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

Repair of Acute Respiratory Distress Syndrome in COVID-19 by Stromal Cells (REALIST-COVID Trial): A Multicentre, Randomised, Controlled Trial

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At a Glance Commentary

Current scientific knowledge on the subject: Mesenchymal stomal cells (MSCs) have been tested as a possible therapy for ARDS due to their anti-inflammatory, pro-resolution and antimicrobial effects. Phase 2 studies to date in ARDS related to 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: This study tested the largest dose of MSCs in COVID related ARDS to date. We showed that, 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. While the cohort had high rates of

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corticosteroid prescription, MSCs still drove significant changes in peripheral blood transcriptome, but this did not translate to clinical efficacy.

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

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Abstract

Rationale

Mesenchymal stromal cells (MSCs) may modulate inflammation, promoting repair in COVID-19-related Acute Respiratory Distress Syndrome (ARDS).

Objectives

We investigated safety and efficacy of ORBCEL-C (CD362-enriched, umbilical cord-derived MSCs) in COVID-related ARDS.

Methods

This multicentre, randomised, double-blind, allocation concealed, placebo-controlled trial (NCT03042143) randomised patients with moderate-to-severe COVID-related ARDS to receive ORBCEL-C (400million cells) or placebo (Plasma-Lyte148).

Measurements

The primary safety and efficacy outcomes were incidence of serious adverse events and oxygenation index at day 7 respectively. Secondary outcomes included respiratory compliance, driving pressure, PaO_2/FiO_2 ratio and SOFA score. Clinical outcomes relating to duration of ventilation, length of intensive care unit 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 day 0, 4 and 7.

Main Results

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60 participants were recruited (final analysis n=30 ORBCEL-C, n=29 placebo: 1 in placebo group withdrew consent). 6 serious adverse events occurred in the ORBCEL-C and 3 in the placebo group, RR 2.9(0.6-13.2)p=0.25. Day 7 mean[SD] oxygenation index did not differ (ORBCEL-C 98.3[57.2], placebo 96.6[67.3]). There were no differences in secondary surrogate outcomes, nor mortality at day 28, day 90, 1 or 2 years. There was no difference in prevalence of interstitial lung disease at 1year nor significant medical events up to 2 years. ORBCEL-C modulated the peripheral blood transcriptome.

Conclusion

ORBCEL-C MSCs were safe in moderate-to-severe COVID-related ARDS, but did not improve surrogates of pulmonary organ dysfunction.

Abstract word count: 250

Keywords

Acute Respiratory Distress Syndrome; COVID-19; Mesenchymal Stromal Cells; Clinical trial

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Introduction

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

Mesenchymal stromal cells (MSCs) are a potential novel therapeutic in ARDS due to 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 MSCs can repair lung injury, restore alveolar fluid clearance, and improve lung compliance and oxygenation (16-18). Prior to the COVID-19 pandemic, MSCs had been investigated in early phase clinical trials in ARDS, which suggested MSCs were safe in this patient population (19-24). Since this trial began, several trials have investigated MSC products in patients with COVID-19 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-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

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advantage of being more homogeneous and better characterised than MSCs isolated by the traditional method of plastic adherence. As a source of MSCs, umbilical cord is cost-efficient, readily available (usually disposed of as a waste product) and not associated with risks to the donor.

Prior to the REALIST research programme, 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, NCT02585622) and liver disease (ORBCEL-C, MERLIN, NCT02997878). In the phase 1 REALIST dose-finding study, patients with ARDS (unrelated to COVID-19) received escalating doses of a single intravenous of ORBCEL-C MSCs (3 cohorts of 3 patients receiving 100, 200 or 400 $x10^6$ MSCs). There was no dose limiting toxicity in any dose cohort (34). The REALIST phase 2 trial aims to investigate the safety and efficacy of a single intravenous infusion of 400 x 10^6 ORBCEL-C MSCs in patients with moderate to severe ARDS. In this study we report the effect of ORBCEL-C on lung physiological measurements, clinical outcomes, whole blood transcriptome and 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 was a multicentre randomised, double-blind, allocation concealed, placebo-controlled trial of ORBCEL-C MSCs in patients with ARDS due to COVID-19, in twelve intensive care units across the United Kingdom (UK). Full details of the study design are published (37), and are available in the Online Data Supplement. The trial was sponsored by Belfast Heath and Social Care Trust and approved by North-East York research ethics

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committee (18/NE/0006) and the Medicines and Health products Regulatory Agency (EudraCT Number: 2017-000584-33). Trial registration NCT03042143.

In brief, following informed consent eligible patients who were mechanically ventilated within 72 hours of onset of moderate to severe ARDS due to COVID-19, were randomised (1:1) to receive a single intravenous infusion of either ORBCEL-C (400 x10^6 CD362 enriched umbilical cord derived MSCs in 200ml Plasma-Lyte 148, see Online Data Supplement for manufacturing details including cell viability assessment), or placebo (Plasma-Lyte 148, 200ml). 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 Data Supplement. The primary efficacy outcome was oxygenation index at day 7 (calculated as: mean airway pressure [cmH_2O] x FiO_2 x 100)/PaO_2[kPa]). Oxygenation index (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 ARDS (38) and is widely reported as a surrogate outcome in ARDS trials (20, 39-41). Secondary surrogate outcomes included indices of pulmonary and non-pulmonary organ dysfunction. OI at days 4 and 14; respiratory compliance, driving pressure, and PaO_2/FiO_2 (PF) ratio on days 4, 7, and 14; and organ failure as measured by the Sequential Organ Failure Assessment (SOFA) score on days 4, 7, and 14. Clinical outcome measures included extubation, reintubation, ventilator-free-days (VFDs) to day-28, duration of ventilation, length of intensive care unit (ICU), and hospital stay, as well as 28- and 90-day mortality. Long-term follow-up was conducted for mortality, significant medical events, and for evidence of

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pulmonary dysfunction and interstitial lung disease on clinically indicated thoracic CT scans and pulmonary function tests.

Whole blood total RNA-sequencing was carried out on samples collected at day 0, 4 and 7 (see Online Data Supplement for details). Other exploratory translational studies detailed in the trial protocol were not conducted in this cohort, due to the UK's regulatory requirement to process all research samples from COVID 19-infected patients in a containment level (CL) 3 laboratory environment during this phase of the pandemic.

Statistical Analysis

Based on our data from a previous study in ARDS, the mean [SD] OI at day 7 in patients with ARDS is 62 [51] cmH_2O/kPa (42). A sample size of 56 subjects (randomised 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 in oxygenation index at day 7, the primary efficacy outcome, of 39 cmH_2O/kPa in OI between groups (38). In previous UK multicentre studies in the critically ill, less than 3% withdrew consent or were lost to follow-up (43, 44), therefore the sample size was inflated to 60 patients to allow for a drop-out 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) based on severity of inflammation (C-Reactive Protein and ferritin (45)), and PF Ratio. Statistical analysis was conducting using STATA v15.1. The statistical analysis plan, which was finalised before recruitment to the study completed, is provided in the Online Data Supplement.

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Results

Patients

From 2nd April 2020 to 4th December 2020, 193 patients were screened for eligibility, of whom 133 were excluded (Figure 1). Screening and recruitment data at each site are provided in Figure E1 in the Online Data Supplement. 60 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 ORBCEL-C group and 29 in 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 SOFA score. Corticosteroid use was similar in both groups (90% in ORBCEL-C, 89.7% in placebo group). Approximately half of patients in each group received anti-viral therapy. No patients in this trial received Interleukin-6 receptor antagonists or convalescent plasma. Concomitant medications and adjuvant therapies are detailed in Table E1a/b in the Online Data Supplement. There were no differences between groups in the time of study drug administration measured from intensive care unit admission, mechanical ventilation initiation, or thaw of the study drug (Table E2, Online Data Supplement).

Primary safety outcomes

There were 6 serious adverse events in the ORBCEL-C group and 3 in the placebo group; risk ratio 2.9; 95% confidence interval 0.6 to 13.2; p=0.25 (Table 2). Study drug administration was well-tolerated with no significant difference between the groups in post-

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infusion (up to 5 hours) haemodynamics, arterial blood gases, PEEP, plateau pressure or temperature (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 pre-specified infusion-related adverse event, and classified as an adverse reaction. One patient in the placebo group had pyrexia reported as an adverse reaction. No other adverse events were considered to be related to the study drug. A summary of adverse event classifications (Table E3a) and detailed listing of all adverse events reported (Table E3b) are provided in the online supplement. Thromboembolic events reported as adverse events are detailed separately (Tables E4a and E4b). During 1-year follow up, one additional instance of pulmonary emboli 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, found no difference in OI at day 7 between the ORBCEL-C group (mean 98.3 [SD 57.2]) and placebo group (mean 96.6 [SD 67.3]; mean difference 1.8; 95% confidence interval 30.7 to 34.4; p=0.91) (Table 3). Adjusted analysis (for baseline age, PF Ratio, APACHE II, vasopressor use and site) did not alter this finding (Table E5a/b in Online Data Supplement). Observed values for OI at day 7 were available for n=18/30 in ORBCEL-C group and n=13/29 in placebo group. Reasons for missing OI at day 7 are listed in Table E5c in the Online Data Supplement and 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

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PCR-confirmed COVID-19 did not alter the findings (Tables E5d and E5e in the Online Data Supplement).

There were no important differences between ORBCEL-C and placebo treated groups in secondary surrogate outcomes of pulmonary and non-pulmonary organ function (Figure 2, Figure E3, Table E6). Clinical outcomes are reported (Table 4), although the study was underpowered for these. While 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 [IQR 13 to 30]) compared to the placebo group (12 days [IQR 7 to 20]; hazard ratio 0.5; 95% confidence interval 0.3 to 0.9; p=0.017) (Table 4, Figure 3). Mortality at day 28 or 90 was similar between groups [28-day mortality ORBCEL-C n=5 (16.7%), placebo n=6 (20.7%), risk ratio 0.8, 95% confidence intervals 0.3 to 2.4, p=0.69; 90-day mortality ORBCEL-C n=7 (23.3%), placebo n=8 (27.6%), risk ratio 0.8, 95% confidence intervals 0.4 to 2.0, p=0.71]. ORBCEL-C had no effect on oxygenation index at day 7 according to *a priori* defined subgroups for baseline oxygenation (PF ratio) and inflammation (C-Reactive protein and ferritin) (Table E7). ORBCEL-C treatment was associated with a statistically significant higher number of VFDs in the group with PF ratio >20kPa at baseline but not those with more impaired oxygenation (Table E8).

