ORIGINAL ARTICLE

Repair of Acute Respiratory Distress Syndrome in COVID-19 by Stromal Cells (REALIST-COVID Trial)

A Multicenter, Randomized, Controlled Clinical Trial

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Abstract

Rationale: Mesenchymal stromal cells (MSCs) may modulate inflammation, promoting repair in coronavirus disease (COVID-19)–related acute respiratory distress syndrome (ARDS).

Objectives: We investigated the safety and efficacy of ORBCEL-C (CD362 [cluster of differentiation 362]–enriched, umbilical cord–derived MSCs) in COVID-19–related ARDS.

Methods: In this multicenter, randomized, double-blind, allocation-concealed, placebo-controlled trial (NCT 03042143), patients with moderate to severe COVID-19–related ARDS were randomized to receive ORBCEL-C (400 million cells) or placebo (Plasma-Lyte 148). The primary safety and efficacy outcomes were the incidence of serious adverse events and oxygenation index at Day 7, respectively. Secondary outcomes included respiratory compliance, driving pressure, Pa_{O_2}:FI_{O_2} ratio, and Sequential Organ Failure Assessment score. Clinical outcomes relating to duration of ventilation, lengths of ICU and hospital stays, and mortality were collected. Long-term follow-up included diagnosis of interstitial lung disease at 1 year and significant medical events and mortality at 2 years. Transcriptomic analysis was performed on whole blood at Days 0, 4, and 7.

Measurements and Main Results: Sixty participants were recruited (final analysis: $n = 30$ received ORBCEL-C, $n = 29$ received placebo; 1 participant in the placebo group withdrew consent). Six serious adverse events occurred in the ORBCEL-C group and three in the placebo group (risk ratio, 2.9 [95% confidence interval, 0.6–13.2]; $P = 0.25$). Day 7 mean (SD) oxygenation index did not differ (ORBCEL-C, 98.3 [57.2] cm H_2O/kPa; placebo, 96.6 [67.3] cm H_2O/kPa). There were no differences in secondary surrogate outcomes or in mortality at Day 28, Day 90, 1 year, or 2 years. There was no difference in the prevalence of interstitial lung disease at 1 year or significant medical events up to 2 years. ORBCEL-C modulated the peripheral blood transcriptome.

Conclusion: ORBCEL-C MSCs were safe in subjects with moderate to severe COVID-19–related ARDS but did not improve surrogates of pulmonary organ dysfunction.

Keywords: acute respiratory distress syndrome; coronavirus disease; mesenchymal stromal cells; clinical trial

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Author Contributions: D.F.M. and C.M.O'K. conceived the study. All authors made substantial contributions to the protocol development and/or conduct of the study. C.C. and C.M. are the trial statisticians and have verified the clinical trial data included in this report. J.R. and M.S.-H. conducted the transcriptomic analysis and its interpretation and have verified data related to this. L.A.-R. and G.A. conceived and contributed to the conduct and interpretation of the deconvolution analysis. D.F.M., E.A.G. and C.M.O'K. prepared the first draft of the manuscript. All authors contributed to the writing of the report and reviewed and approved the final version.

Data sharing statement: Data will be available to researchers on request subject to sponsor approval.

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Acute respiratory distress syndrome (ARDS) is characterized by acute hypoxemic respiratory failure with bilateral radiographic opacities, not fully explained by cardiac failure or fluid overload, due to inflammation-mediated destruction of the epithelial–endothelial barrier (1, 2). Incidences of ARDS in hospitalized patients with coronavirus disease (COVID-19) of 17–68% have been reported (3). Although supportive therapy has been the mainstay of treatment for ARDS due to COVID-19 (4), recent trials have demonstrated that corticosteroids and IL-6 receptor antagonists reduce mortality (5–7). However, mortality remains unacceptably high (6), with a continued need to identify therapeutic agents in COVID-19–related ARDS (8).

Mesenchymal stromal cells (MSCs) are a potential novel therapeutic in ARDS because of their pleiotropic immunomodulatory and reparative properties (9, 10). Mechanisms of MSC actions include paracrine secretion of immunomodulatory factors (11, 12) and an ability to transfer functional mitochondria to damaged cells (including alveolar epithelial cells [13] and immune cells [14, 15]). Preclinical studies have demonstrated that MSCs can repair lung injury, restore alveolar fluid clearance, and improve lung compliance and oxygenation (16–18). Before the COVID-19 pandemic, MSCs had been investigated in early-phase clinical trials in ARDS, which suggested that MSCs were safe in this patient population (19–24). Since this trial began, several trials have investigated MSC products in patients with COVID-19–related ARDS and have similarly supported their safety (25–27).

Variation in manufacturing techniques can alter MSC function (28–30), so that MSC products are not necessarily equivalent. ORBCEL-C consists of a population of CD362 (cluster of differentiation 362)–enriched allogeneic umbilical cord–derived MSCs, which have shown potent activity in *in vitro* and *in vivo* models of ARDS (28, 31–33). In addition to high efficacy, this product offers the advantage of being more homogeneous and better characterized than MSCs isolated using the traditional method of plastic adherence. As a source of MSCs, the umbilical cord is cost efficient, readily available (usually disposed of as a waste product), and not associated with risks to the donor.

Before the REALIST (Repair of Acute Respiratory Distress Syndrome by Stromal Cell Administration) research program, ORBCEL-C had not previously been investigated in humans, though clinical trials of similar MSC products are now underway in patients with diabetic kidney disease (ORBCEL-M; NEPHSTROM [Novel Stromal Cell Therapy for Diabetic Kidney Disease], NCT 02585622) and liver disease (ORBCEL-C; MERLIN [Selected Mesenchymal Stromal Cells to Reduce Inflammation in Patients with PSC and AIH], NCT 02997878). In the phase 1 REALIST dose-finding study, patients with ARDS (unrelated to COVID-19) received escalating doses of a single intravenous infusion of ORBCEL-C MSCs (three cohorts of three patients receiving 100×10^6, 200×10^6, or 400×10^6 MSCs). There was no dose-limiting toxicity in any dose cohort (34). The aim of the REALIST phase 2 trial is to investigate the safety and efficacy of a single intravenous infusion of 400×10^6 ORBCEL-C MSCs in patients with moderate to severe ARDS. In this study, we assessed the effect of ORBCEL-C on lung physiological measurements, clinical outcomes, and the whole-blood

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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.

At a Glance Commentary

Scientific Knowledge on the Subject: Mesenchymal stromal cells (MSCs) have been tested as a possible therapy for acute respiratory distress syndrome (ARDS) because of their antiinflammatory, proresolution, and antimicrobial effects. Phase 2 studies to date in ARDS related to coronavirus disease (COVID-19) have shown conflicting results. An insufficient MSC dose and inhibition of MSC activity by corticosteroids used in the treatment of COVID-19–related ARDS have been suggested as reasons for lack of efficacy, and the longer term effects of MSCs in this population have not been studied.

What This Study Adds to the Field: We tested the largest dose of MSCs in COVID-19–related ARDS to date. Although well tolerated, this higher dose of MSCs did not affect surrogate markers of pulmonary dysfunction and was associated with a longer duration of ventilation. The longer duration of ventilation in MSC-treated patients was not associated with an increased incidence of interstitial lung disease at 12-month follow-up. Mortality was similar in MSC- and placebo-treated patients. Although the cohort had high rates of corticosteroid prescription, MSCs still drove significant changes in peripheral blood transcriptome, but this did not translate to clinical efficacy.

transcriptome, with long-term follow-up, in a cohort of patients with ARDS due to COVID-19. Recruitment to an additional cohort of patients with ARDS unrelated to COVID-19 is ongoing and will be reported separately. Some of these results have been reported in abstract form at conference meetings (35, 36).

