate to Severe Acute Respiratory Distress Syndrome from COVID-19

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Abstract

Rationale: There are limited therapeutic options for patients with coronavirus disease (COVID-19)-related acute respiratory distress syndrome with inflammation-mediated lung injury. Mesenchymal stromal cells offer promise as immunomodulatory agents.

Objectives: Evaluation of efficacy and safety of allogeneic mesenchymal cells in mechanically-ventilated patients with moderate or severe COVID-19–induced respiratory failure.

Methods: Patients were randomized to two infusions of 2 million cells/kg or sham infusions, in addition to the standard of care. We hypothesized that cell therapy would be superior to sham control for the primary endpoint of 30-day mortality. The key secondary endpoint was ventilator-free survival within 60 days, accounting for deaths and withdrawals in a ranked analysis.

Measurements and Main Results: At the third interim analysis, the data and safety monitoring board recommended that the trial halt enrollment as the prespecified mortality reduction from 40% to 23% was unlikely to be achieved ($n = 222$ out of planned 300). Thirty-day mortality was 37.5% (42/112) in cell recipients versus 42.7% (47/110) in control patients (relative risk [RR], 0.88; 95% confidence interval, 0.64–1.21; $P = 0.43$). There were no significant differences in days alive off ventilation within 60 days (median rank, 117.3 [interquartile range, 60.0–169.5] in cell patients and 102.0 [interquartile range, 54.0–162.5] in control subjects; higher is better). Resolution or improvement of acute respiratory distress syndrome at 30 days was observed in 51/104 (49.0%) cell recipients and 46/106 (43.4%) control patients (odds ratio, 1.36; 95% confidence interval, 0.57–3.21). There were no infusion-related toxicities and overall serious adverse events over 30 days were similar.

Conclusions: Mesenchymal cells, while safe, did not improve 30-day survival or 60-day ventilator-free days in patients with moderate and/or severe COVID-19–related acute respiratory distress syndrome.

Keywords: mechanical ventilation; SARS-CoV-2; clinical trial; survival; stem cells

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At a Glance Commentary

Scientific Knowledge on the Subject: Coronavirus disease (COVID-19)-induced acute respiratory distress syndrome (ARDS) remains a highly lethal and morbid condition with limited therapeutic options. Pre- and early clinical data have shown that mesenchymal stromal cells (MSCs) may improve lung injury and associated inflammation through a variety of mechanisms, including immune modulation, alveolar fluid clearance, bacterial clearance, regulation of pulmonary vascular endothelial permeability, and suppression of apoptosis.

What This Study Adds to the Field: In our trial, MSC therapy, compared with sham control, in patients with moderate to severe COVID-19–related ARDS did not produce the hypothesized reduction in 30-day mortality. During the pandemic, new insights have emerged into the existence of different inflammatory subtypes and the importance of age within the overall COVID-19–related ARDS population that might underlie a differential response to such immunomodulatory therapy. Future clinical trials are needed to identify the potential benefits of MSCs and differential dosing on the basis of immune status and age in susceptible phenotypes.

Although there have been advances in the treatment of hospitalized patients with coronavirus disease (COVID-19), a therapeutic gap persists for patients with acute respiratory distress syndrome (ARDS) secondary to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection. SARS-CoV-2 directly infects epithelial cells and immune cells in the lung, leading to direct cytotoxic effects and lung injury with secondary inflammation (1). As the disease process progresses to respiratory failure, many patients develop a hyperinflammatory state characterized by increased concentrations of inflammatory mediators, including cytokines and chemokines such as IL-2, tumor necrosis factor, and macrophage chemoattractant protein, as well as elevated inflammatory biomarkers, including C-reactive protein (CRP) and ferritin (2). The hyperinflammatory state associated with COVID-19 has drawn comparisons with cytokine release syndrome, graft-versus-host disease, and hemophagocytic lymphohistiocytosis (1).

It has become increasingly clear that ARDS-related mortality in patients with COVID-19 is associated with increasing age and the accompanying dysregulated inflammatory response. Consequently, it has been postulated that targeted immunomodulation may mollify or reverse the hyperinflammatory state associated with COVID-19 and reduce ARDS-related mortality (3). Indeed, dexamethasone has been shown to reduce mortality in patients with hypoxemia hospitalized with COVID-19 receiving mechanical ventilation or oxygen (4, 5). However, other single-target immunomodulatory agents have demonstrated only mixed results (6, 7), accentuating the need to evaluate cellular-based therapies, which may have multiple targets.

Preclinical and early clinical data have shown that mesenchymal stromal cells (MSCs) may improve lung injury and associated inflammation through a variety of mechanisms, ranging from paracrine secretion of antiinflammatory cytokines and growth factors to production of antimicrobial peptides and restoration of epithelial bioenergetics through the transfer of mitochondria (8, 9). Adult-derived expanded MSCs have benefits in pediatric steroid-refractory acute graft-vs-host disease (SR-aGVHD) (10) and preclinical models of acute lung injury (11). MSCs have demonstrated safety in phase I/IIa trials of ARDS (12, 13). A small study of nonmechanically ventilated patients with COVID-19 pneumonia reported rapid recovery and improved inflammatory markers after treatment with MSCs (14). These data led to the design of a trial evaluating MSCs (using a lower dose of remestemcel-L than has been used clinically in pediatric SR-aGVHD) in patients with COVID-19–induced moderate or severe ARDS, the results of which are reported here.

Trial Registration: ClinicalTrials.gov (NCT04371393).

Methods

Study Design and Participants

This randomized, parallel, sham infusion-controlled trial evaluating MSCs in patients

A complete list of trial members may be found in the supplement.