Long-term follow up is summarised 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. 41 survivors were remotely followed up for significant medical events at 1 year (n=20 in ORBCEL-C, n=21 in placebo group). No further patients had died at the 2-year timepoint. 31 survivors were remotely followed up for significant medical events at 2 years (n=17 in ORBCEL-C, n=14 in placebo group). One patient in placebo group withdrew consent for follow up at 2 years.

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Reported significant medical events were similar in both groups (Table 5 and Table E9 for description of significant medical events). Clinically indicated thoracic CTs were available for 13 patients (ORBCEL-C n=5/20, placebo n=8/21). CT evidence of interstitial lung disease was similar between groups. Pulmonary function test reports were available for 11/20 ORBCEL-C patients and 9/21 in the placebo group. Diffusing capacity for carbon monoxide was reduced in both groups, with median percentage predicted value of 62% (57 to 71.5) in the placebo group compared to 76% (IQR 74 to 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 (Figure E4). There were no trends to differences in renal or liver function test abnormalities in response to ORBCEL-C (Table E10). Routine clinical measurements of coagulation (prothrombin time, activated partial thromboplastin time, and fibrinogen) were similar between groups (Figure E5).

Transcriptomic analyses

Analyses framework is shown in the Online Data Supplement. At baseline, there were no differentially expressed genes (DEGS) between ORBCEL-C and placebo groups (Figure 4A). Within the ORBICEL-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 Figure 4B, 4C), with only 48 concordant genes between the groups (Figure 4D, Figure E6). The Ingenuity Pathway Analysis (IPA) of DEGS over time (Figure 4E) highlight that the ORBCEL-C group affected pathways involved in senescence mechanisms (such as upregulated unfolded protein responses, and P53 signalling, and down regulated Sirtuin signalling). In contrast, the IPA of

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DEGS over time in the placebo group highlight mainly immune responses to viral infection pathways (such as up regulated B cell signalling, upregulation of the interferon regulatory factor (IRF) family of transcription factors).

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

Discussion

The key finding of this study is that a single dose of $400x10^6$ ORBCEL-C was safe and well tolerated in the population of patients with COVID-ARDS. However, there were no differences in the primary efficacy outcome of oxygenation index at day 7, or in other secondary surrogate outcomes of systemic and pulmonary organ function at day 4, 7 and 14. While 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.

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This phase 2 trial aimed to assess the safety of ORBCEL-C in a population of patients with COVID-19 ARDS. ORBCEL-C therapy was safe and well tolerated, with no significant differences between groups in the primary safety outcome, or in the incidence adverse events. In this study adverse events which were expected (and related to the underlying condition) were not reported unless considered by the site investigator to be associated with the 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, however as thromboembolism is recognised to be associated with COVID-19 disease (48, 49) these events may have been considered related to the underlying disease and not reported as an adverse event.

No significant safety concerns in relation to MSC infusion in COVID respiratory failure were identified in this study nor in other clinical trials to date (25-27). The detailed long-term follow up showing similar 2 year mortality rates in both groups, and similar overall incidence of significant medical events (defined as events occurring after the 90-day adverse event reporting window which would otherwise fulfil the criteria for serious adverse events) supports the safety of ORBCEL-C. We note (non-statistically significant) 3 diagnoses of malignancy in the 2 year follow up period in the MSC treated group. While long-term follow up in critically ill patients is challenging (50), and indeed achieving follow-up in > 90 % of patients at 1 year and > 75% at 2 years 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 COVID-19 patients at 1 year including vital status with some limited data on parenchymal lung disease (27, 51). Prior to the COVID-19 pandemic, a meta-analysis evaluating MSC administration in a range of clinical conditions (including 55

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randomised controlled trials and 2696 patients) reported MSCs were associated with a risk of fever, but found no association with non-fever infusional toxicity, malignancy, infection, thromboembolism or death (52). The safety outcomes and long-term follow up in this trial adds to the body of evidence supporting the safety of MSCs in patients with COVID ARDS.

While 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 only seen 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 CTs 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 protocolised screening for evidence of interstitial lung disease.

There have now been several RCTs investigating MSCs in COVID-19. Kirkham *et al* report a meta-analysis of eight RCTs (including 165 patients treated with MSCs, and 151 control patients) investigating 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% CI 0.42 to 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 which had a control group mortality of 80%, thus the population was not comparable to this study (56). More recently, Bowdish *and colleagues* reported a commercially funded trial investigating two infusions (2×10^6

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cells/kg/dose) of Remestemecel-L (a bone marrow derived MSC product) in n=222 patients with moderate to severe COVID-19 ARDS and found no difference in the primary outcome of mortality at 30 days (27). Similarly, Monsel *and colleagues* investigated MSC administration in forty-five patients with mild to severe ARDS (three infusions of 1 x 10^6 MSCs/kg/dose over 5 days) and found no difference in the primary outcome of change in the PF ratio between day 0 and day 7 (26). These later two trials recruited a population of patients with COVID-19 ARDS comparable to those recruited to the REALIST trial, though Monsel *et al* 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 x 10^6 cells/kg). However, it is difficult to compare the dosing schedule of MSC products between trials as it is well recognised that variation in MSC source and manufacturing may lead to variation in their function (28, 29), so that MSC products are not equipotent.

Like in the REALIST study, corticosteroid use in both Monsel and Bowdish trials was high (approximately 80%) (26, 27). Evidence regarding steroid and MSC interactions is conflicting. In pre-clinical experimental models steroids have been reported to reduce MSCs' immunomodulatory and anti-inflammatory effects and inhibit 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 outcome from ARDS.

Despite the lack of signal towards clinical efficacy even with a higher MSC dose in the REALIST trial, our study provides evidence for potential biological activity of ORBCEL-C

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in the setting of COVID-19 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 pathobiology of ARDS such as senescence (59), unfolding protein response (60) and DDOST biosynthesis (which is implicated in Advanced Glycosylation End Product pathways (61, 62)), and are testable hypothesis in future studies.

That the MSC product in the REALIST trial had biological activity, but did not translate to efficacy, raises questions regarding the optimisation of MSC administration to achieve a therapeutic effect. Higher doses (up to 10 x 10^6 MSCs/kg) have been tolerated in similar populations of patients with non-COVID-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 have been well-tolerated in patients with COVID-19 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. While pre-clinical evidence suggests administration early in the time course of ARDS development is beneficial (33), a clinical study reported by Shi *et al* suggests benefit at a later stage in the disease course (64). One hundred patients with established lung damage due to COVID-19 were randomised to receive either 40 x 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 six-minute walk distance (64). Subgroup analyses have suggested there may be differential effects of MSCs in different patient groups with trends towards mortality benefit in younger patients (less than 65 years)

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and diabetic patients (27), and in the REALIST trial there was a trend towards increased ventilator free days in less hypoxic patients (PF ratio >20), however 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 following administration (30), factors such as timing of administration and disease severity could have important impact on their biological activity. Thus, the lack of efficacy in patients with COVID-19 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 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 which had been 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 (NHSBT). Cell viability was not further assessed at the time of study drug preparation at the cell therapy facility as 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 3) the thaw and dilute procedure at the cell therapy facility followed a strictly standardised procedure which replicated the thaw process used during the cell viability assessment at the central manufacturing site. This is in contrast to the recently published study where cell therapy varied by site (20), where a further manufacturing step was undertaken to wash the MSCs and reduce the DMSO content, and this was found to adversely impact cell viability.

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A limitation of this study included that data for the primary efficacy outcome, oxygenation index 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 minimise the impact of missing data. Furthermore, to minimise the risk of bias introduced by imputation, multiple methods of imputation were used and consistently demonstrated no difference in the primary outcome.

In conclusion, this phase 2 randomised controlled trial demonstrated that ORBCEL-C MSCs were safe, 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 programme is ongoing and currently investigating ORBCEL-C MSCs in a cohort of patients with ARDS unrelated to COVID-19.

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Data sharing statement

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

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Figure legends

Figure 1: Consort Flow Chart

Figure 2: Oxygenation Index (OI, cm H_2O/kPa) over time from baseline (day 0) to day 14. Mean (SD) for observed values are presented. Reasons oxygenation index data not available at the specified timepoint are presented below the graph. *Mean Airway Pressure not recorded therefore not possible to obtain oxygenation index.

Figure 3: Kaplan Meier curve for duration of ventilation.
Data are presented separately for all patients (A), survivors (B), and non survivors (D).

Figure 4: Transcriptomic analysis of peripheral whole blood samples at day 0 (n=19 ORBCEL-C, n=23 placebo), day 4 (n=14 ORBCEL-C, n=14 placebo) and day 7 (n=18 ORBCEL-C and n=12 placebo).

A: Volcano plot comparing differentially expressed genes (DEGs) in the ORBCEL-C and placebo group at baseline (Day 0).
B: Venn diagram depicting the overlap of DEGs at day 7 compared to day 0 in the ORBCEL-C group (white) and the placebo group (grey).
C & D: Volcano plots comparing the time-dependent effects on DEGs (day 7 vs day 0) in the ORBCEL-C group (B) and the placebo group (C).
For A, C & D Padj <0.01, Log2 Fold Change (Log2FC) cut off -1<>1.
E: Heatmap of the top canonical pathways identified by Ingenuity Pathway Analysis (IPA) in both treatment groups.

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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, years	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)
Predicted body weight, 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			
Caucasian	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 (LIS)	2.8 (0.4)	2.8 (0.5)	2.8 (0.4)

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Table 1: Baseline Characteristics			
First Qualifying P/F Ratio	17.6 (4.1)	19.4 (4.9)	18.5 (4.6)
Worst PaO_2/FiO_2 ratio (Day 0/24 hrs prior to randomisation)	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 (mmHg)	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 (cmH_2O)	10.9 (2.6)	10.2 (2.7)	10.6 (2.7)
Plateau Pressure (cmH_2O)	24.0 (4.0) n=29	23.6 (4.4) n=27	23.8 (4.2) n=56
Driving Pressure (cmH_2O)	13.1 (4.4) n=29	13.3 (5.0) n=27	13.2 (4.6) n=56
Respiratory Compliance (ml/cmH_2O)	39.4 (22.3) n=29	39.6 (21.0) n=27	39.5 (21.5) n=56
Tidal Volume (ml/kg PBW)	7.1 (2.0)	7.5 (3.0)	7.3 (2.5)
Mode of ventilation			

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Table 1: Baseline Characteristics			
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%)
Mean (SD) or n (%) presented.			