Methods

Study Design and Population

REALIST-COVID (NCT 03042143) was a multicenter randomized, double-blind, allocation-concealed, placebo-controlled trial of ORBCEL-C MSCs in patients with ARDS due to COVID-19 in 12 ICUs across the United Kingdom. Full details of the study design have been published (37) and are available in the online supplement. The trial was sponsored by Belfast Health and Social Care Trust and approved by the North-East York research ethics committee (18/NE/0006) and the Medicines and Healthcare Products Regulatory Agency (European Union Drug Regulating Authorities Clinical Trials Database number 2017-000584-33).

In brief, after informed consent was obtained, eligible patients who were mechanically ventilated within 72 hours of the onset of moderate to severe ARDS due to COVID-19 were randomized (1:1) to receive a single intravenous infusion of either ORBCEL-C (400×10^6 CD362-enriched umbilical cord–derived MSCs in 200 ml Plasma-Lyte 148; *see* the online supplement for manufacturing details, including cell viability assessment) or placebo (Baxter Healthcare Ltd.) (200 ml Plasma-Lyte 148). All other aspects of care were according to standard critical care guidelines (4).

Trial outcomes included both primary safety and primary efficacy outcomes. The primary safety outcome was the incidence of serious adverse events. Adverse events were collected until Day 90, and details of adverse event reporting are available in the online supplement. The primary efficacy outcome was oxygenation index (OI) at Day 7 (calculated as [mean airway pressure (cm H_2O) $\times$ FI_{O_2} $\times$ 100]/Pa_{O_2} [kPa]). OI is a physiological index of the severity of ARDS that measures both impaired oxygenation and the amount of mechanical support delivered. OI independently predicts outcome in patients with ARDS (38) and is widely reported as a surrogate outcome in ARDS trials (20, 39–41). Secondary surrogate outcomes included indices of pulmonary and nonpulmonary organ dysfunction: OI at Days 4 and 14; respiratory compliance, driving pressure, and Pa_{O_2}:FI_{O_2} (PF) ratio on Days 4, 7, and 14; and organ failure as measured using the Sequential Organ Failure Assessment score on Days 4, 7, and 14. Clinical outcome measures included extubation, reintubation, ventilator-free days (VFDs) to Day 28, duration of ventilation, lengths of ICU and hospital stays, and 28- and 90-day mortality. Long-term follow-up was conducted for mortality, significant medical events, and evidence of pulmonary dysfunction and interstitial lung disease on clinically indicated thoracic computerized tomography (CT) scans and pulmonary function tests.

Whole-blood total RNA sequencing was performed on samples collected at Days 0, 4, and 7 (*see* the online supplement for details). Other exploratory translational studies detailed in the trial protocol were not conducted in this cohort, because of the United Kingdom's regulatory requirement to process all research samples from patients with COVID-19 in a containment level 3 laboratory environment during this phase of the pandemic.

Statistical Analysis

On the basis of our data from a previous study in ARDS, the mean (SD) OI at Day 7 in patients with ARDS is 62 (51) cm H_2O/kPa (42). A sample size of 56 subjects (randomized 1:1) would have 80% power at a two-tailed significance level of 0.05 using a two-sample *t* test to detect a clinically significant difference between groups in OI at Day 7, the primary efficacy outcome, of 39 cm H_2O/kPa (38). In previous United Kingdom multicenter studies in the critically ill, fewer than 3% of subjects withdrew consent or were lost to follow-up (43, 44); therefore the sample size was inflated to 60 patients to allow for a dropout rate of 5%.

The primary efficacy analysis was on an intention-to-treat basis and was conducted on the last available data carried forward (imputed values). Sensitivity analyses were conducted by imputing extreme values (minimum and maximum) and mean substitution. *A priori*–defined subgroup analyses were undertaken for the primary efficacy outcome and selected secondary outcomes (VFDs at Day 28 and 28-day mortality) on the basis of the severity of inflammation (C-reactive protein and ferritin [45]), as well as PF ratio. Statistical analysis was conducting using Stata version 15.1 (StataCorp LLC). The statistical analysis plan, which was finalized before recruitment to the study was completed, is provided in the online supplement.

Results

Patients

From April 2, 2020, to December 4, 2020, 193 patients were screened for eligibility, of whom 133 were excluded (Figure 1). Screening and recruitment data at each site are described in Figure E1 in the online