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Data sharing statement: Mesoblast is interested in sharing clinical data from the trial with scientific researchers in the interest of advancing public health. Qualified external researchers can request deidentified participant data to conduct research. These deidentified data will only be available for request after all patient follow-up is completed and a period of 12 months has elapsed after U.S. Food and Drug Administration and European Medicines Agency approval. Proposals will be reviewed and approved by Mesoblast and the representative of the Coordinating Center. After approval of a proposal, data can be shared through a secure online platform after signing a data access agreement.

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This article has a related editorial.

This article has an online supplement, which is accessible from this issue's table of contents at www.atsjournals.org.

with SARS-CoV-2–related ARDS was conducted under an investigational new drug application. A coordinating center, independent event adjudication committee, and data and safety monitoring board oversaw trial progress. A central institutional review board approved the protocol, and informed consent was obtained from all patients or their legally authorized representatives.

The target population was adults with SARS-CoV-2, confirmed by real-time reverse transcription PCR assay, who required mechanical ventilation for moderate or severe ARDS (modified Berlin criteria) (15). Patients needed to have bilateral opacities on a chest radiograph or computerized tomographic scan, respiratory failure not fully explained by cardiac failure or fluid overload, and moderate to severe impairment of oxygenation assessed by the Pa_{O_2}/FI_{O_2} ratio. The severity of ARDS was defined as moderate if Pa_{O_2}/FI_{O_2} was $>$100 mm Hg and $\leq$200 mm Hg and severe if Pa_{O_2}/FI_{O_2} was $\leq$100 mm Hg, both with ventilator settings that included positive end-expiratory pressure $\geq$5 cm H_2O. In addition, patients were required to have a CRP concentration $>$4 mg/dl, an Acute Physiology and Chronic Health Evaluation (APACHE) score greater than 5, and creatinine clearance $\geq$30 ml/min.

Patients were excluded if they were receiving extracorporeal membrane oxygenation, had evidence of bacterial pneumonia, were massively obese (body mass index above 55), had untreated HIV, malignancy within 12 months of active treatment, or elevated liver function tests (LFTs) (greater than 8$\times$ the upper limit of normal). In addition, patients were excluded if they had been intubated for more than 72 hours at the time of randomization or had a prior history of respiratory disease requiring supplemental oxygen.

Randomization and Masking

Patients were randomized in a 1:1 ratio using a web-based system, and randomization was stratified by clinical center and ARDS severity. A random permuted block design with block sizes of two and four was used. Patients and investigators were blinded, except for the site's research pharmacist and one unblinded trial statistician. The infusion bags were masked to their content by the research pharmacist.

Procedures

Patients were randomized to receive intravenous infusion of MSCs (remestemcel-L) plus standard of care versus placebo (PlasmaLyte) plus standard of care. Patients received two infusions of the study product during the first week, with the second infusion 4 days after the first infusion ($\pm$1 d). The MSC dose was 2×10^6 MSC/kg of body weight. This dose regimen was adapted from that used in patients with SR-aGVHD in which 2×10^6 MSC/kg of body weight were infused twice weekly for 4 weeks; both aGVHD and COVID-19 ARDS have in common excessive T-cell proliferation and infiltration accompanied by damage to gut and pulmonary epithelial surfaces (10). The rationale for using a shorter dosing period with COVID-19–related ARDS was on the basis of a pilot trial that used this dosing regimen in a series of 11 adult patients with COVID-19 with moderate to severe ARDS on mechanical ventilation, 9 of whom were extubated and discharged from the ICU within 28 days of MSC initiation (16). In this trial, the MSCs were cryopreserved and stored at or below -135°C in liquid nitrogen vapor phase until use. It was held at distribution centers until requested by a treating hospital and transported to the hospital in controlled vapor shippers on a just-in-time basis to ensure the quality of the product at the time of treatment. The viability of the batches administered in the trial ranged from 78% to 93%. *See* the Appendix in the data supplement for manufacturing and cell viability.

The protocol included guidelines for SARS-CoV-2 ARDS management, such as ventilator settings, proning, sedation, pain management, and concomitant medications, including the use of off-label or investigational antiviral agents and investigational anti–IL-6 agents. The use of dexamethasone and remdesivir became the standard of care during the trial.

Outcomes

Patients were followed for 12 months after randomization. The primary endpoint was a reduction in all-cause mortality within 30 days after randomization. The key secondary endpoint was days alive off mechanical ventilation within 60 days after randomization. Secondary endpoints included mortality at 7, 14, 60, and 90 days and 12 months, resolution and/or improvement of ARDS, and clinical improvement at Days 7, 14, 21, and 30 after randomization. ARDS resolution or improvement was defined as being alive with a decrease in ARDS scale severity. Clinical improvement was defined as discharge or an improvement by two points on a seven-point ordinal scale that ranged from one (death) to seven (nonhospitalized status with the resumption of normal activities).

Total and ICU length of stay (LOS) after randomization for the index hospitalization, readmissions, and the total number of days in hospital within 60 days after randomization were assessed. Secondary safety endpoints included any infusion-related toxicity (hypersensitivity reaction within 2 h of administration) and the incidence of serious adverse events within 30 days after randomization. Pulmonary symptoms and vital status were assessed at 6 and 12 months.

Statistical Analysis

The trial was designed early in the pandemic when 30-day mortality for patients with ARDS was between 40% and 60% (17). The sample size was on the basis of a relatively high treatment effect because we needed a trial that could be accomplished in a tractable period of time, given the desperate need for new therapies for this high-mortality condition and the limited availability of cell products at the time. In addition, before the start of enrollment, the design was further modified from a phase II trial with a one-sided test of the primary endpoint to a phase II/III design with a two-sided test to facilitate registration and a more expeditious route to bring the therapy to patients, if efficacious. A sample size of 150 patients in each group ensured approximately 84% power to detect a difference of 17% from an assumed control rate of 40% mortality by 30 days after randomization using a two-sided, 0.05-level test of independent proportions. Three interim analyses for stopping accrual early for efficacy or futility when 30%, 45%, and 60% of patients had reached the primary endpoint were prespecified using Bayesian predictive probabilities (more detail on assumptions and operating characteristics are provided in the Supplemental Appendix).