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Table 2: Safety outcomes

	ORBCEL-C	Placebo	Relative Risk (95% Confidence Intervals)	p-value
Total Adverse Events	23* 15 (50%)†	16* 10 (34.5%)†	1.5 (0.8 to 2.7)	0.29
Adverse Reactions	1* 1 (3.3%)†	1* 1 (3.4%)†	0.97 (0.06 to 14.7)	1.00
Total Serious Adverse Events	6* 6 (20%)†	3* 2 (6.9%)†	2.9 (0.6 to 13.2)	0.25
Serious Adverse Reactions (SAR)	0*	0*	-	-
Suspected Unexpected Serious Adverse Reactions (SUSAR)	0*	0*	-	-

*Number of events

†Number of patients (% based on total number of patients in each group).

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Table 3: Oxygenation Index (cmH_2O/kPa) 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) presented for intention-to-treat population. *Primary Unadjusted Analysis				

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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 1st successful extubation (days)[*#]		22.0 (16.0-43.0)	17.0 (9.0-25.0)	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][II]	3.5 (0.0-11.0)	7.0 (0.0-18.0)	-	0.27
	Mean (SD) [††]	6.1 (7.3)	8.9 (8.9)	-2.7 (-6.9 to 1.5)	0.20
Duration of Ventilation (Days)[*,†]	All	19.0 (13.0-30.0)	12.0 (7.0-20.0)	0.5 (0.3 to 0.9)	0.017
	Survivors	20.0 (16.0- 31.0) n=25	10.0 (7.0-20.0) n=22	0.4 (0.2 to 0.8)	0.007
	Non-Survivors	13.0 (9.0-25.0) n=5	12.0 (7.0-32.0) n=7	1.4 (0.4 to 4.8)	0.62
Length of ICU stay (Days)[*,†]		24.0 (18.0-37.0)	18.0 (11.0-32.0)	0.6 (0.3 to 1.0)	0.064
Length of hospital stay (Days)[*,†]		36.0 (26.0-53.0)	26.0 (17.0-39.0)	0.7 (0.4 to 1.2)	0.17
28 day mortality[§]		5 (16.7%)	6 (20.7%)	0.8 (0.3 to 2.4)	0.69
90 day mortality[§]		7 (23.3%)	8 (27.6%)	0.8 (0.4 to 2.0)	0.71

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Mean (SD), median(IQR) or n(%) presented

*hazard ratio presented

†Censored at day 90

‡Number of patients with at least one occurrence.

§Relative Risk presented, p-value from chi-square (or fishers exact) presented.

IIMedian [IQR] and p-value from Wilcoxon rank sum presented

††Mean (SD) presented for treatment arms and mean difference (95% confidence interval), p-value from 2-sample t-test.

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Table 5: Long-term follow-up – mortality, significant medical events and clinically indicated pulmonary function testing and Thoracic CT		
	ORBCEL-C	**Placebo**
Total no. patients in primary analysis	30	29
D28 Mortality (n,%)	5/30 (16.7)	6/29 (20.7)
D90 Mortality (n,%)	7/30 (23)	8/29 (27.5)
Loss to follow up[#]	2	0
1 year mortality (n,%)	8/28 (28.6)	8/29 (27.6)
2 year mortality (n,%)	8/28 (28.6)	8/29 (27.6)
Significant Medical Events		
Number followed up at 1 year	20	21
Number followed up at 2 years	20	20
No. of events	14	11
No. of patients (n,%)	11/20 (55)	9/21[$] (43)
Pulmonary Function Testing[*] - % predicted		
No. available (n,%)	11/20 (55)	9/21 (43)
Timing[**] (Days, median [IQR])	174 (119 to 291)	180 (123 to 230)
Forced expiratory volume in 1 sec (Median [IQR])	84 (74.5 to 92.5)	75 (73 to 86)
Forced vital capacity (Median [IQR])	75 (68.5 to 87.0)	73 (69 to 81)
Carbon monoxide diffusion capacity (Median [IQR])	n=10 76 (74 to 91.5)	n=8 62 (57 to 71.5)
Pulmonary Function Testing[*] – Number less than lower limit of normal		
Forced expiratory volume in 1 sec < 80% predicted (n,%)	5/11 (46)	5/9 (56)

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Forced vital capacity <80% predicted (n,%)	6/11 (55)	6/9 (67)
Carbon monoxide diffusion capacity <80% predicted (n,%)	6/10 (60)	8/8 (100)
Thoracic CT[*]		
No. available (n,%)	5/20 (25)	8/21 (38)
Timing[**] (Days, median [IQR])	181 (157 to 198)	203 (95.5 to 233)
Evidence of interstitial lung disease (n,%)	4/5 (80)	6/8 (75)
Evidence of VTE (n,%)	0/5 (0)	1/8 (13)[***]

[#]For the two patients lost to follow up in the ORBCEL-C group the last available follow up information was at day-90.

[*]CT and pulmonary function testing data were collected from clinically indicated investigations performed between day-28 and 1 year (+/- 30 days).

[**]Time from study drug administration to most recent PFTs or CT imaging.

[***]One additional VTE detected on clinically indicated Thoracic CT during follow up between day 28 and 1 year (which was not reported by the site investigator as a safety event as they considered it to be due to underlying disease – see Table 4a/4b).

[&]For n=1 in the placebo follow up for SMEs was only to year 1 as the patient withdrew consent for SME follow up at year 2.

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Figure 1

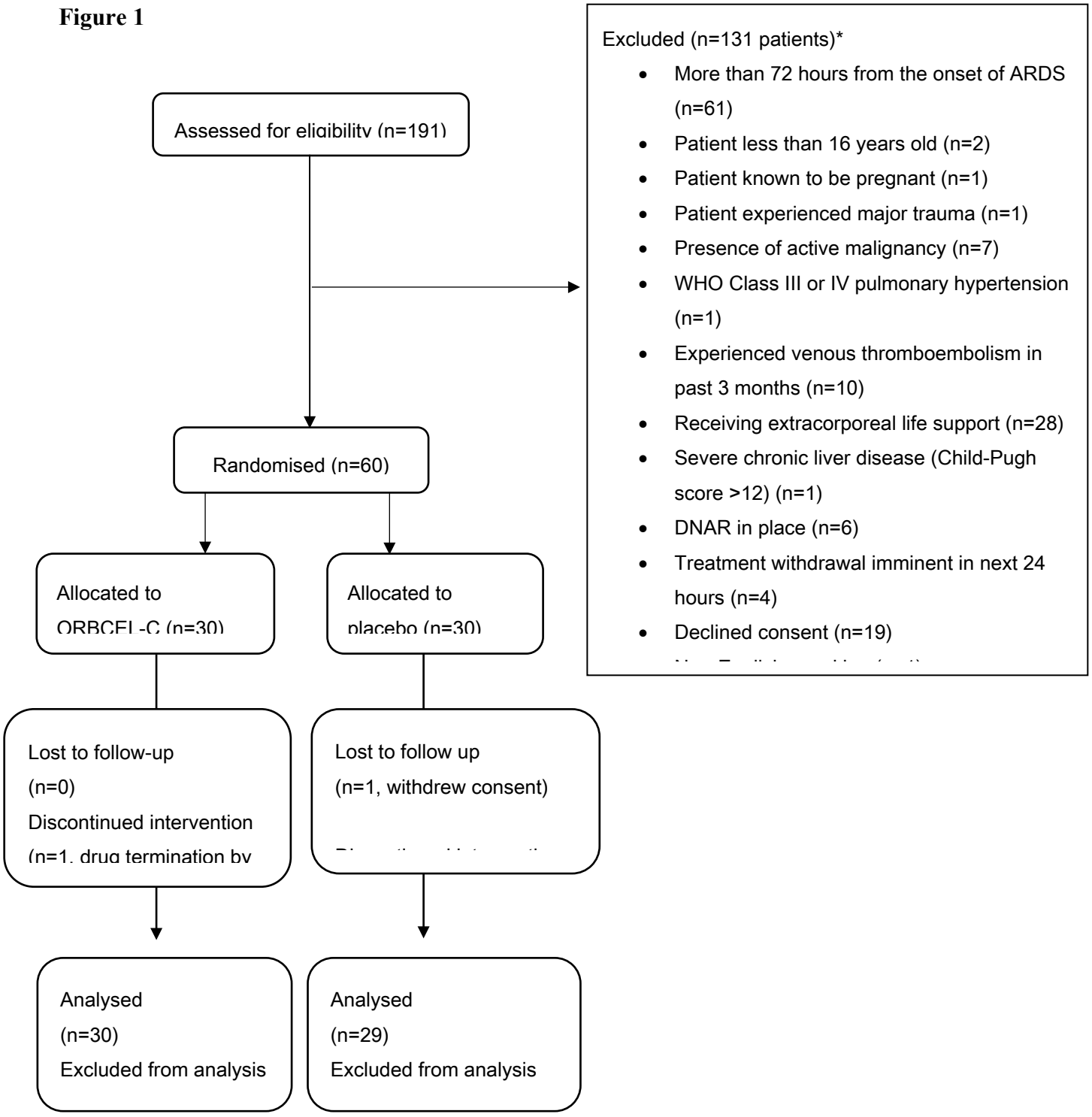

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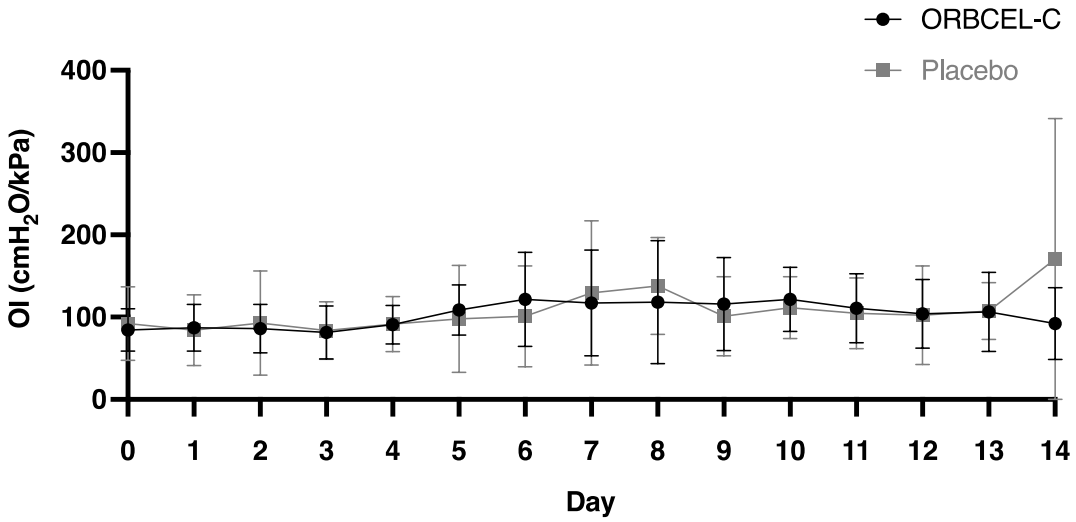
Figure 2