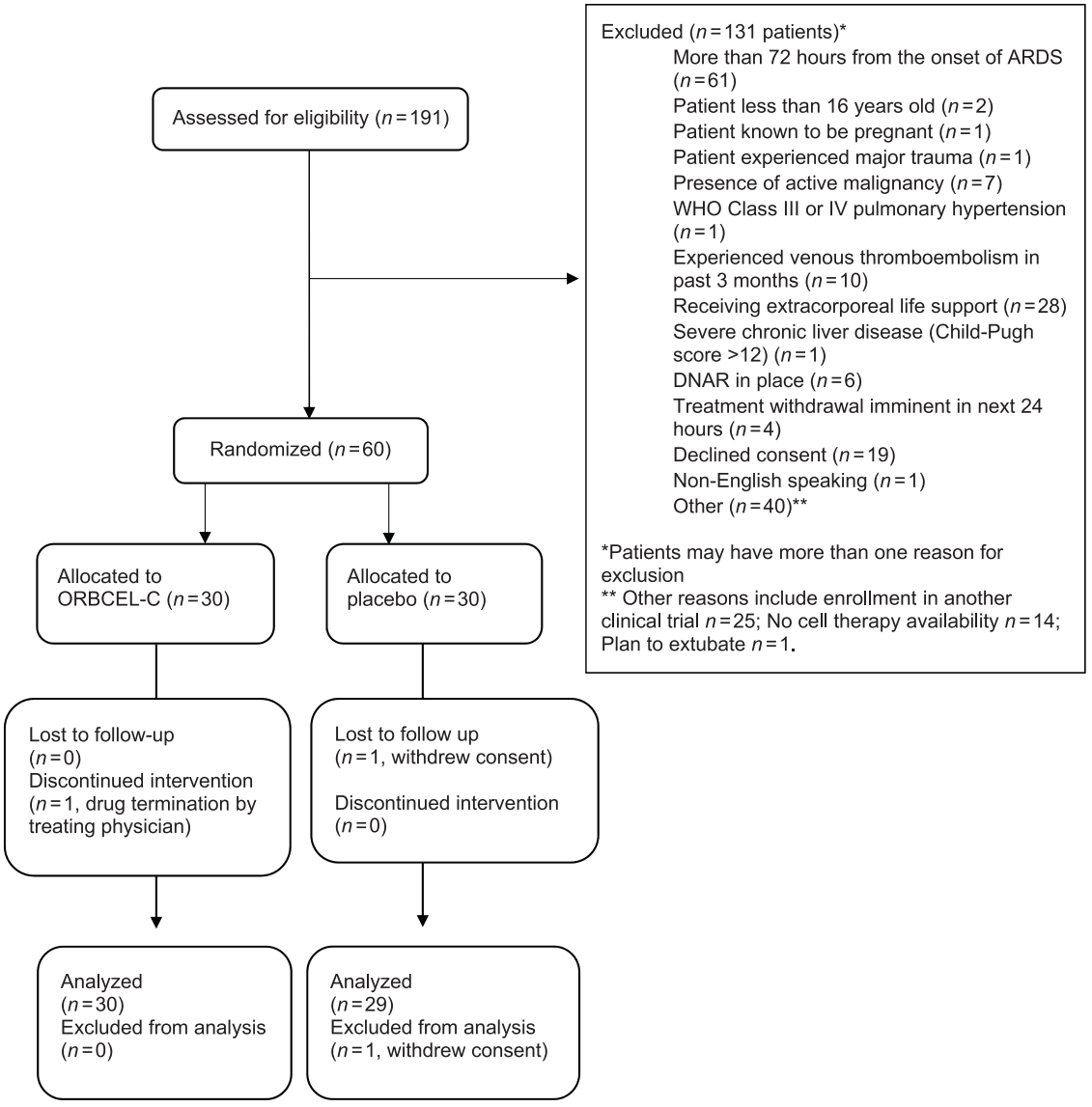


Figure 1. Consolidated Standards of Reporting Trials flowchart. ARDS = acute respiratory distress syndrome; DNAR = do not attempt resuscitation; WHO = World Health Organization.

supplement. Sixty patients were recruited, with 30 allocated to each group (Figure 1). One patient in the placebo group subsequently withdrew consent and was excluded from the analysis. The primary outcome was therefore available for 59 patients (30 in the ORBCEL-C group and 29 in the placebo group).

Baseline patient characteristics and supportive care were similar between the groups (Table 1), and patients were typical of a critically ill population with COVID-19 (6), with predominant respiratory failure indicated by the low PF ratio and high OI and comparatively low Sequential Organ Failure Assessment score. Corticosteroid use was similar in both groups (90% in the ORBCEL-C group, 89.7% in placebo group). Approximately half of patients in each group received antiviral therapy. No patients in this trial received IL-6 receptor antagonists or convalescent plasma. Concomitant medications and adjuvant therapies are detailed in Tables E1a and E1b. There were no differences between groups in the time of study drug administration measured from ICU admission, mechanical ventilation initiation, or thaw of the study drug (*see* Table E2).

Primary Safety Outcomes

There were six serious adverse events in the ORBCEL-C group and three in the placebo group (risk ratio, 2.9 [95% confidence interval, 0.6–13.2]; $P = 0.25$) (Table 2). Study drug administration was well tolerated, with no significant difference between the groups in postinfusion (up to 5 h) hemodynamics, arterial blood gases, positive end-expiratory pressure, plateau pressure, or temperature (*see* Figure E2). The incidence of adverse events was similar in both groups. No serious adverse events were reported related to the study drug. One patient in the ORBCEL-C group had pyrexia within 24 hours of the study drug administration, reported as a prespecified infusion-related adverse event and classified as an adverse reaction. One patient in the placebo group had pyrexia,

Table 1. Baseline Characteristics

	ORBCEL-C ($n = 30$)	Placebo ($N = 29$)	Total ($N = 59$)
Gender, male	24 (80.0%)	20 (69.0%)	44 (74.6%)
Age, yr	58.4 (9.2)	58.4 (12.5)	58.4 (10.8)
Weight, kg	93.1 (21.9)	92.8 (19.5)	93.0 (20.6)
Height, cm	168.8 (10.6)	168.4 (10.1)	168.6 (10.3)
PBW, kg	64.1 (10.7)	63.2 (10.8)	63.6 (10.7)
Temperature, °C	37.1 (1.2)	36.7 (1.0)	36.9 (1.1)
COVID-19 diagnosis			
Clinical	2 (6.7%)	3 (10.3%)	5 (8.5%)
Assay	28 (93.3%)	26 (89.7%)	54 (91.5%)
Ethnicity			
White	23 (76.7%)	20 (69.0%)	43 (72.9%)
Black	3 (10.0%)	2 (6.9%)	5 (8.5%)
Asian	3 (10.0%)	6 (20.7%)	9 (15.3%)
Unknown	0 (0.0%)	0 (0.0%)	0 (0.0%)
Other	1 (3.3%)	1 (3.5%)	2 (3.4%)
Baseline severity			
APACHE II score	13.2 (5.3)	13.7 (4.6)	13.5 (4.9)
Murray lung injury score	2.8 (0.4)	2.8 (0.5)	2.8 (0.4)
First qualifying PF ratio	17.6 (4.1)	19.4 (4.9)	18.5 (4.6)
Worst PF ratio on Day 0 (24 h before randomization)	15.2 (4.2)	16.1 (5.4)	15.7 (4.8)
Total SOFA score	7.7 (3.4) ($n = 29$)	7.9 (3.1) ($n = 27$)	7.8 (3.2) ($n = 56$)
Oxygenation index	84.3 (26.0) ($n = 29$)	92.2 (44.6) ($n = 27$)	88.1 (36.0) ($n = 56$)
Lowest mean arterial pressure, mm Hg	69.7 (8.8)	70.6 (8.6)	70.1 (8.6)
Vasopressor use	17 (56.7%)	15 (51.7%)	32 (54.2%)
Ferritin	1394.5 (617.4) ($n = 13$)	1728.8 (997.0) ($n = 13$)	1561.7 (830.2) ($n = 26$)
CRP	145.3 (109.6)	136.6 (103.0) ($n = 28$)	141.1 (105.6) ($n = 58$)
Ventilatory parameters			
PEEP, cm H_2O	10.9 (2.6)	10.2 (2.7)	10.6 (2.7)
Plateau pressure, cm H_2O	24.0 (4.0) ($n = 29$)	23.6 (4.4) ($n = 27$)	23.8 (4.2) ($n = 56$)
Driving pressure, cm H_2O	13.1 (4.4) ($n = 29$)	13.3 (5.0) ($n = 27$)	13.2 (4.6) ($n = 56$)
Respiratory compliance, ml/cm H_2O	39.4 (22.3) ($n = 29$)	39.6 (21.0) ($n = 27$)	39.5 (21.5) ($n = 56$)
V_T, ml/kg PBW	7.1 (2.0)	7.5 (3.0)	7.3 (2.5)
Mode of ventilation			
SIMV	30 (100.0%)	27 (93.1%)	57 (96.6%)
PS	0 (0.0%)	2 (6.9%)	2 (3.4%)
Adjunctive therapies			
Airway pressure release ventilation	0 (0.0%)	1 (3.4%)	1 (1.7%)
High-frequency oscillatory ventilation	1 (3.3%)	0 (0.0%)	1 (1.7%)
Neuromuscular-blocking drugs	15 (50.0%)	12 (41.4%)	27 (45.8%)
Nitric oxide	1 (3.3%)	2 (6.9%)	3 (5.1%)
Prone position	8 (26.7%)	4 (13.8%)	12 (20.3%)

Definition of abbreviations: APACHE = Acute Physiology and Chronic Health Evaluation; COVID-19 = coronavirus disease; CRP = C-reactive protein; PBW = predicted body weight; PEEP = positive end-expiratory pressure; PF = Pa_{O_2}:$F_{I_{O_2}}$; PS = pressure support; SIMV = synchronized intermittent mandatory ventilation; SOFA = Sequential Organ Failure Assessment.
Mean (SD) or *n* (%) is presented.

reported as an adverse reaction. No other adverse events were considered to be related to the study drug. A summary of adverse event classifications (*see* Table E3a) and a detailed list of all adverse events reported (*see* Table E3b) are provided in the online supplement. Thromboembolic events reported as adverse events are detailed separately (*see* Tables E4a and E4b). During 1-year follow-up, one additional instance of pulmonary embolus was identified on thoracic CT in the placebo group.