The primary efficacy analysis compared the proportion of patients who died by 30 days after randomization between groups using a two-sided, 0.05-level test for independent proportions. Missing values because of early withdrawal were imputed as deaths. The key secondary efficacy endpoint was assessed using a two-sided, 0.05-level Wilcoxon rank-sum test. Patients surviving to Day 60 were ranked according to their number of ventilator-free

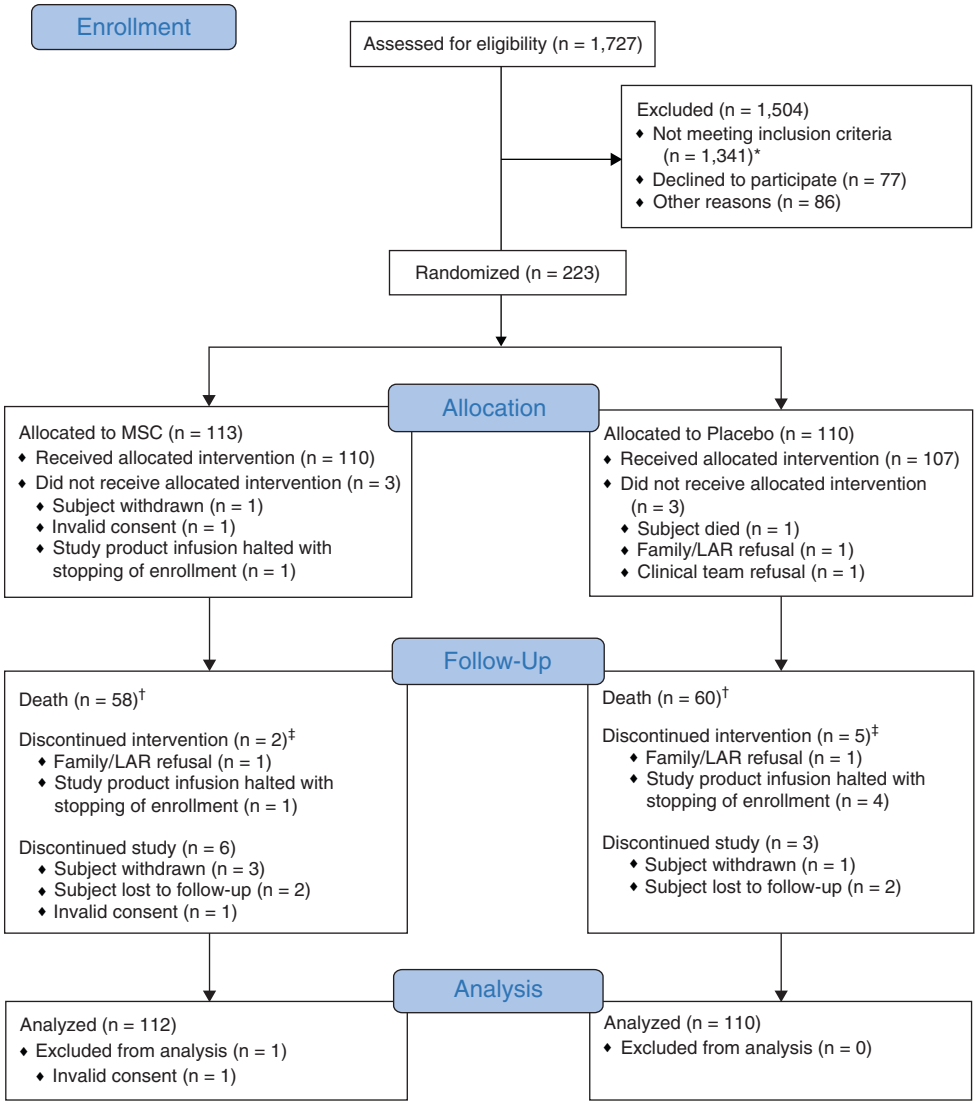

Figure 1. CONSORT (Consolidated Standards of Reporting Trials) diagram. *The most common reasons for not meeting inclusion/exclusion criteria included not having moderate and/or severe acute respiratory distress syndrome or requiring mechanical ventilator support ($n = 533$) and intubation greater than 72 hours ($n = 208$). †Three subjects discontinued intervention because of death (MSC = 1 and placebo = 2). ‡Discontinued intervention means the second infusion was not administered. LAR = legally authorized representative; MSC = mesenchymal stromal cell.

days. Patients who withdrew or had an unknown extubation date were assigned ranks lower than the lowest observed rank, in order on the basis of the proportion of known days alive assessed for intubation and free from mechanical ventilation. Patients who died before Day 60 were assigned the lowest ranks, in order on the basis of the time of death. Differences in rank between groups were assessed using the Hodges-Lehmann estimate of location shift (18).

Survival at 7 and 14 days after randomization was assessed as above. Survival (60 and 90 d and 12 mo) was estimated using the Kaplan-Meier method, and comparisons were made with the log-rank test. Clinical improvement and resolution and/or improvement in ARDS were assessed using mixed effect logistic regression. LOS during the index hospitalization (including ICU d) and LOS through Day 60, including readmission days, were compared using Wilcoxon rank-sum tests. Deaths, withdrawals, and patients with incomplete data were ranked according to the worst-rank method described above. Group differences in readmission rates through Day 60 and serious adverse event rates through Day 30 were evaluated using Poisson regression with robust variance estimation.

Subgroup analyses of the primary endpoint in key clinical subgroups were prespecified when the number of patients in the strata was at least 20. With the exception of ARDS severity, for which a formal interaction test was prespecified, subgroup analyses are descriptive and presented as relative risks with 95% confidence intervals (CIs).