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

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Figure 3

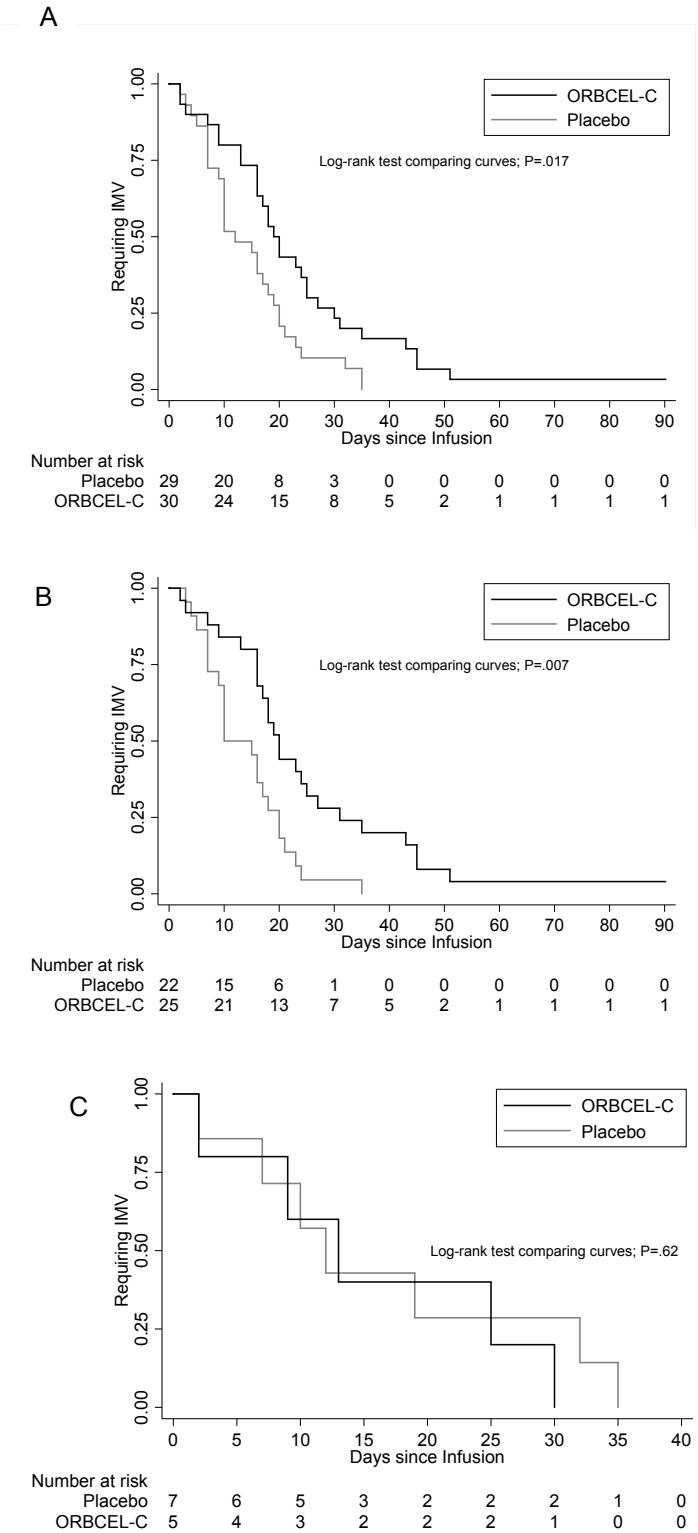

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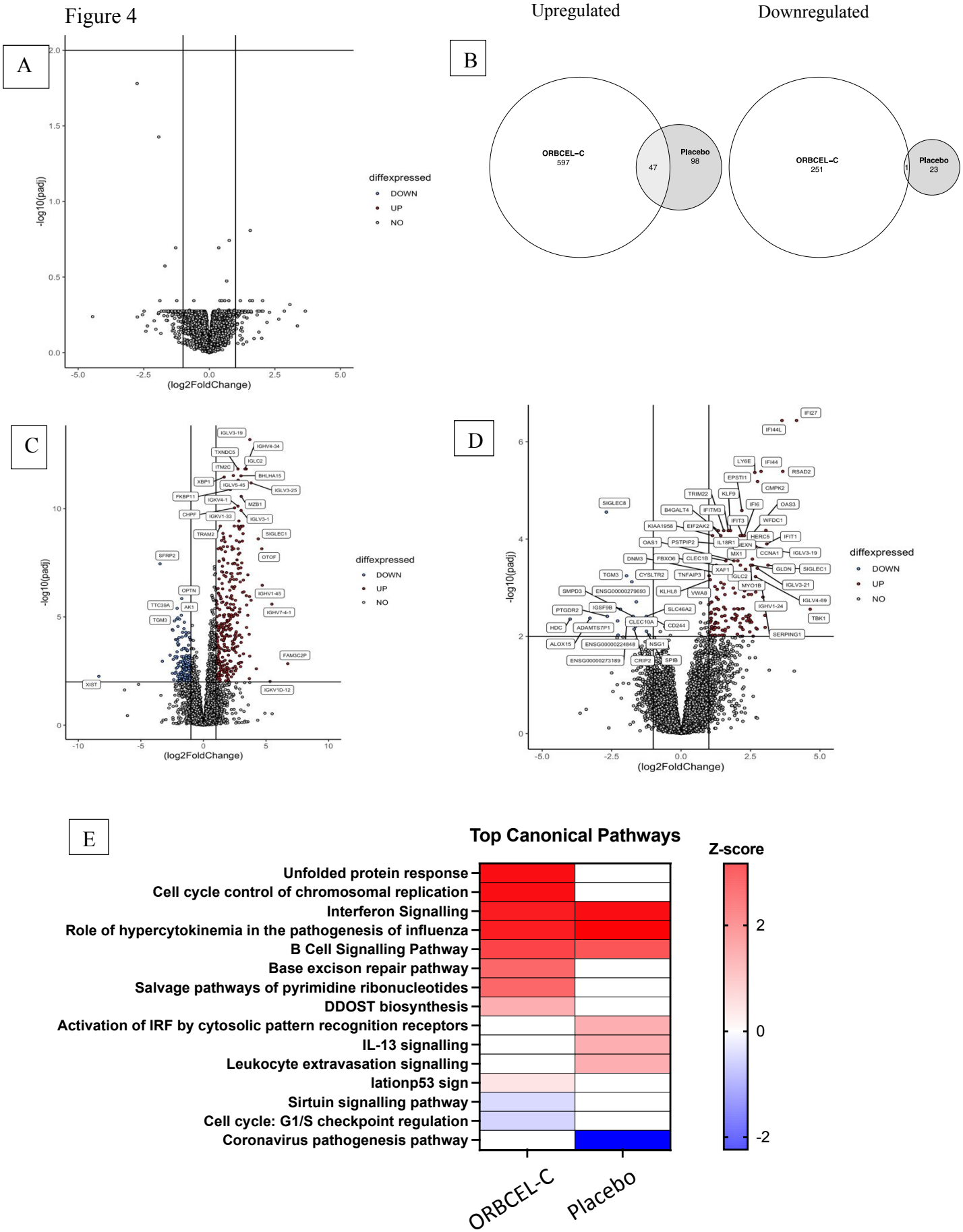
Figure 4



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Repair of Acute Respiratory Distress Syndrome in COVID-19 by Stromal Cells (REALIST-COVID Trial): A Multicentre, Randomised, Controlled Trial

Ellen A Gorman, Jennifer Rynne, Hannah J Gardiner, Anthony J Rostron, Jonathan Bannard-Smith, Andrew M Bentley, David Brealey, Christina Campbell, Gerard Curley, Mike Clarke, Ahilanadan Dushianthan, Phillip Hopkins, Colette Jackson, Kallirroi Kefela, Anna Krasnodembskaya, John G Laffey, Cliona McDowell, Margaret McFarland, Jamie McFerran, Peter McGuigan, Gavin D Perkins, Jonathan Silversides, Jon Smythe, Jacqui Thompson, William S Tunnicliffe, Ingeborg DM Welters, Laura Amado-Rodríguez, Guillermo Albaiceta, Barry Williams, Manu Shankar-Hari, Daniel F McAuley, Cecilia M O'Kane.

ONLINE DATA SUPPLEMENT

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Supplemental Methods

Hospital sites participating in the study

Guy's and St Thomas' Hospital, Guys' and Saint Thomas's NHS Foundation Trust
Kings College Hospital, King's College Hospital NHS Foundation Trust
University College Hospital, University College London Hospital NHS Foundation Trust
Heartlands, University Hospital Birmingham NHS Foundation Trust
Queen Elizabeth Hospital, University Hospital Birmingham NHS Foundation Trust
Belfast City Hospital, Royal Victoria Hospital, and Mater Infirmorum, Belfast Health and Social Care Trust
Liverpool Royal Infirmary, Liverpool and Broadgreen University Hospitals NHS Trust
Manchester Royal Infirmary, Manchester University NHS Foundation Trust
Southampton Hospital, University Hospital Southampton NHS Foundation Trust
Sunderland Royal Hospital, South Tyneside and Sunderland NHS Foundation Trust
Wythenshawe Hospital, Manchester University NHS Foundation Trust
Edinburgh Royal Infirmary

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Study participants

Eligible patients were mechanically ventilated within 72 hours of the onset of moderate to severe Acute Respiratory Distress Syndrome (ARDS, defined by the Berlin criteria (1)), with COVID-19 confirmed either by PCR or clinical diagnosis. Full inclusion and exclusion criteria are detailed below. Patients or their legal representatives provided informed consent.