Primary Efficacy Outcomes

The primary unadjusted analysis for the primary efficacy outcome, using imputed data from the last available data carried forward, revealed no difference in OI at Day 7 between the ORBCEL-C group (mean, 98.3 [SD 57.2] cm H_2O/kPa) and the placebo group (mean, 96.6 [SD 67.3] cm H_2O/kPa; mean difference, 1.8 cm H_2O/kPa [95% confidence interval, 30.7–34.4 cm H_2O/kPa]; $P = 0.91$) (Table 3). Adjusted analysis (for baseline age, PF ratio, Acute Physiology and Chronic Health Evaluation II score, vasopressor use, and site) did not alter this

Table 2. Safety Outcomes

	ORBCEL-C	Placebo	Relative Risk (95% Confidence Interval)	*P* Value
Total adverse events	23* 15 (50)†	16* 10 (34.5)†	1.5 (0.8–2.7)	0.29
Adverse reactions	1* 1 (3.3)†	1* 1 (3.4)†	0.97 (0.06–14.7)	1.00
Total serious adverse events	6* 6 (20)†	3* 2 (6.9)†	2.9 (0.6–13.2)	0.25
Serious adverse reactions	0*	0*	—	—
Suspected unexpected serious adverse reactions	0*	0*	—	—

*Number of events.
†Number of patients (percentage based on total number of patients in each group).

finding (*see* Tables E5a and E5b). Observed values for OI at Day 7 were available for 18 of 30 subjects in the ORBCEL-C group and 13 of 29 in the placebo group. Reasons for missing OI at Day 7 are listed in Table E5c; most commonly this was due to moving to pressure support ventilation. Sensitivity analysis (using multiple imputations and analysis using observed values), per protocol analysis, and analysis in the population with PCR-confirmed COVID-19 did not alter the findings (*see* Tables E5d and E5e).

There were no important differences between the ORBCEL-C and placebo groups in secondary surrogate outcomes of pulmonary and nonpulmonary organ function (Figure 2; *see* Figure E3 and Table E6). Clinical outcomes are reported in Table 4, although the study was underpowered for these. Although there was no difference in the rate of successful extubation, there was an increase in the duration of ventilation in the ORBCEL-C group (19 days [interquartile range (IQR), 13–30 days]) compared with the placebo group (12 days [IQR, 7–20 days]; hazard ratio, 0.5 [95% confidence interval, 0.3–0.9]; $P = 0.017$) (Table 4, Figure 3). Mortality at Day 28 was similar between groups (ORBCEL-C, $n = 5$ [16.7%]; placebo, $n = 6$ [20.7%]; risk ratio, 0.8 [95% confidence interval, 0.3–2.4]; $P = 0.69$], as was 90-day mortality (ORBCEL-C, $n = 7$ [23.3%]; placebo, $n = 8$ [27.6%]; risk ratio, 0.8 [95% confidence interval, 0.4–2.0]; $P = 0.71$).

ORBCEL-C had no effect on OI at Day 7 according to *a priori*–defined subgroups for baseline oxygenation (PF ratio) and inflammation (C-reactive protein and ferritin) (*see* Table E7). ORBCEL-C treatment was associated with a statistically significant higher number of VFDs in the group with PF ratios >20 kPa at baseline but not those with more impaired oxygenation (*see* Table E8).

Long-term follow-up is summarized in Table 5. One patient in the ORBCEL-C group died between Day 90 and 1 year. Two patients in ORBCEL-C group were lost to follow-up at 1 year. Forty-one survivors were remotely followed up for significant medical events at 1 year ($n = 20$ in the ORBCEL-C group, $n = 21$ in the placebo group). No further patients died at the 2-year time point. Fourty survivors were remotely followed up for significant medical events at 2 years ($n = 20$ in the ORBCEL-C group, $n = 20$ in the placebo group). One patient in the placebo group withdrew consent for follow-up at 2 years.

Reported significant medical events were similar in both groups (Table 5; *see* Table E9 for descriptions of significant medical events). Clinically indicated thoracic CT examinations were available for 13 patients (ORBCEL-C, $n = 5$ of 20; placebo, $n = 8$ of 21). CT evidence of interstitial lung disease was similar between groups. Pulmonary function test reports were available for 11 of 20 ORBCEL-C patients and 9 of 21 in the placebo group. $D_{L_{CO}}$ was reduced in both groups, with a median percentage predicted value of 62% (IQR, 57–71.5%) in the placebo group compared with 76% (IQR, 74–91.5%) in the ORBCEL-C group.

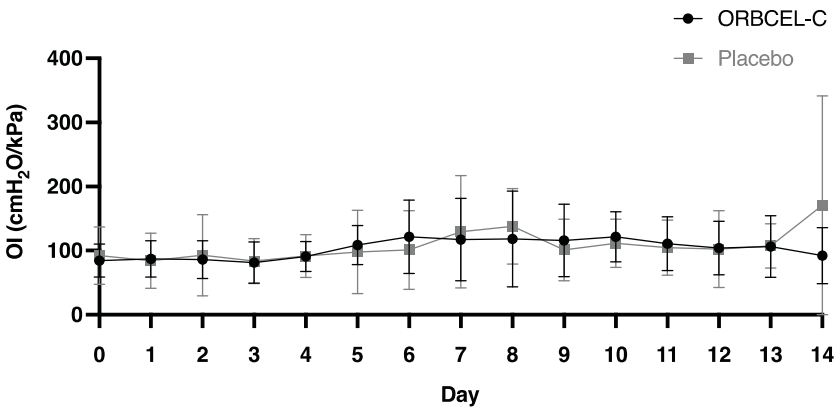
Laboratory Analysis

C-reactive protein, total peripheral white blood cell counts, and neutrophil counts were similar in both groups (*see* Figure E4). There were no trends toward differences in renal or liver function test abnormalities in response to ORBCEL-C (*see* Table E10).

Table 3. Oxygenation Index at Day 7

	ORBCEL-C ($n = 30$)	Placebo ($n = 29$)	Mean Difference (95% Confidence Interval)	*P* Value
Imputed				
Last value carried forward*	98.3 (57.2)	96.6 (67.3)	1.8 (−30.7 to 34.3)	0.91
Minimum value	91.0 (60.3)	87.7 (71.2)	3.3 (−31.0 to 37.7)	0.85
Maximum value	111.8 (55.3)	131.1 (87.4)	−19.3 (−57.3 to 18.7)	0.31
Mean substitution	100.0 (55.3)	103.5 (65.0)	−3.5 (−35.0 to 27.9)	0.82
Observed values	117.4 (64.5) ($n = 18$)	129.4 (87.8) ($n = 13$)	−12.0 (−67.8 to 43.9)	0.66

Mean (SD) oxygenation index (cm H_2O/kPa) is presented for the intention-to-treat population.
*Primary unadjusted analysis.