Safety endpoints (infusional toxicities and serious adverse events) were assessed in

Table 1. Patient Characteristics at Baseline

Patient Characteristic	MSC ($n = 112$)	Placebo ($n = 110$)
Age (yr), mean ± SD	61.8 ± 13.0	59.6 ± 13.8
<45, *n* (%)	12/112 (10.7)	15/110 (13.6)
45–65, *n* (%)	46/112 (41.1)	52/110 (47.3)
>65, *n* (%)	54/112 (48.2)	43/110 (39.1)
Female, *n* (%)	33/112 (29.5)	35/110 (31.8)
Race, *n* (%)		
White	46/112 (41.1)	53/110 (48.2)
Black	21/112 (18.8)	15/110 (13.6)
Other*	45/112 (40.2)	42/110 (38.2)
Ethnicity, Hispanic or Latino, *n* (%)	41/112 (36.6)	47/110 (42.7)
BMI, *n* (%)		
<30	47/112 (42.0)	38/110 (34.5)
30–40	49/112 (43.8)	48/110 (43.6)
⩾40	16/112 (14.3)	24/110 (21.8)
Coexisting illness, *n* (%)		
Hypertension	65/110 (59.1)	63/107 (58.9)
Diabetes mellitus	46/109 (42.2)	42/107 (39.3)
Renal disease	14/109 (12.8)	13/107 (12.1)
Prior pulmonary disease	18/109 (16.5)	15/106 (14.2)
Heart failure	6/109 (5.5)	4/108 (3.7)
Cancer	12/109 (11.0)	11/106 (10.4)
History of smoking/e-cigarette/vaping	35/101 (34.7)	31/101 (30.7)
COVID-19 medications, *n* (%)		
Remdesivir	76/112 (67.9)	74/110 (67.3)
Convalescent plasma	25/112 (22.3)	30/110 (27.3)
IL-6 inhibitor	6/112 (5.4)	5/110 (4.5)
Glucocorticoid	92/112 (82.1)	96/110 (87.3)
Adjunctive medications, *n* (%)		
Antibiotics	92/112 (82.1)	83/110 (75.5)
Anticoagulation	103/112 (92.0)	106/110 (96.4)
Antiplatelets	22/112 (19.6)	20/110 (18.2)
ACE inhibitor or ARB	4/112 (3.6)	1/110 (0.9)
Neuromuscular blockade or paralytic	75/112 (67.0)	71/110 (64.5)
Pulmonary vasodilators	16/112 (14.3)	15/110 (13.6)
Disease severity, *n* (%)		
Moderate	79/112 (70.5)	76/110 (69.1)
Severe	33/112 (29.5)	34/110 (30.9)
SOFA score, *n* (%)	6.6 ± 2.1	6.7 ± 1.9
D on ventilation before randomization, median (IQR)	1.0 (1.0–2.0)	1.0 (1.0–2.0)
Use of prone ventilation, *n* (%)	47/112 (42.0)	50/110 (45.5)
Labs, median (IQR)		
C-Reactive protein, mg/dl	13.0 (6.6–21.5)	15.2 (8.8–22.3)
IL-6, pg/ml	58.2 (23.2–248.9)	56.0 (22.1–205.2)
IL-8, pg/ml	22.4 (16.5–36.5)	24.9 (14.5–33.6)
Creatinine, mg/dl	0.9 (0.7–1.3)	1.0 (0.7–1.4)
Blood urea nitrogen, mg/dl	26.0 (19.0–37.0)	28.0 (21.0–40.0)
Lymphocytes, %	7.0 (4.5–9.8)	5.5 (3.7–9.5)
Neutrophil–lymphocyte ratio, %	12.1 (8.5–19.3)	15.3 (8.6–25.8)
Aspartate aminotransferase, U/L	38.0 (29.0–61.0)	32.0 (26.0–52.5)
Alanine aminotransferase, U/L	30.0 (21.5–46.0)	33.0 (22.0–55.0)

Definition of abbreviations: ACE = angiotensin-converting-enzyme; ARB = angiotensin receptor blocker; BMI = body mass index; COVID-19 = coronavirus disease; IQR = interquartile range; MSC = mesenchymal stromal cell; SOFA = Sequential Organ Failure Assessment.
Categorical measures are presented as number/number observed (%), and continuous measures are presented as median (IQR) or mean ± SD.
*"Other" race includes seven unknowns in the MSC group and six in the placebo group.

all randomized subjects who received any amount of study product. All other endpoints were evaluated in the intent-to-treat population. There was no formal correction of the type I error rate for multiple testing, as prespecified. As such, point estimates of treatment effects for secondary endpoints are presented with 95% CIs that have not been adjusted for multiplicity. Analyses were conducted using SAS version 9.4 (SAS Institute, Inc.).

Results

Patients

From April 30 to December 14, 2020, 223 patients were randomized across 20 U.S. sites (Figure 1). At the third interim analysis, randomization, but not follow-up, was halted by the data safety monitoring board because of a low predicted probability of observing any benefit of MSCs for the primary endpoint. The final analysis included 222 randomized patients (112 in the MSC and 110 in the control group). One patient was excluded from the study because the patient's spokesperson, who signed the consent, was determined not to be a legally authorized representative. The mean age was 61.8 ± 13.0 years in the MSC group and 59.6 ± 13.8 years in the control group, with approximately 30% of patients categorized with severe ARDS (Table 1). Concomitant therapies included remdesivir in two-thirds of patients, corticosteroids in over 80% of patients, and anticoagulation in over 90% of patients at the time of enrollment. Median CRP was 13.0 in MSC recipients and 15.2 in control patients. Among MSC recipients, 107 (95.5%) received both infusions, 3 (2.7%) received only the first infusion, and 2 (1.8%) received no infusion. In the control group, 100 (90.9%) patients received both infusions, 7 (6.4%) received only one infusion, and 3 (2.7%) received none (Table E1 in the supplemental appendix).