Eligibility criteria
Inclusion criteria 1. Moderate to severe ARDS as defined by the Berlin definition a. Onset within 1 week of identified insult. b. Within the same 24-hour time period i. Hypoxic respiratory failure (PaO_2/FiO_2 ratio ≤ 27 kPa on PEEP ≥ 5 cm H_20) ii. Bilateral infiltrates on chest X-ray consistent with pulmonary oedema not explained by another pulmonary pathology. iii. Respiratory failure not fully explained by cardiac failure or fluid overload. 2. The patient is receiving invasive mechanical ventilation. 3. COVID-19 based on clinical diagnosis or PCR test.
Exclusion criteria 1. More than 72 hours from the onset of ARDS. 2. Age <16 years. 3. Patient is known to be pregnant. 4. Major trauma in prior 5 days. 5. Presence of any active malignancy (other than non-melanoma skin cancer) that required treatment within the last year. 6. WHO Class III or IV pulmonary hypertension. 7. Venous thromboembolism currently receiving anti-coagulation or within the past 3 months 8. Currently receiving extracorporeal life support (ECLS). 9. Severe chronic liver disease with Child-Pugh score >12. 10. DNAR (Do Not Attempt Resuscitation) order in place. 11. Treatment withdrawal imminent within 24 hours. 12. Consent declined. 13. Prisoners. 14. Non-English speaking patients or those who do not adequately understand verbal or written information unless an interpreter is available. 15. Previously enrolled in the REALIST trial.

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Randomisation and Masking

Participants were randomised (1:1) to receive either ORBCEL-C (400 x 10^6 cells in 200mls) or placebo (Plasma-Lyte 148, 200mls), stratified by recruitment centre and vasopressor use. Patients were randomised by the research staff at the clinical site via an automated centralised 24-hour telephone or web-based randomisation system (CHaRT, Centre for Healthcare Randomised Trials, University of Aberdeen). The randomisation sequence was generated by the Northern Ireland Clinical Trials Unit statistician using NQuery Advisor using variable block sizes and saved in a restricted section of the trial management folder, which can only be accessed by the clinical trials unit statistician and not those who enrol or assign interventions. The investigator, treating physician, other members of the site research team and participants were blinded. Cell therapy facility, clinical trials pharmacists and designated members of the clinical trials unit were unblinded to facilitate preparation of ORBCEL-C and distribution to cell therapy facilities serving the clinical sites. To maintain blinding, placebo was prepared in identical bags to the bags used to infuse the ORBCEL-C MSCs. The infusion bag was masked by an amber opaque bag in the cell therapy facility, and an opaque infusion set was used for MSC/placebo administration.

Study drug manufacture

The ORBCEL-C cellular product consisted of allogeneic donor anti-CD362 antibody enriched human UC-derived mesenchymal stromal cells. It was manufactured to Good Manufacturing Practice standards as previously described (2). In brief (taken directly from reference 2), it was manufactured from three donors, and MSCs from different donors were not pooled during the manufacturing process. Cells isolated from umbilical cord tissue were first enriched for CD362 prior to culture in flasks to passage 2 or 3. Cells were subsequently expanded to passage 4 (P4) with Terumo Quantum® cell expansion system. Cells harvested at P4 were re-suspended in Plasma-Lyte 148 buffer containing 2.5% Human Albumin Solution and an equal volume of CryoStor® CS10 cryoprotectant (DMSO 10%, BioLife Solutions, final DMSO concentration 5%) to achieve a concentration of cells of 10 x 10^6 cells per ml. Cryopreserved drug product was stored at fixed cell doses of either 100 x10^6, 200 x10^6 or 400 x10^6 cells in 10, 20 or 40 ml volumes respectively. The cryopreserved drug product was used within its validated shelf life (24 month validation at the onset of the trial, subsequently validated and extended to 36 months).

Final drug product release testing included appearance, morphology, sterility testing, endotoxin testing, post thaw cell count (trypan blue manual count) , cell viability testing (minimum threshold >70% post thaw) and flow cytometry to confirm cell phenotype fulfils criteria of MSCs described by the international society for cellular therapy (ISCT) (minimum of 90% positive for MSC markers (CD90, CD73 and CD105) and cell purity exceeding 95% (based on maximum values of 5% expression of markers CD45, CD34 and HLA-DR)) (3). Cells also underwent mycoplasma analysis and karyotyping. Validation work has confirmed that a cell viability >70% is maintained for at least 6 hours following the thaw procedure.

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Following manufacture, quality control testing and certification by a Qualified Person (QP), ORBCEL-C stock was distributed to cell therapy facilities (CTF) located in proximity to clinical sites for storage until required (taken from reference 2).

Placebo was Plasma-Lyte-148 200ml, an isotonic buffered crystalloid solution (4), Baxter Healthcare Ltd. The cell product was thawed at CTFs and diluted with Plasma-Lyte 148 to a total volume of 200mls for intravenous administration. Following dilution, the final DMSO concentration in the cellular infusion was 1%. To maintain blinding, care was taken to ensure the placebo was not released earlier than the typical time needed for preparation of the cell therapy product from the frozen cellular drug product. The study drug was transported to the clinical site at 2-8^{o}C. Both the cellular product and the placebo had an equivalent 6-hour expiry date, which for the cellular product was the beginning of the thaw process.

Procedures

All patients received intravenous chlorphenamine 10mg before study drug administration, to reduce any histamine-mediated effects of dimethyl sulfoxide (DMSO) in the cell cryopreservant (5). The cells or matched placebo were administered over 30-90 minutes via a dedicated infusion line, through a standard blood component administration set with a 200 micron in-line filter, and within 6 hours of the start of the thaw process. Patients were monitored throughout the infusion and for five hours following completion of the infusion. All other aspects of care were according to standard critical care guidelines (6), including lung protective ventilation, and at the discretion of the treating physician. A schedule of assessments is provided below.

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Schedule of assessments

	Day 0	Day 1	Day 2-3	Day 4	Day 5-6	Day 7	Day 8-13	Day 14	Day 15-28	Day 90 (+/- 14 days)	1 Year (+/- 30 days)	2 Year (+/- 30 days)
Eligibility assessment	X											
Informed consent	X											
Enrolment/ Randomisation	X											
Baseline data	X											
Daily data		X	X	X	X	X	X	X				
Chlorphenamine administration		X										
Study drug administration		X										
Adverse events		X	X	X	X	X	X	X	X	X		
Mortality									X	X	X	X
Medical Event											X	X

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Adverse event reporting

Adverse events were reported to day-90. Events expected in this critically ill population were not reported as adverse events unless considered to be related to the study drug or unexpectedly severe or frequent.

The following pre-specified adverse events occurring within 6 hours of the start of infusion were collected

1. An increase in vasopressor dose greater than or equal to the following:
    a. Norepinephrine: 0.1 mcg/kg/min
    b. Epinephrine: 0.1 mcg/kg/min
    c. Commencement of any vasopressor including norepinephrine, epinephrine, vasopressin, phenylephrine, and dopamine
2. New ventricular tachycardia, ventricular fibrillation or asystole
3. New cardiac arrhythmia requiring cardioversion
4. Hypoxemia requiring an increase in FiO_2 of 0.2 or more and an increase in PEEP of 5 or more to maintain SpO_2 in the target range
5. Clinical scenario consistent with transfusion incompatibility or transfusion related infection (e.g. urticaria, new bronchospasm).

The following pre-specified adverse events occurring within 24 hours of the start of infusion were collected:

1. Any death
2. Any cardiac arrest
3. Temperatures recorded as >38.5ºC or temperatures that are recorded as >38.5ºC prior to study drug administration and have increased by ≥1ºC.

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Procedures for long-term follow up

Follow up for survival status and significant medical events

At hospital discharge patients' contact information and primary care details were recorded and stored centrally at the Northern Ireland Clinical Trials Unit (NICTU). A thank you note was sent to the patient to thank them for participation in the study and to remind them the trial team would contact them in the future for follow up. Mortality status was obtained from general practice and hospital records where possible and was collected by staff at NICTU at 1- and 2-year timepoints (+/- 30 days). Where possible the patient's status was established prior to direct contact with the patient.

Survivors were followed up for significant medical events at 1 and 2 years via patient telephone interview, medical record review, and/or contact with the primary care provider. Follow up was conducted centrally by two research physicians on behalf of NICTU, and supported by recruiting sites through review of hospital records. Where a patient was not contactable (after reasonable efforts to contact were made), medical records and/or contact with the primary care provider was used as the primary source of follow up information. Where a patient was not contactable, and no information was available on medical records or from a primary care provider, the patient was recorded as lost to follow up.

Significant medical events occurring up to the 1- and 2-year (+/- 30 days) timepoints are reported. A significant medical event was defined as any event which fulfilled criteria for a serious adverse event occurring beyond the adverse event reporting period (which was up to day 90). In addition, if evidence of interstitial lung disease was found, it was reported as a significant medical event if it was considered to be clinically significant (eg associated with new symptoms, treatment or the patient was under the care of (or referred to) a respiratory physician) and/or there was evidence of severe disease on computerised tomography (CT) imaging. A significant medical event was not recorded on the basis of abnormal pulmonary function tests alone. Significant medical events were reviewed by the Chief Investigator for causality and expectedness, and any event considered to be related to the study drug administration would have been reported as a serious adverse reaction and usual expediated adverse event reporting procedures would have been followed.

Extended follow up for evidence of pulmonary dysfunction and interstitial lung disease

Results of clinically indicated thoracic CT and pulmonary function tests (PFTs) occurring between day 28 and 1 year (+/-30 days) were collected and reviewed by a blinded assessor for evidence of pulmonary dysfunction and interstitial lung disease. Data collected included: thoracic CT reports, time from study drug administration to CT and pulmonary function testing (where more than one investigation had been performed, the time to the most recent investigation was used); percentage predicted forced vital capacity, forced expiratory volume in 1 second, and diffusion capacity for carbon monoxide where available.