ORBCEL-C (n)	29	27	26	18	17	17	19	18	20	16	16	16	14	13	11
PS	0	2	3	10	10	10	8	8	5	8	8	8	11	12	12
Dead	0	0	1	1	1	1	1	1	1	1	2	2	2	2	3
Not available*	1	1	0	0	0	0	0	1	1	2	0	1	0	0	0
No Ventilation	0	0	0	1	2	2	2	2	3	3	3	2	2	1	1
ICU Discharge	0	0	0	0	0	0	0	0	0	0	1	1	1	2	3
Total	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30

Placebo (n)	27	26	27	24	21	17	14	13	10	8	7	8	9	5	4
PS	2	2	2	4	5	7	10	12	12	12	12	7	9	10	11
Dead	0	0	0	1	1	1	1	1	1	2	2	3	3	4	4
Not available*	0	1	0	0	1	2	2	0	0	1	1	1	0	1	0
No Ventilation	0	0	0	0	1	2	1	2	5	5	5	6	2	3	3
ICU Discharge	0	0	0	0	0	0	1	1	1	1	2	4	6	6	7
Total	29	29	29	29	29	29	29	29	29	29	29	29	29	29	29

Figure 2. Oxygenation index (OI; cm H_2O/kPa) over time from baseline (Day 0) to Day 14. Mean (SD) for observed values is presented. Reasons OI data were not available at the specified time point are presented below the graph. *Mean airway pressure was not recorded, therefore it was not possible to obtain OI. PS = pressure support.

Routine clinical measurements of coagulation (prothrombin time, activated partial thromboplastin time, and fibrinogen) were similar between groups (*see* Figure E5).

Transcriptomic Analyses

The analytic framework is shown in the online supplement. At baseline, there were no differentially expressed genes (DEGs) between the ORBCEL-C and placebo groups (Figure 4A). Within the ORBCEL-C group, and within the placebo group, over time (Day 7 vs. Day 0), there were significantly more DEGs in the ORBCEL-C group (896 vs. 169; Figures 4B and 4C), with only 48 concordant genes between the groups (Figures 4D and E6). The Ingenuity Pathway Analysis (IPA, Qiagen) of DEGs over time (Figure 4E) highlights that ORBCEL-C affected pathways involved in senescence mechanisms (such as upregulated unfolded protein responses, P53 signaling, and downregulated sirtuin signaling). In contrast, the IPA of DEGs over time in the placebo group highlighted mainly immune responses to viral infection pathways (such as upregulated B-cell signaling and upregulation of the IRF [IFN regulatory factor] family of transcription factors).

We then used established methods to deconvolute the whole-blood transcriptome to determine the impact of allocation on proportions of leukocyte subsets (46). In the ORBCEL-C group, proportions of total B cells and natural killer cell subsets reduced over time (*see* Figure E7). In contrast, in the placebo group, proportions of total T cells, monocytes, and plasmacytoid dendritic cell subsets increased over time (*see* Figure E7), consistent with the IPA described above.

Discussion

The key finding of this study is that a single dose of 400×10^6 ORBCEL-C was safe and well tolerated in a population of patients with COVID-19–related ARDS. However, there were no differences in the primary efficacy outcome of OI at Day 7 and in other secondary surrogate outcomes of systemic and pulmonary organ function at Days 4, 7, and 14. Although the study was underpowered for clinical outcomes, a prolonged duration of ventilation was reported in survivors in the ORBCEL-C group, but long-term follow-up of survivors showed no increased incidence of pulmonary disorders or other significant medical events. Whole-blood transcriptome analyses provided evidence for biological activity of ORBCEL-C MSCs.

The aim of this phase 2 trial was to assess the safety of ORBCEL-C in a population of patients with COVID-19–related ARDS. ORBCEL-C therapy was safe and well tolerated, with no significant differences between groups in the primary safety outcome or in the incidence of adverse events.

Table 4. Clinical Outcomes

	ORBCEL-C ($n = 30$)	Placebo ($n = 29$)	Mean Difference, Hazard Ratio, or Relative Risk (95% Confidence Interval)	*P* Value
Time to first successful extubation, d*†	22.0 (16.0 to 43.0)	17.0 (9.0 to 25.0)	0.6 (0.3 to 1.1)	0.079
Incidence of extubation‡§	24 (80.0%)	22 (75.9%)	1.1 (0.8 to 1.4)	0.70
Incidence of reintubation‡§	2 (8.3%)	3 (13.6%)	0.6 (0.1 to 3.3)	0.56
Ventilation-free days at Day 28				
Median (IQR)‖	3.5 (0.0 to 11.0)	7.0 (0.0 to 18.0)	—	0.27
Mean (SD)¶	6.1 (7.3)	8.9 (8.9)	−2.7 (−6.9 to 1.5)	0.20
Duration of ventilation, d*†				
All	19.0 (13.0 to 30.0)	12.0 (7.0 to 20.0)	0.5 (0.3 to 0.9)	0.017
Survivors	20.0 (16.0 to 31.0) ($n = 25$)	10.0 (7.0 to 20.0) ($n = 22$)	0.4 (0.2 to 0.8)	0.007
Nonsurvivors	13.0 (9.0 to 25.0) ($n = 5$)	12.0 (7.0 to 32.0) ($n = 7$)	1.4 (0.4 to 4.8)	0.62
Length of ICU stay, d*†	24.0 (18.0 to 37.0)	18.0 (11.0 to 32.0)	0.6 (0.3 to 1.0)	0.064
Length of hospital stay, d*†	36.0 (26.0 to 53.0)	26.0 (17.0 to 39.0)	0.7 (0.4 to 1.2)	0.17
28-d mortality§	5 (16.7%)	6 (20.7%)	0.8 (0.3 to 2.4)	0.69
90-d mortality§	7 (23.3%)	8 (27.6%)	0.8 (0.4 to 2.0)	0.71

Definition of abbreviation: IQR = interquartile range.
Mean (SD), median (IQR), or *n* (%) is presented.
*Hazard ratio is presented.
†Censored at Day 90.
‡Number of patients with at least one occurrence.
§Relative risk is presented, with *P* value from chi-square (or Fisher exact) test.
‖Median (IQR) and *P* value from Wilcoxon rank sum test are presented.
¶Mean (SD) is presented for treatment arms and mean difference (95% confidence interval), with *P* value from two-sample *t* test.

In this study, adverse events that were expected (and related to the underlying condition) were not reported unless considered by the site investigator to be associated with study drug administration or unexpectedly severe or frequent. This approach to reporting of adverse events (47) may not capture the true incidence of specific events. For instance, we report the number of thromboembolic events reported as safety events, but as thromboembolism is recognized to be associated with COVID-19 (48, 49), these events may have been considered related to the underlying disease and not reported as adverse events.

No significant safety concerns in relation to MSC infusion in COVID respiratory failure were identified in this study or in other clinical trials to date (25–27). The detailed long-term follow-up showing similar 2-year mortality rates in both groups and a similar overall incidence of significant medical events (defined as events occurring after the 90-day adverse event reporting window that would otherwise fulfill the criteria for serious adverse events) supports the safety of ORBCEL-C. We note three diagnoses of malignancy in the 2-year follow-up period in the MSC-treated group (statistically nonsignificant). Although long-term follow-up in critically ill patients is challenging (50), and indeed achieving follow-up in >90% of patients is a strength of this study, we advocate this in other MSC trials to understand any potential long-term adverse outcomes. Two other studies have reported safety outcomes of MSCs in patients with COVID-19 at 1 year, including vital status, with some limited data on parenchymal lung disease (27, 51). Before the COVID-19 pandemic, a meta-analysis evaluating MSC administration in a range of clinical conditions (including 55 randomized controlled trials and 2,696 patients) showed that MSCs were associated with a risk of fever but demonstrated no association with nonfever infusional toxicity, malignancy, infection, thromboembolism, or death (52). The safety outcomes and long-term follow-up in this trial add to the body of evidence supporting the safety of MSCs in patients with COVID-19–related ARDS.