Mortality

At 30 days after randomization, 42 (37.5%) patients with MSC compared with 47 (42.7%) control patients had died (relative risk (RR), 0.88; 95% CI, 0.64–1.21; $P = 0.43$) (Table 2). One MSC patient withdrew before infusion and was imputed as a death. Figure 2A depicts survival at 60 and 90 days (hazard ratio, 0.89; 95% CI, 0.62–1.28).

Days Alive off Mechanical Ventilatory Support

At 60 days, the number of patients alive free from ventilator support was 49/110 (44.5%) in the MSC and 47/110 (42.7%) in the control group. Among survivors, the median days on the ventilator within 60 days was 13.5 (IQR, 7.5–35) in MSC and 14.5 (interquartile range (IQR), 9–37) in control patients. In a ranked

Table 2. Mortality, Hospitalizations, and Adverse Events

	MSC (*n* = 112)		Placebo (*n* = 110)		
Mortality*	*n* (%)		*n* (%)		Relative Risk (95% CI)
Primary endpoint, died by D 30	42 (37.5)		47 (42.7)		0.88 (0.64–1.21)
Died by D 7	6 (5.4)		5 (4.5)		1.18 (0.37–3.75)
Died by D 14	21 (18.8)		24 (21.8)		0.86 (0.51–1.45)
	MSC† (*n* = *44*; Patient D = *1,604*)		Placebo† (*n* = *47*; Patient D = *1,511*)		
	Patients, *n* (%)	Events (Rate per 30 Pt D), *n*	Patients, *n* (%)	Events (Rate per 30 Pt D), *n*	Relative Rate (95% CI)
Readmissions at 60 d	2 (4.5)	4 (0.075)	3 (6.4)	3 (0.060)	1.26 (0.22–7.29)
Serious AEs at 30 d	MSC‡ (*n* = *110*; Patient D = *2,807*)		Placebo‡ (*n* = *107*; Patient D = *2,646*)		Relative Rate (95% CI)
	Patients, *n* (%)	Events (Rate per 30 Pt D), *n*	Patients, *n* (%)	Events (Rate per 30 Pt D), *n*	
Neoplasm/tumorigenesis	1 (0.9)	1 (0.011)	0	0	—
Cardiac arrhythmias, sustained ventricular arrhythmia	1 (0.9)	1 (0.011)	1 (0.9)	1 (0.011)	0.94 (0.06–14.81)
Cardiac arrhythmias, sustained supraventricular arrhythmia	14 (12.7)	14 (0.150)	6 (5.6)	6 (0.068)	2.20 (0.86–5.60)
Cardiac arrhythmias, type not specified	1 (0.9)	1 (0.011)	0	0	—
Deterioration of respiratory status	31 (28.2)	31 (0.331)	26 (24.3)	30 (0.340)	0.97 (0.62–1.53)
Hepatic dysfunction	2 (1.8)	2 (0.021)	1 (0.9)	1 (0.011)	1.89 (0.17–20.63)
Major infection, localized	16 (14.5)	18 (0.192)	21 (19.6)	21 (0.238)	0.81 (0.44–1.50)
Major Infection, sepsis	16 (14.5)	17 (0.182)	17 (15.9)	18 (0.204)	0.89 (0.46–1.72)
Multisystem organ failure	2 (1.8)	2 (0.021)	4 (3.7)	4 (0.045)	0.47 (0.09–2.56)
Myocardial infarction	0	0	2 (1.9)	2 (0.023)	—
Pleural effusion	1 (0.9)	1 (0.011)	3 (2.8)	4 (0.045)	0.24 (0.02–2.30)
Psychiatric episode	1 (0.9)	1 (0.011)	0	0	—
Renal dysfunction, acute renal dysfunction	26 (23.6)	26 (0.278)	25 (23.4)	26 (0.295)	0.94 (0.57–1.57)
Thromboembolic event, ischemic stroke	2 (1.8)	2 (0.021)	1 (0.9)	1 (0.011)	1.89 (0.17–20.63)
Thromboembolic event, systemic thromboembolism	1 (0.9)	1 (0.011)	2 (1.9)	2 (0.023)	0.47 (0.04–5.11)
Thromboembolic event, venous thromboembolism	7 (6.4)	7 (0.075)	3 (2.8)	3 (0.034)	2.20 (0.59–8.22)
Vasodilatory state	7 (6.4)	7 (0.075)	8 (7.5)	8 (0.091)	0.82 (0.31–2.21)
Other AE	19 (17.3)	28 (0.299)	15 (14.0)	18 (0.204)	1.47 (0.72–2.99)
Pneumothorax	6 (5.5)	7 (0.075)	6 (5.6)	8 (0.091)	0.82 (0.26–2.65)
All serious AEs	68 (61.8)	167 (1.785)	70 (65.4)	153 (1.735)	1.03 (0.75–1.41)

Definition of abbreviations: AE = adverse event; CI = confidence interval; MSC = mesenchymal stromal cell.
*Per protocol, one patient in the MSC arm who withdrew before 30 days was imputed as a death.
†A total of 129 patients was excluded from readmission analyses (66 in the MSC group and 63 in the placebo group) because they died during the index admission or were not discharged by Day 60. Two additional patients (both in the MSC arm) are excluded for early withdrawal or unavailable index hospitalization discharge data.
‡Safety endpoints are analyzed using the safety population, which is defined as all randomized subjects who received any amount of study product.

analysis incorporating deaths and withdrawals, there were no significant between-group differences in days alive without ventilator support (Figures 2B and 2C).