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Transcriptomic analysis

Sample collection and RNA isolation

Peripheral whole blood samples were collected into PAXgene Blood RNA tubes (PreAnalytiX) and stored at -20 to -80 °C. RNA was isolated according to the manufacturer's instructions using an automated QIAcube system (Qiagen). Total RNA quality and quantity was assessed using the Tapestation 4150 (Agilent) and the Qubit fluorometer (Life Technologies), respectively, according to the manufacturer's instructions.

RNA-sequencing

RNA strand-specific library preparation with globin depletion and poly(A) selection was performed using the NEBNext Globin and rRNA depletion kit, Poly(A) mRNA magnetic isolation module, and the Ultra II directional RNA library prep kit (New England Biolabs) with unique dual index primers, according to the NEB protocol. 100 ng of RNA were used as input for library preparation. RNA-sequencing was performed on a NovaSeq (Illumina) platform (150bp, paired end sequencing) to a depth of 25M paired-end reads per sample, carried out by Genewiz (Azenta Life Sciences).

Data analyses

Cancer cloud genomics (CGC) cloud resource was used for initial data analysis. *FastQC* was used to quality check the files and assess sequencing success *(7)*. The raw sequencing FASTQ files were trimmed of sequencing adapters using *Trimmomatic* (8). Reads were aligned to the transcriptome and transcript abundances were estimated using *Salmon Quant* (8), using the human transcriptome file and gene annotation file from GENCODE (v38). Salmon quant files were imported to RStudio for all downstream analyses. Differential gene expression analysis was performed using *DeSeq2* (9). Treatment groups were compared at baseline and differential gene expression was performed on each group separately comparing changes over time. Treatment groups were compared at baseline and differential gene expression was performed on each group separately comparing changes over time. Genes with a Log2 fold change of >1 or <-1, and an adjusted p-value of <0.01, were considered to be differentially expressed and statistically significant (consistent with approaches used by others (10, 11). *ImmunoStates* was used for deconvolution of cell population proportions (12). Ingenuity pathway analysis (IPA) was performed to identify the top canonical pathways in each treatment group over time (13). For visualisation of the data using volcano plots, boxplots, principle component analysis (PCA) plots and heatmaps, the *Ggplot2* and *gplots* packages were used (14, 15). *Eulerr* was used to generate Venn diagrams (16).

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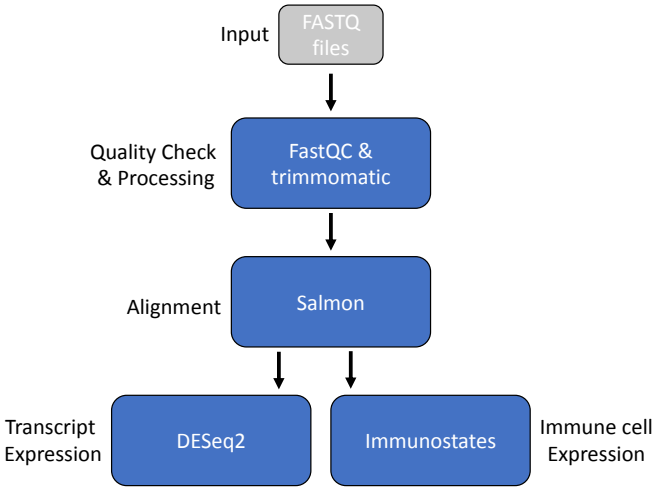
Transcriptomic Analysis Framework



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Supplemental Tables

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Table E1a: Concomitant Medication during hospital stay				
			ORBCEL-C N=30	Placebo N=29
Concomitant Medications (at any point during hospital stay)*	Anti-viral Medications	Yes	15 (50.0%)	16 (55.2%)
		Keletra (Lopinavir/Ritonavir)	2 (6.7%)	3 (10.3%)
		Remedesivir	11 (36.7%)	14 (48.3%)
		Other	2 (6.7%%)	2 (6.9%)
	Experimental Treatments	Yes	4 (13.3%)	4 (13.8%)
		IL-6 blockade	0 (0.0%)	0 (0.0%)
		IL-1 blockade	0 (0.0%)	0 (0.0%)
		IFN	0 (0.0%)	0 (0.0%)
		Macrolides	0 (0.0%)	0 (0.0%)
		Convalescent Plasma	0 (0.0%)	0 (0.0%)
		Heparin/LMWH (not used for prevention/treatment of thrombosis)	3 (10.0%)	4 (13.8%)
		Other	2 (6.7%)	0 (0.0%)
	Steroids	Yes	27 (90.0%)	26 (89.7%)
		Hydrocortisone	4 (13.3%)	7 (24.1%)
		Dexamethasone	24 (80.0%)	23 (79.3%)
		Methylprednisolone	5 (16.7%)	7 (24.1%)
		Prednisolone	2 (6.7%)	4 (13.8%)
		Other	0 (0.0%)	0 (0.0%)
No. (%) presented. *Patients may have received more than one concomitant medication				

Table E1b: Adjuvant therapies day 1 to day 14.			
	Orbcel-C n=30	Placebo n=29	Total
Airway Pressure Release Ventilation	5 (16.7%)	6 (20.7%)	11 (18.6%)
High-frequency oscillatory ventilation	2 (6.7%)	0 (0.0%)	2 (3.4%)
Neuromuscular Blocking Drugs	23 (76.7%)	23 (79.3%)	46 (78.0%)
Inhaled Vasodilator	3 (10.0%)	6 (20.7%)	9 (15.3%)
Prone position	20 (66.7%)	16 (55.2%)	36 (61.0%)
ECMO/$ECCO_2R$	1 (3.3%)	2 (6.9%)	3 (5.1%)
No. (%) presented.			

Table E2: Timing of study drug administration		
	Treatment Group	
	Orbcel-C n=30	Placebo n=29
Duration between thaw and commencement of IMP administration (Minutes)	172.7 (55.6)	179.9 (45.4)
Duration between thaw and completion of IMP administration (Minutes)	212.1 (54.4)	222.8 (45.4)
Duration of infusion (Minutes)	39.4 (10.3) 37.5 (30 to 45)	42.9 (11.6) 40 (30 to 50)
Duration between ICU admission and completion of IMP administration (Days)	3.2 (2.9)	2.5 (1.3)
Duration between commencement of IMV and completion of IMP administration (Days)	1.9 (0.7)	2.0 (0.8)
Mean (SD) or Median (IQR) reported		

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Table E3a: Classification of Adverse Events						
	Number of events		Number of patients*		Risk Ratio (95% Confidence Interval	p-value
	ORBCEL-C	Placebo	ORBCEL-C	Placebo		
Adverse Events						
Cardiac disorders	1	1	1 (3.3%)	1 (3.4%)	0.97 (0.06 to 14.7)	1.00
Gastrointestinal disorders	0	1	0 (0.0%)	1 (3.4%)	0 (-)	0.49
General disorders and administration site conditions	1	0	1 (3.3%)	0 (0.0%)	-	1.00
Respiratory, thoracic and mediastinal disorders	3	0	3 (10.0%)	0 (0.0%)	-	0.24
Vascular disorders	1	1	1 (3.3%)	1 (3.4%)	0.97 (0.06 to 14.7)	1.00
Blood and lymphatic system disorders	4	1	4 (13.3%)	1 (3.4%)	3.9 (0.5 to 32.6)	0.35
Serious Adverse Events						
Cardiac disorders	2	3	2 (6.7%)	3 (10.3%)	0.64 (0.1 to 3.6)	0.67
Gastrointestinal disorders	0	3	0 (0.0%)	3 (10.3%)	0 (-)	0.11
General disorders and administration site conditions	1	0	1 (3.3%)	0 (0.0%)	-	1.00
Hepatobiliary disorders	1	1	1 (3.3%)	1 (3.4%)	1.0 (0.06 to 14.7)	1.00
Infections and infestations	4	3	2 (6.7%)	3 (10.3%)	0.64 (0.1 to 3.6)	0.67
Injury, poisoning and procedural complications	0	1	0 (0.0%)	1 (3.4%)	0 (-)	0.49
Investigations	1	2	1 (3.3%)	2 (6.9%)	0.48 (0.05 to 5.0)	0.61
Nervous system disorders	3	0	3 (10.0%)	0 (0.0%)	-	0.24
Renal and urinary disorders	1	0	1 (3.3%)	0 (0.0%)	-	1.00
Respiratory, thoracic and mediastinal disorders	3	1	3 (10.0%)	1 (3.4%)	2.9 (0.3 to 26.3)	0.61
Skin and subcutaneous tissue disorders	1	0	1 (3.3%)	0 (0.0%)	-	1.00
Vascular disorders	2	1	2 (6.7%)	1 (3.4%)	1.9 (0.2 to 19.5)	1.00
*No. (%) for number of patients based on total number of patients in each group.						