Although the finding of an increased duration of ventilation in the ORBCEL-C–treated group raises the question of harm, this should be interpreted with caution. First, the trial was not designed to have statistical power to evaluate clinical outcomes, and with a small sample size, firm conclusions should not be made (53). Second, the signal of increased duration of ventilation in the ORBCEL-C group was seen only in survivors and may have reflected both a more severe disease state and an increased risk of barotrauma (54). The similar frequency in both groups of interstitial lung disease on clinically indicated thoracic CT within 12 months of follow-up is reassuring, as is the lack of reduced gas transfer factor in the ORBCEL-C group (diffusing capacity in the placebo group is in fact lower). We acknowledge, though, that follow-up was limited by lack of protocolized screening for evidence of interstitial lung disease.

There have now been several randomized controlled trials investigating MSCs in COVID-19. Kirkham and colleagues reported a meta-analysis of eight randomized controlled trials (including 165 patients treated with MSCs and 151 control patients) investigating the administration of MSCs to patients with severe or critical COVID-19 (55). MSCs were reported to reduce the relative risk of death (risk ratio, 0.63 [95% confidence interval, 0.42–0.94]) but showed no difference in the absolute risk of death. Of note, the relative risk of death analysis was heavily weighted by one study that had a control group mortality rate of

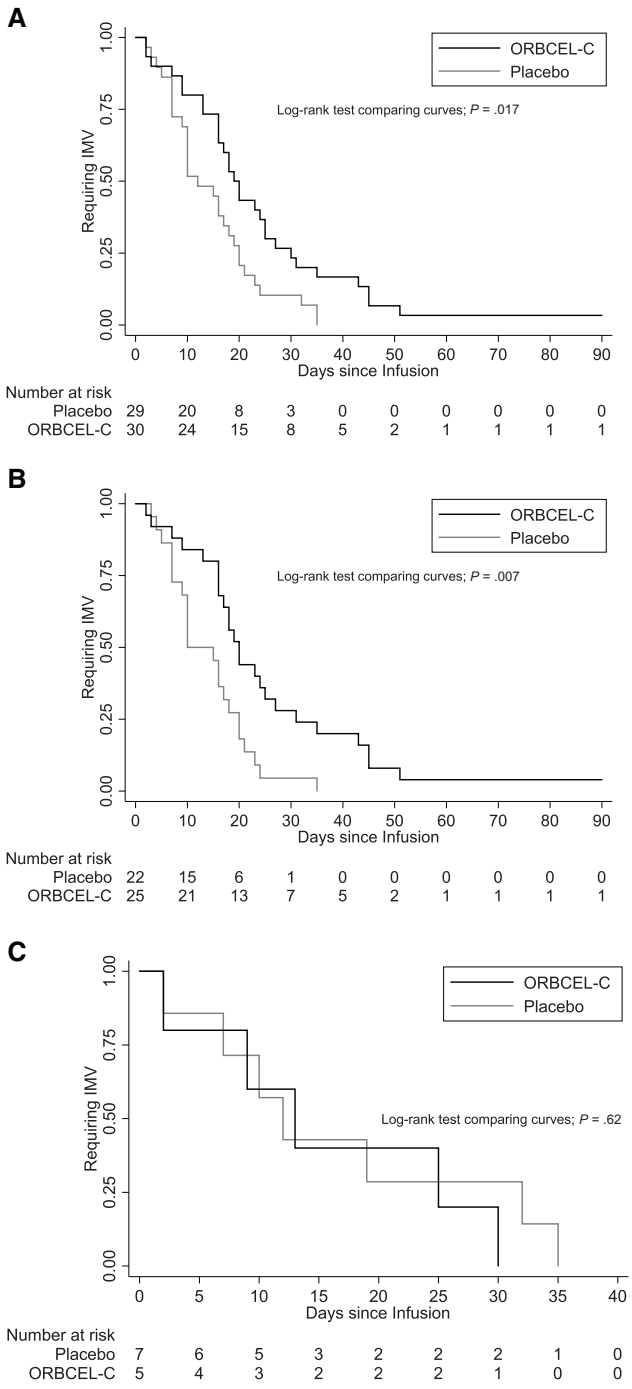


Figure 3. Kaplan-Meier curve for duration of ventilation. (*A–C*) Data are presented separately for all patients (*A*), survivors (*B*), and nonsurvivors (*C*). IMV = invasive mechanical ventilation.

80%, thus the population was not comparable with that of this study (56). More recently, Bowdish and colleagues reported a commercially funded trial investigating two infusions (2×10^6 cells/kg/dose) of remestemcel-L (a bone marrow–derived MSC product) in 222 patients with moderate to severe COVID-19–related ARDS and found no difference in the primary outcome of mortality at 30 days (27). Similarly, Monsel and colleagues investigated MSC administration in 45 patients with mild to severe ARDS (three infusions of 1×10^6 MSCs/kg/dose over 5 d) and found no difference in the primary outcome of change in the PF ratio between Day 0 and Day 7 (26). These latter two trials recruited populations of patients with COVID-19–related ARDS comparable with those recruited for the REALIST trial, though Monsel and colleagues also recruited patients with mild ARDS. Of the trials reported in COVID-19, REALIST administered the greatest dose of MSCs (at an approximate dose of 6×10^6 cells/kg). However, it is difficult to compare the dosing schedule of MSC products among trials, as it is well recognized that variations in MSC source and manufacturing may lead to variation in their function (28, 29) so MSC products are not equipotent.

As in the REALIST study, corticosteroid use in the studies of both Monsel and colleagues and Bowdish and colleagues was high (approximately 80%) (26, 27). Evidence regarding steroid and MSC interactions is conflicting. In preclinical experimental models, steroids have been reported to reduce MSCs' immunomodulatory and antiinflammatory effects and inhibit the cell cycle, promoting apoptosis (57). However, in patients, they retained immunomodulatory activity in graft-versus-host disease in the presence of steroids (58). Our transcriptomic data support the concept that MSCs remained biologically active in the presence of corticosteroids, suggesting that the steroids did not cause significant cytotoxicity, but our data do not exclude the possibility that steroids inhibited the pathways by which MSCs might improve outcomes of ARDS.

Despite the lack of a signal of clinical efficacy, even with a higher MSC dose, in the REALIST trial, our study provides evidence for potential biological activity of ORBCEL-C in the setting of COVID-19–related ARDS treated with dexamethasone. Specifically, we observed differences in enriched immunological mechanisms over time between the ORBCEL-C group and the placebo group, despite similar transcriptional profiles at baseline. Several of the pathways identified have been associated with the pathobiology of ARDS, such as senescence (59), unfolding protein response (60), and dolichyl-diphosphooligosaccharide–protein glycosyltransferase biosynthesis (which is implicated in advanced glycosylation end product pathways [61, 62]), and are testable hypotheses in future studies.