Clinical Improvement and Resolution/Improvement of ARDS

At 30 days, 37/111 (33.3%) MSC patients and 34/110 (30.9%) control patients showed a two-point improvement from baseline on the seven-point ordinal scale or were discharged alive (OR, 1.17; 95% CI, 0.42–3.22) (Figure E1A, supplemental appendix). Resolution or improvement of ARDS at 30 days was observed in 51/104 (49.0%) MSC recipients and 46/106 (43.4%) in control patients (OR, 1.36; 95% CI, 0.57–3.21) (Figure E1B, supplemental appendix).

Adverse Events and Hospitalizations

There was no between-group difference in serious adverse events over 30 days (Table 2). No infusion-related toxicity events were observed during the course of the study. In a ranked analysis, there was no difference between groups in the index LOS or ICU days (Figures E2 and E3, supplemental appendix). Among survivors, median LOS was 25 days (IQR, 15–43) in the MSC group versus 26 days (IQR, 19–47) in the control group, with median ICU days of 18 (IQR, 11–30) and 21 (IQR, 12–36), respectively. There was no between-group difference in readmissions (Table 2) or the expanded total LOS through 60 days (Figure E4, supplemental appendix).

Twelve-Month Mortality and Pulmonary Symptoms

At 12 months, mortality remained similar between groups (hazard ratio, 0.91; 95% CI, 0.63–1.30) (Figure E5, supplemental

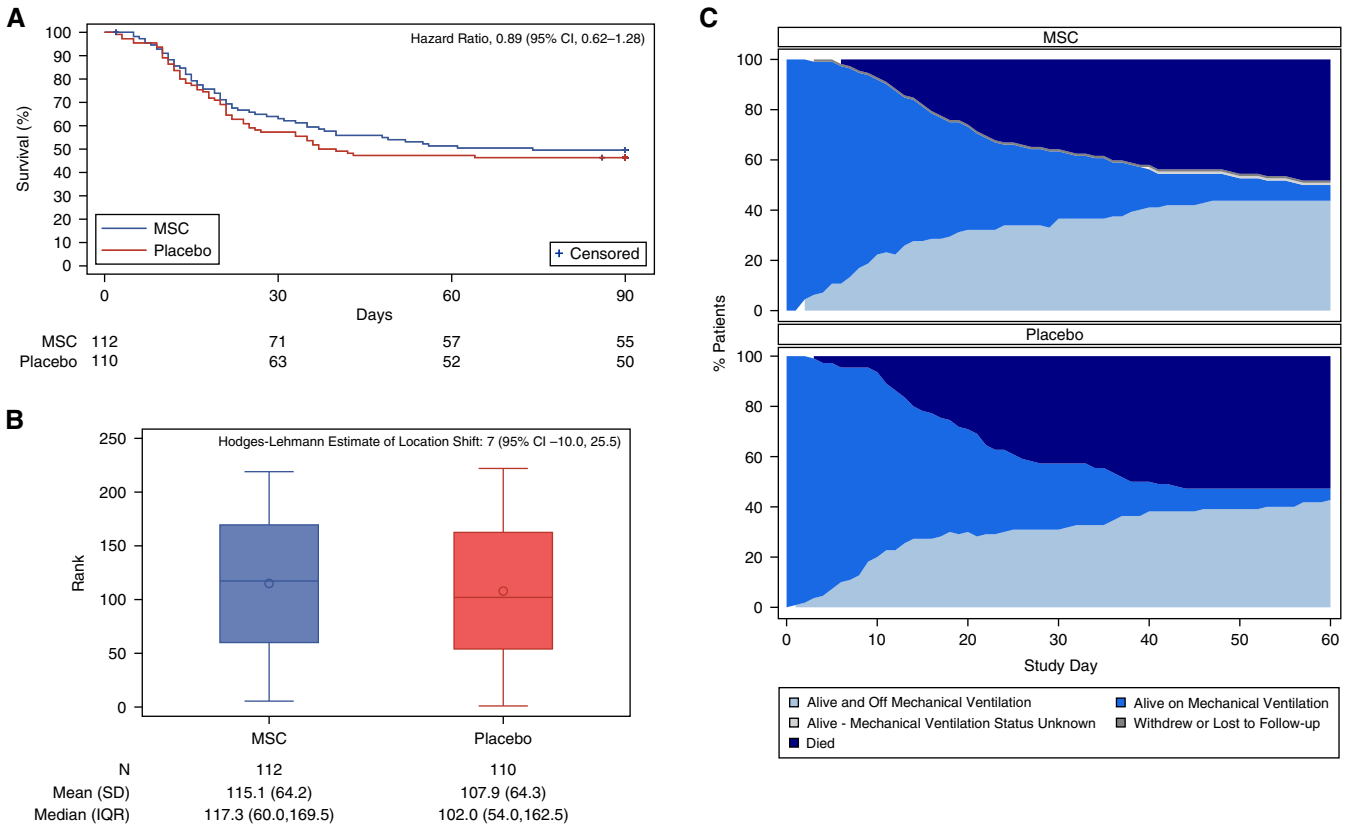

Figure 2. Survival and key secondary endpoint. (*A*) Depicts survival by randomization group. The tick marks show the censoring of data. (*B*) Depicts the distributions of rank by randomization group from the rank-based assessment of the key secondary endpoint, days alive, and free of mechanical ventilation at Day 60. Higher ranks correspond to better outcomes (i.e., fewer d on mechanical ventilation), and deaths were assigned the worst ranks in order of time of death. (*C*) Depicts the proportion of patients alive and off mechanical ventilation, alive on mechanical ventilation, alive with unknown ventilation status, withdrawn, or died, by randomization group over each study day from randomization through Day 60. CI = confidence interval; IQR = interquartile range; MSC = mesenchymal stromal cell.

appendix) and relatively unchanged from mortality at 90 days. By 12 months, the incidence of asthma, chronic obstructive pulmonary disease, emphysema, and pulmonary fibrosis was low across both groups (Table E2, supplemental appendix). At 12 months, none of the survivors was supported with invasive or noninvasive mechanical ventilation. At this time point, the use of supplementary oxygen was greater in the MSC group, but the numbers are small, and the confidence interval spans one (7/46 [15.2%] vs. 2/47 [4.3%]; RR, 3.58; 95% CI, 0.78–16.32) (Table E3, supplemental appendix).