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Table E3b: Full listing of Adverse Events reported							
Allocation	AE term	System Organ Class	Severity	Causality	Expectedness	Serious	Study Day*
Orbcel-C	Internal Jugular Venous thrombosis	Blood and lymphatic system disorders	Mild (Grade 1)	Not related	N/A	No	19
Orbcel-C	Drop in platelet level from 143 to 66	Blood and lymphatic system disorders	Mild (Grade 1)	Unlikely	N/A	No	12
Orbcel-C	Anaemia	Blood and lymphatic system disorders	Moderate (Grade 2)	Not related	N/A	No	4
Orbcel-C	Small clot in left subclavian vein	Blood and lymphatic system disorders	Mild (Grade 1)	Unlikely	N/A	No	38
Orbcel-C	SVT and fast AF	Cardiac disorders	Moderate (Grade 2)	Not related	N/A	Yes	4
Orbcel-C	Non-sustained Ventricular tachycardia	Cardiac disorders	Moderate (Grade 2)	Not related	N/A	No	17
Orbcel-C	Multi Organ Failure	General disorders and administration site conditions	Death (Grade 5)	Unlikely	N/A	Yes	13
Orbcel-C	Deranged LFTs	Hepatobiliary disorders	Mild (Grade 1)	Not related	N/A	No	3
Orbcel-C	Antibiotic resistant Pseudomonas aeruginosa on vascular line tip	Infections and infestations	Moderate (Grade 2)	Not related	N/A	No	10
Orbcel-C	Vancomycin resistant E. faecium in urine	Infections and infestations	Moderate (Grade 2)	Not related	N/A	No	13
Orbcel-C	Antibiotic resistant Klebsiella aerogenes in bronchial lavage sample	Infections and infestations	Moderate (Grade 2)	Not related	N/A	No	7
Orbcel-C	Pyrexia	Infections and infestations	Mild (Grade 1)	Possibly	Expected	No	2
Orbcel-C	Deranged liver enzymes	Investigations	Moderate (Grade 2)	Not related	N/A	No	1
Orbcel-C	Right sided watershed infarcts	Nervous system disorders	Severe (Grade 3)	Not related	N/A	No	24
Orbcel-C	Myoclonic Jerks	Nervous system disorders	Mild (Grade 1)	Not related	N/A	No	3
Orbcel-C	Bilateral tremor	Nervous system disorders	Mild (Grade 1)	Not related	N/A	No	76
Orbcel-C	Acute on chronic kidney injury requiring haemofiltration	Renal and urinary disorders	Severe (Grade 3)	Not related	N/A	No	1
Orbcel-C	Pneumomediastinum	Respiratory, thoracic and mediastinal disorders	Death (Grade 5)	Not related	N/A	Yes	1
Orbcel-C	Haemorrhagic ARDS	Respiratory, thoracic and mediastinal disorders	Moderate (Grade 2)	Not related	N/A	Yes	12
Orbcel-C	Respiratory Deterioration	Respiratory, thoracic and mediastinal disorders	Severe (Grade 3)	Unlikely	N/A	Yes	11
Orbcel-C	Rash	Skin and subcutaneous tissue disorders	Mild (Grade 1)	Unlikely	N/A	No	16
Orbcel-C	Pulmonary Embolism	Vascular disorders	Severe (Grade 3)	Not related	N/A	No	5
Orbcel-C	Pulmonary embolism with non-occlusive thrombi in the right lower lobe pulmonary artery.	Vascular disorders	Mild (Grade 1)	Unlikely	N/A	Yes	15
Placebo	Haemoptysis	Blood and lymphatic system disorders	Moderate (Grade 2)	Not related	N/A	No	5
Placebo	Prolonged QTc	Cardiac disorders	Mild (Grade 1)	Not related	N/A	No	27
Placebo	STEMI	Cardiac disorders	Moderate (Grade 2)	Not related	N/A	Yes	27
Placebo	AF	Cardiac disorders	Moderate (Grade 2)	Not related	N/A	No	2
Placebo	Upper GI Bleed	Gastrointestinal disorders	Moderate (Grade 2)	Not related	N/A	No	7

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Placebo	Small bowel hernia with massively distended loops of small bowel	Gastrointestinal disorders	Life-threatening (Grade 4)	Not related	N/A	Yes	10
Placebo	PR Bleed - Enteritis	Gastrointestinal disorders	Mild (Grade 1)	Not related	N/A	No	9
Placebo	Acute Liver Failure	Hepatobiliary disorders	Moderate (Grade 2)	Not related	N/A	No	17
Placebo	Ventilator Aquired Pneumonia diagnosis - Klebsiella in sputum	Infections and infestations	Life-threatening (Grade 4)	Unlikely	N/A	No	9
Placebo	CMV viraemia	Infections and infestations	Moderate (Grade 2)	Not related	N/A	No	16
Placebo	Pyrexic	Infections and infestations	Mild (Grade 1)	Possibly	Expected	No	2
Placebo	Ulcerated lesion on the posterior tracheal wall	Injury, poisoning and procedural complications	Mild (Grade 1)	Not related	N/A	No	28
Placebo	Fibrotic lung disease secondary to covid 19	Investigations	Moderate (Grade 2)	Not related	N/A	No	27
Placebo	Baseline temperature unrecordable-patient being actively warmed with bair hugger-15mins into IMP infusion patient temperature 33.9	Investigations	Mild (Grade 1)	Unlikely	N/A	No	1
Placebo	Bilateral pneumothoraces and pneumomediastinum secondary to barotrauma	Respiratory, thoracic and mediastinal disorders	Moderate (Grade 2)	Not related	N/A	No	16
Placebo	Right Lower Lobe Pulmonary Emboli	Vascular disorders	Moderate (Grade 2)	Not related	N/A	Yes	28

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Table E4a: Venous Thromboembolism (VTE) and Pulmonary Emboli reported as safety event				
	No. of Events		No. of Patients	
	ORBCEL-C	Placebo	ORBCEL-C	Placebo
Deep Vein Thrombosis*	2	0	2	0
Pulmonary Embolism	2	1	2	1
*includes internal jugular venous thrombosis and left subclavian vein thrombus				

Table E4b: Detailed assessment of Venous Thromboembolism (VTE) and Pulmonary Emboli reported as safety event						
Event Description	Treatment group	System Organ Class	Time of event	Causality	Expectedness	Classification
Right lower lobe pulmonary emboli	Placebo	Vascular disorders	Day 28	Not related	Unexpected	Moderate (Grade 2)
Pulmonary emboli	ORBCEL-C	Vascular disorders	Day 5	Not related	Unexpected	Severe (Grade 3)
Pulmonary embolism with non-occlusive thrombi in right lower lobe pulmonary artery	ORBCEL-C	Vascular disorders	Day 15	Unlikely	Unexpected	Mild (Grade 1)
Left subclavian vein thrombus	ORBCEL-C	Blood and lymphatic system disorders	Day 38	Unlikely	Unexpected	Mild (Grade 1)
Internal jugular vein thrombus	ORBCEL-C	Blood and lymphatic system disorders	Day 19	Not related	Unexpected	Mild (Grade 1)

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Table E5a: Adjusted analysis for Oxygenation Index (cmH_2O/kPa) at day 7				
	ORBCEL-C n=30	Placebo n=29	Mean Difference (95% CI)	p-value
Imputed				
Last value carried forward	96.0 (11.6)	98.9 (11.8)	-2.9 (-36.6 to 30.8)	0.86
Minimum value	88.7 (12.3)	90.0 (12.5)	-1.3 (-36.9 to 34.3)	0.94
Maximum value	109.9 (13.5)	133.0 (13.8)	-23.1 (-62.2 to 16.1)	0.24
Mean substitution	97.6 (11.4)	106.0 (11.2)	-8.5 (-40.9 to 24.0)	0.60
Observed values	108.6 (17.0)	141.7 (20.3)	-33.1 (-89.8 to 23.6)	0.24
Mean (SE) presented for the adjusted analysis (adjusted for baseline age, PF Ratio, APACHE II and vasopressor use) in the intention-to-treat population.				

Table E5b: Post-hoc adjusted analysis for Oxygenation Index (cmH_2O/kPa) at day 7 (to include site)				
	ORBCEL-C n=30	Placebo n=29	Mean Difference (95% CI)	p-value
Last value carried forward	98.3 (10.4)	96.6 (12.5)	1.2 (-32.6 to 35.0)	0.94
Observed values	108.1 (20.2)	142.3 (24.6)	-34.2 (-107.8 to 39.5)	0.34
Mean (SE) presented for the adjusted analysis (adjusted for baseline age, PF Ratio, APACHE II, vasopressor use, and site) in the intention-to-treat population.				

Table E5c: Reasons for missing observed data for Oxygenation Index at day 7		
	ORBCEL-C	Placebo
Death	1	1
Extubation	2	2
Pressure support ventilation	8	12
ICU discharge	0	1
Missing data	1	0

Table E5d: Secondary analysis of oxygenation index (cmH_2O/kPa) at day 7 in a per protocol population								
	Unadjusted, Mean (SD)		Mean Difference (95% CI)	p-value	Adjusted, Mean (SE)‡		Mean Difference (95% CI)	p-value
	ORBCEL-C n=29*	Placebo n=29			ORBCEL-C	Placebo		
Observed values	118.5 (66.4) n=17	129.4 (87.8) n=13	-10.9 (-68.5 to 46.7)	0.70	108.7 (18.0)	142.3 (20.8)	-33.6 (-92.9 to 25.6)	0.25
Imputed values	98.3 (58.2)	96.6 (67.3)	1.7 (-31.4 to 34.8)	0.92	95.9 (12.0)	98.9 (11.9)	-3.0 (-37.3 to 31.4)	0.86
Per protocol analyses which includes only those patients who completed the study drug infusion. *n=1 in ORBCEL-C group had study drug administration terminated early by the treating physician as sediment was noted in the infusion filter, therefore n=29 were included in the per-protocol analysis. ‡Adjusted for baseline age, PF Ratio, APACHE II and Vasopressor Use								

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Table E5e: Additional analysis of oxygenation index (cmH_2O/kPa) at day 7 in patients with confirmed PCR COVID-19								
	Unadjusted, Mean (SD)		Mean Difference (95% CI)	p-value	Adjusted, Mean (SE)*		Mean Difference (95% Confidence Interval)	p-value
	ORBCEL-C n=30	Placebo n=27			ORBCEL-C	Placebo		
Observed values	117.4 (64.5) n=18	129.4 (87.8) n=13	-12.0 (-67.8 to 43.9)	0.66	108.6 (17.0)	141.7 (20.3)	-33.1 (-89.8 to 23.6)	0.24
Imputed Values								
Last value carried forward	98.3 (57.2)	99.6 (68.5)	-1.3 (-34.7 to 32.1)	0.94	96.4 (11.7)	101.7 (12.4)	-5.3 (-39.8 to 29.2)	0.76
Minimum value	91.0 (60.3)	91.0 (72.4)	0.04 (-35.2 to 35.3)	1.00	89.3 (12.4)	92.9 (13.1)	-3.6 (-40.1 to 32.9)	0.84
Maximum value	111.8 (55.3)	134.7 (88.4)	-23.0 (-61.7 to 15.8)	0.24	108.9 (13.0)	138.0 (13.7)	-29.2 (-67.5 to 9.2)	0.13
Mean substitution	100.0 (55.3)	106.6 (65.6)	-6.6 (-38.7 to 25.5)	0.68	97.7 (11.2)	109.1 (11.8)	-11.4 (-44.3 to 21.5)	0.49
*Adjusted for age, PF Ratio, APACHE II and Vasopressor Use								