That the MSC product in the REALIST trial had biological activity but did not translate to efficacy raises questions

Table 5. Long-Term Follow-Up: Mortality, Significant Medical Events, and Clinically Indicated Pulmonary Function Testing and Thoracic Computed Tomography

	ORBCEL-C	Placebo
Total number of patients in primary analysis	30	29
Day 28 mortality, *n*/*N* (%)	5/30 (16.7)	6/29 (20.7)
Day 90 mortality, *n*/*N* (%)	7/30 (23)	8/29 (27.5)
Loss to follow-up*	2	0
1-yr mortality, *n*/*N* (%)	8/28 (28.6)	8/29 (27.6)
2-yr mortality, *n*/*N* (%)	8/28 (28.6)	8/29 (27.6)
SMEs		
Number followed up at 1 yr	20	21
Number followed up at 2 yr	20	20
Number of events	14	11
Number of patients, *n*/*N* (%)	11/20 (55)	9/21† (43)
Pulmonary function testing,‡ % predicted		
Number available, *n*/*N* (%)	11/20 (55)	9/21 (43)
Timing,§ d, median (IQR)	174 (119–291)	180 (123–230)
FEV_1, L, median (IQR)	84 (74.5–92.5)	75 (73–86)
FVC, L, median, (IQR)	75 (68.5–87.0)	73 (69–81)
D_{LCO}, median (IQR)	76 (74–91.5) ($n = 10$)	62 (57–71.5) ($n = 8$)
Pulmonary function testing,‡ number less than lower limit of normal		
$FEV_1 < 80\%$ predicted, *n*/*N* (%)	5/11 (46)	5/9 (56)
$FVC < 80\%$ predicted, *n*/*N* (%)	6/11 (55)	6/9 (67)
$D_{LCO} < 80\%$ predicted, *n*/*N* (%)	6/10 (60)	8/8 (100)
Thoracic CT‡		
Number available, *n*/*N* (%)	5/20 (25)	8/21 (38)
Timing,§ d, median (IQR)	181 (157–198)	203 (95.5–233)
Evidence of interstitial lung disease, *n*/*N* (%)	4/5 (80)	6/8 (75)
Evidence of VTE, *n*/*N* (%)	0/5 (0)	1/8 (13)‖

Definition of abbreviations: CT = computerized tomography; IQR = interquartile range; SME = significant medical event; VTE = venous thromboembolism.
*For the two patients lost to follow-up in the ORBCEL-C group, the last available follow-up information was at Day 90.
†For $n = 1$ in the placebo group, follow-up for SMEs was only to Year 1, as the patient withdrew consent for SME follow-up at Year 2.
‡CT and pulmonary function testing data were collected from clinically indicated investigations performed between Day 28 and 1 year (±30 d).
§Time from study drug administration to most recent pulmonary function testing or CT imaging.
‖One additional VTE was detected on clinically indicated thoracic CT during follow-up between Day 28 and 1 yr (which was not reported by the site investigator as a safety event, as it was considered to be due to underlying disease; *see* Tables E4a and E4b).

regarding the optimization of MSC administration to achieve a therapeutic effect. Higher doses (up to 10×10^6 MSCs/kg) have been tolerated in similar populations of patients with non–COVID-19–related ARDS (19–21) but have been associated with procoagulant effects when administered to healthy volunteers in a human model of endotoxemia (63). Repeated administration of lower MSC doses has been well tolerated in patients with COVID-19–related ARDS, as outlined in the aforementioned studies (26, 27), and it is possible that repeated administration of higher MSC doses, such as used in the REALIST trial, may be needed to achieve therapeutic efficacy. The optimal time of administration remains unclear. Although preclinical evidence suggests that administration early in the time course of ARDS development is beneficial (33), a clinical study reported by Shi and colleagues suggests benefit at a later stage in the disease course (64). One hundred patients with established lung damage due to COVID-19 were randomized to receive either 40×10^6 MSCs or placebo during their convalescence (median, 45 days from symptom onset) and there was a reduction in solid-component lung lesions on CT at Day 28 and improved 6-minute-walk distance (64). Subgroup analyses have suggested that there may be differential effects of MSCs in different patient groups, with trends toward a mortality benefit in younger patients (<65 yr) and patients with diabetes (27), and in the REALIST trial, there was a trend toward increased VFDs in less hypoxic patients (PF ratio > 20), but given the small sample size in these analyses, further exploration of these findings is required. As MSCs are responsive to their microenvironment and may express different phenotypes after administration (30), factors such as the timing of administration and disease severity could have an important impact on their biological activity. Thus, the lack of efficacy in patients with COVID-19–related ARDS does not preclude benefit in patients with ARDS due to other causes, and further work is required to understand the optimal patient populations that will benefit from MSCs.

The lack of an established potency assay for MSCs is an area of unmet need within the wider field of MSC therapy. The cells used in the REALIST study had to meet a range of specific criteria (including cell surface markers, sterility, and viability) after manufacture to allow product release. There is no evidence to suggest these cells are dissimilar to previously manufactured batches that were investigated in preclinical *in vitro* or *in vivo* studies (28, 31–33). Cell viability assessment in this study was assessed after freeze–thaw cycles at the central manufacturing site (Cellular and Molecular Therapies Division, National Health Service Blood and Transplant Service). Cell viability was not further assessed at the time of study drug preparation at the cell therapy facility, because *1*) no further manufacturing steps (such as a wash to remove DMSO) were conducted at the cell therapy facility; *2*) many sites did not have technical experience to carry out a cell viability assessment; and *3*) the thaw-and-dilute procedure at the cell therapy facility followed a strictly standardized procedure that replicated the thaw process used during the cell viability assessment at the central manufacturing site. This is in contrast to a recently published study in which cell therapy varied by site (20), in which a further manufacturing step was undertaken to wash the MSCs and reduce the DMSO content, and this was found to adversely affect cell viability.

A limitation of this study is that data for the primary efficacy outcome, OI at Day 7, were not available for all participants. In most cases, this was unavoidable (related to extubation, death, and mode of ventilation), and mitigations were in place, using imputed data, to minimize the impact of missing data. Furthermore, to minimize the risk of bias