Subgroups

In a subgroup analysis stratified by ARDS severity, the RR of 30-day death with MSCs compared with sham control was 0.87 (95% CI, 0.57–1.31) in patients with moderate ARDS and 0.91 (95% CI, 0.55–1.50) in those with severe ARDS. Figure 3 depicts descriptive prespecified subgroup analyses, such as age, ethnicity, and diabetes, of the primary endpoint (*see* Figure E6, supplemental appendix, for a similar analysis of 90-day mortality).

Discussion

Patients with COVID-19–related ARDS have only a limited array of therapeutic options and, as this trial demonstrates, continue to experience very high mortality rates. This need has galvanized an intensive search for new candidate therapeutics and motivated the current effort to evaluate a fixed MSC dosing regimen for this condition. This trial that was halted at the third interim analysis for futility showed no significant difference in 30-day mortality with the use of MSCs versus sham control nor days off mechanical ventilation within 60 days of randomization. In terms of safety, there were no infusion-related toxicities or differences in serious adverse events over 30 days after randomization. Survival out to 12 months was not different between the MSC and sham control groups.

MSCs have potential therapeutic applications for the treatment of ARDS through a variety of mechanisms, including immune modulation, alveolar fluid clearance, bacterial clearance, regulation of pulmonary vascular endothelial permeability, and suppression of apoptosis (1). Preclinical models of ARDS have supported the safety and efficacy of MSC therapy for the treatment of lung injury (19–21); however, MSC therapy in patients with ARDS remains investigational. Two phase I trials have reported no safety concerns in administering MSCs to patients with ARDS (13, 22). In a randomized trial of 60 patients, Matthay and colleagues concluded that administration of MSCs was safe in patients with non–COVID-19–related

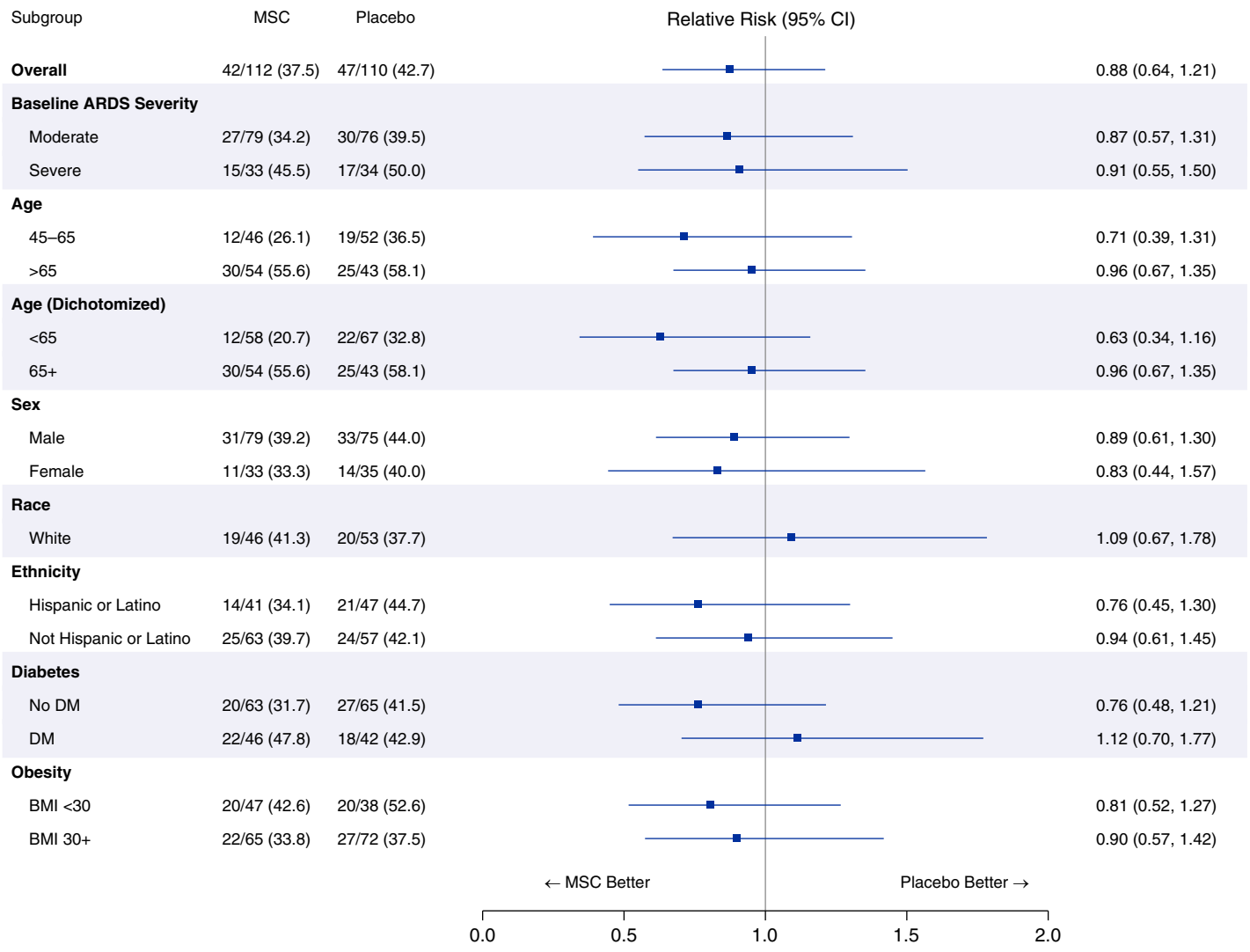

Figure 3. Subgroup analyses of the primary endpoint. Subgroup analyses of the primary endpoint in key clinical subgroups were prespecified. Per protocol, strata in which the number of patients assigned to a specific group is less than 20 were not considered. ARDS = acute respiratory distress syndrome; BMI = body mass index; CI = confidence interval; DM = diabetes mellitus; MSC = mesenchymal stromal cell.

moderate to severe ARDS (12). Overall 28-day mortality did not differ significantly between groups in that trial. However, those results may have been affected by the poor overall viability of MSCs used in that study (36–85%). Preliminary observational studies with MSCs in the setting of COVID-19 ARDS have demonstrated a signal of therapeutic benefit (23, 24).