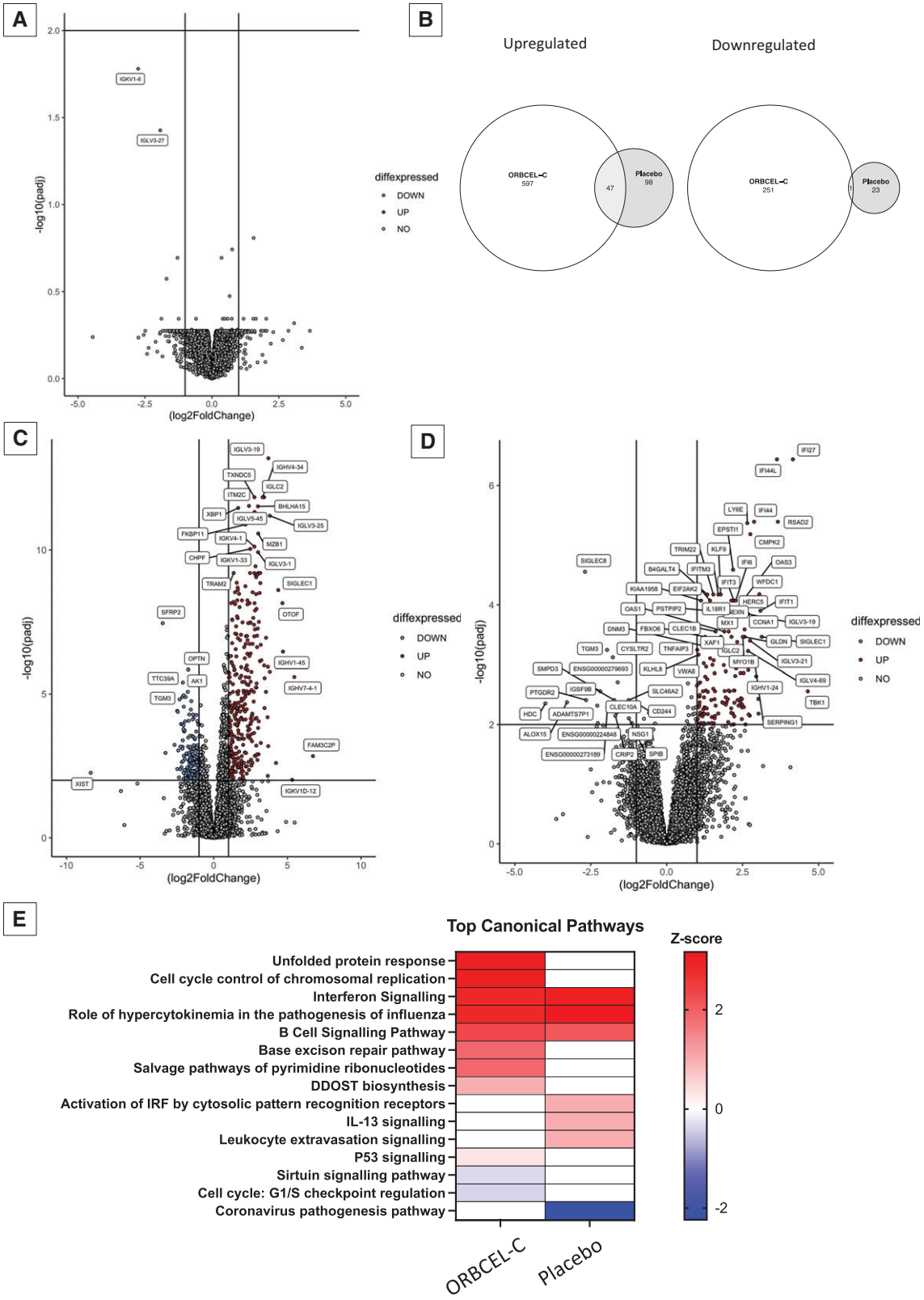


Figure 4. Transcriptomic analysis of peripheral whole-blood samples at Day 0 (ORBCEL-C, $n = 19$; placebo, $n = 23$), Day 4 (ORBCEL-C, $n = 14$; placebo, $n = 14$) and Day 7 (ORBCEL-C, $n = 18$; placebo, $n = 12$). (*A*) Volcano plot comparing differentially expressed genes (DEGs) in the ORBCEL-C and placebo groups at baseline (Day 0). (*B*) Venn diagram depicting the overlap of DEGs at Day 7 compared with Day 0 in the

introduced by imputation, multiple methods of imputation were used and consistently demonstrated no difference in the primary outcome.

Conclusions

This phase 2 randomized controlled trial demonstrated that ORBCEL-C MSCs were safe and well tolerated in a population of patients with moderate to severe ARDS due to COVID-19. This early phase 2 study did not demonstrate improvements in surrogates of pulmonary organ dysfunction in a population of patients with COVID-19–related ARDS. The REALIST research program is ongoing and currently investigating ORBCEL-C MSCs in a cohort of patients with ARDS unrelated to COVID-19. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

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Figure 4. (*Continued*). ORBCEL-C group (white) and the placebo group (gray). (*C* and *D*) Volcano plots comparing the time-dependent effects on DEGs (Day 7 vs. Day 0) in the ORBCEL-C group (*C*) and the placebo group (*D*). For *A*, *C*, and *D*, adjusted $P < 0.01$ and $\log_2$ fold change cutoff >1 and <-1. (*E*) Heatmap of the top canonical pathways identified by Ingenuity Pathway Analysis in both treatment groups. ADAMTS7P1 = ADAMTS7 pseudogene 1; AK1 = adenylate kinase 1; ALOX15 = arachidonate 15-lipoxygenase; B4GALT4 = beta-1,4-galactosyltransferase 4; BHLHA15 = basic helix-loop-helix family member A15; CCNA1 = cyclin A1; CD244 = cluster of differentiation 244; CHPF = chondroitin polymerizing factor; CLEC = C-type lectin domain containing; CMPK2 = cytidine/uridine monophosphate kinase 2; CRIP2 = cysteine rich protein 2; CYSLTR2 = cysteinyl leukotriene receptor 2; DDOST = dolichyl-diphosphooligosaccharide–protein glycosyltransferase; diffexpressed = differentially expressed; DNM3 = dynamin 3; EIF2AK2 = eukaryotic translation initiation factor 2 alpha kinase 2; EPSTI1 = epithelial stromal interaction 1; FAM3C2P = family with sequence similarity 3 member C2, pseudogene; FBXO6 = F-box protein 6; FKBP11 = FKBP prolyl isomerase 11; GLDN = gliomedin; HDC = histidine decarboxylase; HERC5 = HECT and RLD domain containing E3 ubiquitin protein ligase 5; IFI27 = IFN alpha inducible protein; IFIT = IFN induced protein with tetratricopeptide repeats; IGHV = immunoglobulin heavy variable; IGKV = immunoglobulin kappa variable; IGLC2 = immunoglobulin lambda constant 2; IGLV = immunoglobulin lambda variable; IGSF9B = immunoglobulin superfamily member 9B; IRF = IFN regulatory factor; ITM2C = integral membrane protein 2C; KLF9 = KLF transcription factor 9; KLHL8 = Kelch like family member 8; LY6E = lymphocyte antigen 6 family member E; MX1 = MX dynamin like GTPase 1; MYO1B = myosin IB; MZB1 = marginal zone B and B1 cell specific protein; NEXN = nexilin F-actin binding protein; NSG1 = neuronal vesicle trafficking associated 1; OAS = oligoadenylate synthetase; OPTN = optineurin; OTOF = otoferlin; padj = adjusted *P* value; PSTPIP2 = proline-serine-threonine phosphatase interacting protein 2; PTGDR2 = prostaglandin D_2 receptor 2; RSAD2 = radical S-adenosyl methionine domain containing 2; SERPING1 = serpin family G member 1; SFRP2 = secreted frizzled related protein 2; SIGLEC = sialic acid binding immunoglobulin like lectin; SLC46A2 = solute carrier family 46 member 2; SMPD3 = sphingomyelin phosphodiesterase 3; SPIB = Spi-B transcription factor; TBK1 = TANK binding kinase 1; TGM3 = transglutaminase 3; TNFAIP3 = TNF alpha induced protein 3; TRAM2 = translocation associated membrane protein 2; TRIM2 = tripartite motif containing 2; TTC39A = tetratricopeptide repeat domain 39A; TXNDC5 = thioredoxin domain containing 5; VWA8 = von Willebrand factor A domain containing 8; WFDC1 = WAP four-disulfide core domain 1; XAF1 = XIAP associated factor 1; XBP1 = X-box binding protein 1.

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