There are several potential explanations for the results observed in this trial. These include an incomplete knowledge of COVID-19–related ARDS pathophysiology and the rapid evolution of COVID-19–related ventilatory and pharmacologic management practices during the trial. A key exclusion criterion concerned the duration of mechanical ventilation (>72 h) before enrollment. This stipulation was intended to ensure the administration of MSCs during the peak of the cytokine storm and before the development of end-stage, irreversible parenchymal lung damage. However, the timing of endotracheal intubation in the course of illness changed during trial conduct. In the first few months of the pandemic, guidelines recommended intubation and invasive mechanical ventilation when oxygen requirements on the nasal cannula reached 6 L/min (25). This guidance was driven largely by concern for aerosol generation with noninvasive respiratory support and the risk of rapid patient deterioration (26). As the COVID-19 pandemic progressed, concerns that high-flow nasal cannula could result in the dissemination of dangerous concentrations of infected aerosols receded (27). Clinicians also realized there were potential benefits to deferring or delaying mechanical ventilation, including avoidance of sedation, reduction in the time patients are immobilized, and minimization of patient–clinician communication challenges. Within 2–3 months of study initiation, increasing numbers of COVID-19 patients were managed with noninvasive respiratory support for days or weeks before intubation (28). This change in ventilatory management practices that affected a substantial number of enrolled patients meant that those who

failed noninvasive ventilation and eventually required intubation may have progressed to the point of inflammatory parenchymal lung damage that was more developed and possibly less modifiable by the time of enrollment.

An additional factor to consider relates to the potential heterogeneity of the patient population enrolled. With regard to non–COVID-19–induced ARDS, researchers have described two distinct subphenotypes, hyperinflammatory or reactive and hypoinflammatory or uninflamed (29, 30). These subphenotypes differ in biomarker profiles, disease course, and, more importantly, the response to ARDS management strategies and outcomes. The hyperinflammatory subphenotype is characterized by increased concentrations of proinflammatory biomarkers, including IL-6, IL-8, and TNFR-1, decreased serum bicarbonate concentrations, vasopressor dependence, and the presence of sepsis (31). This subphenotype is also associated with higher mortality and fewer ventilator-free and organ failure-free days compared with the hypoinflammatory subphenotype (32). Recently, an exploratory study showed evidence that hyperinflammatory and hypoinflammatory subphenotypes may exist in COVID-19–induced ARDS (33). Chen and colleagues showed a significant survival benefit with corticosteroids in the hyperinflammatory subphenotype but no effect in the hypoinflammatory cohort (34). The proportion of such distinct subphenotypes among patients with COVID-19 enrolled in this trial, and any associated differential treatment effects may have contributed to the neutral findings. Serial inflammatory biomarkers were collected during the study, and planned analyses might help identify ARDS subphenotypes among enrolled patients, which require further study.

In defining these subphenotypes, age may also play a role as reflected in the signal of survival benefit seen in those under 65 years in the forest plot; *see* results section and 90-day mortality in Appendix. The absence of a signal in older patients is consistent with the fact that they have a longer duration of SARS-CoV-2 viral clearance because of age-related maladaptive immune responses that is associated with higher degrees of inflammation in the lungs (35–40). Consequently, it is possible that higher or more prolonged dosing with MSC would be required in older patients relative to younger patients to achieve similar survival benefits and may have affected the results of this trial. Although the lower dose may be sufficient in younger patients, future studies should explore age-related dosing.

This trial has several limitations. Disease management practices during the pandemic changed rapidly, and the protocol could not practically be modified at the same pace. Eligibility criteria reflected the state of clinical knowledge at the time of protocol design and did not account for prospective identification of subphenotypes of patients, which may have helped identify treatment responders. Moreover, as the timing of intubation changed from early in the cytokine storm to later in the disease course, the use of days on a ventilator as a marker of pulmonary parenchymal disease became imperfect. We did not collect information on the duration of noninvasive respiratory management before randomization to characterize this population further. Furthermore, the design of this phase II/III trial was on the basis of the expectation of a relatively high mortality reduction (similar to the one subsequently found in the Randomised Evaluation of COVID-19 Therapy [RECOVERY] trial) (4). Whereas a smaller relative mortality reduction would have been clinically meaningful, such a trial would have required the enrollment of several thousand patients, which would have been challenging within the constraints of the pandemic. Finally, because the trial was halted early, our ability to detect differences in secondary endpoints and subgroup analyses was reduced. Given that ARDS is a highly lethal disease with minimal treatment options, the signals identified in this trial deserve further exploration in future research.

Conclusions

In our trial, MSC therapy, compared with sham control, in patients with moderate to severe COVID-19–related ARDS did not produce the hypothesized reduction in 30-day mortality or improve ventilator-free days over 60 days after randomization. During the pandemic, new insights emerged into the existence of different inflammatory subtypes and the importance of age within the overall COVID-19–related ARDS population, which might underlie a differential response to immunomodulatory therapy. Future research is needed to identify the potential benefits of MSCs in susceptible phenotypes. ■

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