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Table E6: Secondary outcomes pulmonary and non-pulmonary organ function				
	Treatment Group		Mean Difference (95% Confidence Interval)	p-value
	ORBCEL-C n=30	Placebo n=29		
Oxygenation Index (cmH_2O/kPa)				
Day 4	90.9 (23.5) n=17	91.4 (33.8) n=21	0.5 (-19.1 to 20.2)	0.96
Day 14	92.3 (43.9) n=11	170.8 (170.8) n=4	78.5 (-35.9 to 192.9)	0.16
Respiratory compliance (ml/cmH_2O)				
Day 4	30.9 (8.2) n=16	33.5 (19.5) n=19	2.6 (-8.0 to 13.2)	0.62
Day 7	47.4 (45.0) n=18	27.8 (8.7) n=11	-19.6 (-47.9 to 8.7)	0.17
Day 14	26.8 (12.7) n=11	20.2 (4.4) n=3	-6.6 (-23.3 to 10.0)	0.40
Driving Pressure (cmH_2O)				
Day 4	14.7 (3.9) n=16	13.9 (5.2) n=19	-0.8 (-4.0 to 2.4)	0.62
Day 7	13.1 (6.5) n=18	14.5 (3.4) n=11	1.5 (-2.9 to 5.8)	0.49
Day 14	18.0 (6.3) n=11	20.3 (1.5) n=3	2.4 (-5.9 to 10.6)	0.54
PF ratio (PaO_2/FiO_2)				
Day 4	19.6 (4.5) n=29	21.4 (7.2) n=28	1.9 (-1.3 to 5.0)	0.25
Day 7	18.2 (5.9) n=28	19.3 (6.2) n=27	1.2 (-2.1 to 4.5)	0.47
Day 14	26.6 (12.3) n=23	22.6 (6.3) n=17	-4.0 (-10.6 to 2.6)	0.23
Sequential Organ Failure Assessment (SOFA) score				
Day 4	6.0 (3.5) n=26	6.5 (3.5) n=27	0.6 (-1.4 to 2.5)	0.56
Day 7	5.9 (3.7) n=28	5.8 (3.2) n=21	-0.05 (-2.1 to 2.0)	0.96
Day 14	6.2 (3.8) n=21	5.5 (2.4) n=15	-0.7 (-2.9 to 1.6)	0.56
Mean (SD) presented for treatment arms. P-value from 2-sample t-test.				

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Table E7: Subgroup analysis of oxygenation index (cmH_2O/kPa) at day 7

		ORBCEL-C n=30	Placebo n=29	Mean Difference (99% Confidence Intervals)	Interaction Term
CRP (mg/L)	< median	77.7 (28.5) n=14	74.4 (23.5) n=15	3.3 (-56.0 to 62.6)	0.90
	≥ median	116.4 (69.8) n=16	116.8 (92.1) n=13	-0.4 (-60.0 to 59.2)	
Ferritin (ng/ml)	<1500	77.8 (24.0) n=9	87.8 (29.3) n=6	-10.0 (-53.0 to 33.1)	0.070
	≥1500	108.4 (16.0) n=4	77.5 (38.3) n=7	30.9 (-20.3 to 82.1)	
PF ratio (PaO_2/FiO_2)	<20	108.3 (62.4) n=22	83.6 (29.1) n=15	24.6 (-30.4 to 79.6)	0.056
	≥20	71.1 (26.1) n=8	110.4 (91.9) n=14	-39.3 (-112.1 to 33.5)	

Mean (SD) presented.

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Table E8: Subgroup analyses for selected secondary outcomes						
			Treatment Group		Mean Difference/Risk Ratio (99% Confidence Intervals)	Interaction Term
			ORBCEL-C	Placebo		
Ventilation Free Days at day 28*	CRP (mg/L)	< median	4.0 (0.0-15.0) 8.0 (9.2) n=14	7.0 (0.0-18.0) 8.7 (9.1) n=15	-0.7 (-8.8 to 7.3)	0.28
		≥ median	3.5 (0.0-9.5) 4.5 (4.7) n=16	10.0 (0.0-18.0) 9.7 (8.9) n=13	-5.2 (-13.3 to 2.9)	
	Ferritin (ng/ml)	<1500	0.0 (0.0-12.0) 5.3 (7.8) n=9	11.5 (0.0-13.0) 9.8 (8.8) n=6	-4.5 (-16.0 to 7.0)	0.25
		≥1500	9.5 (4.5-10.0) 7.3 (4.9) n=4	0.0 (0.0-10.0) 5.0 (8.0) n=7	2.3 (-11.4 to 15.9)	
	PF ratio (PaO_2/FiO_2)	<20	0.0 (0.0-9.0) 4.3 (6.7) n=22	10.0 (0.0-18.0) 10.0 (8.7) n=15	-5.7 (-12.7 to 1.4)	0.034
		≥20	12.0 (6.5- 15.5) 11.1 (6.9) n=8	4.0 (0.0-18.0) 7.6 (9.2) n=14	3.5 (-5.8 to 12.8)	
28 day mortality**	CRP (mg/L)	< median	2 (14.3%) n=14	5 (33.3%) n=15	0.4 (0.06 to 3.0)	0.16
		≥ median	3 (18.8%) n=16	1 (7.7%) n=13	2.4 (0.1 to 40.7)	
	Ferritin (ng/ml)	<1500	1 (11.1%) n=9	1 (16.7%) n=6	0.7 (0.02 to 19.6)	-
		≥1500	0 (0.0%) n=4	2 (28.6%) n=7	-	
	PF ratio (PaO_2/FiO_2)	<20	5 (22.7%) n=22	3 (20.0%) n=15	1.1 (0.2 to 6.0)	-
		≥20	0 (0.0%) n=8	3 (21.4%) n=14	-	
Mean (SD), median (IQR) or n (%) presented. *Mean difference presented **Risk Ratio presented						

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Table E9: Description of Significant Medical Events		
	ORBCEL-C	Placebo
Number followed up at 1 year	20	21
Number followed up at 2 years	20	20
Total No. of events	14	11
Total No. of patients (n,%)	11/20 (55)	9/21 (43)
Cardiac Disorders	1	0
Infection	2	1
Fracture	2	1
Gastrointestinal disorder	0	1
Neoplasm	3	0
Hyperglycaemia	1	1
Renal and urinary disorder	1	1
Respiratory Disorders (Pulmonary Fibrosis)	4	6
[&]For n=1 in the placebo follow up for SMEs was only to year 1 as the patient withdrew consent for SME follow up at year 2.		

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Table E10: Clinical Laboratory Assessments at day 0, 4, 7, and 14		
	ORBCEL-C n=30	Placebo n=29
AST (U/L)		
Day 0	37.3 (22.7) n=16	43.5 (41.2) n=15
Day 4	61.1 (48.8) n=15	48.7 (36.8) n=13
Day 7	46.1 (28.1) n=16	42.1 (18.4) n=14
Day 14	45.1 (27.1) n=14	64.6 (80.1) n=8
ALT (U/L)		
Day 0	42.2 (22.5) n=20	40.9 (31.3) n=18
Day 4	54.3 (22.4) n=13	59.3 (49.7) n=11
Day 7	59.1 (29.3) n=15	56.4 (36.1) n=8
Day 14	58.5 (43.4) n=11	113.6 (83.4) n=8
ALP (U/L)		
Day 0	91.2 (54.8) n=28	86.5 (41.6) n=27
Day 4	102.7 (49.2) n=23	88.3 (36.9) n=24
Day 7	105.9 (50.7) n=26	85.9 (39.2) n=21
Day 14	125.3 (83.6) n=20	115.9 (64.3) n=17
CRP (mg/L)		
Day 0	145.3 (109.6)	136.6 (103.0) n=28
Day 4	85.2 (99.2) n=23	86.8 (105.6) n=23
Day 7	136.8 (130.0) n=24	94.0 (109.9) n=20
Day 14	101.9 (80.5) n=21	90.5 (71.2) n=16
PT (s)		
Day 0	11.7 (1.4) n=22	12.5 (2.1) n=20
Day 4	11.7 (1.4) n=19	11.7 (1.5) n=14
Day 7	11.5 (1.0) n=17	11.8 (1.7) n=14
Day 14	11.7 (1.0) n=13	10.7 (3.6) n=10
APTT (s)		
Day 0	26.2 (5.6) n=25	29.5 (6.6) n=23
Day 4	28.8 (14.4) n=19	24.7 (8.1) n=18
Day 7	23.9 (8.9) n=19	26.4 (6.5) n=17
Day 14	29.8 (19.6) n=17	25.3 (9.7) n=12
Fibrinogen (g/l)		
Day 0	5.7 (1.7) n=24	5.4 (1.6) n=21
Day 4	5.3 (1.3)	4.7 (1.5)

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	n=19	n=12
Day 7	6.6 (1.9) n=15	5.6 (1.9) n=15
Day 14	6.3 (1.2) n=13	5.8 (1.8) n=12
Hb (g/L)		
Day 0	116.0 (15.7)	116.1 (15.7)
Day 4	108.8 (18.7) n=28	109.4 (16.7) n=25
Day 7	107.3 (19.7) n=29	106.7 (18.2) n=25
Day 14	94.2 (17.0) n=23	94.6 (15.2) n=18
WBC ($x10^9$/L)		
Day 0	12.3 (7.3)	11.7 (5.2)
Day 4	11.7 (4.2) n=28	12.4 (5.8) n=25
Day 7	15.2 (6.3) n=29	13.0 (5.3) n=25
Day 14	12.1 (4.5) n=23	11.2 (3.7) n=18
Neutrophils ($x10^9$/L)		
Day 0	10.9 (7.5)	10.2 (4.9)
Day 4	9.2 (2.9) n=27	10.6 (5.5) n=25
Day 7	13.1 (6.0) n=29	10.7 (5.0) n=25
Day 14	9.1 (4.1) n=22	8.8 (3.4) n=18
Highest Urea (mmol/L)		
Day 0	12.9 (6.9)	11.6 (5.3)
Day 4	13.2 (5.7) n=27	15.2 (6.4) n=25
Day 7	12.1 (3.9) n=28	15.8 (11.0) n=25
Day 14	16.1 (11.3) n=24	15.0 (6.8) n=18
Lowest eGFR (mL/min)		
Day 0	58.8 (27.7) n=28	67.7 (21.0) n=28
Day 4	62.8 (27.4) n=25	68.8 (23.7) n=26
Day 7	68.0 (27.4) n=28	67.9 (25.3) n=23
Day 14	63.5 (34.9) n=24	72.2 (24.4) n=18
Ferritin (ng/ml)		
Day 0	1394.5 (617.4) n=13	1728.8 (997.0) n=13
Day 4	844.7 (357.3) n=6	1617.1(1813.1) n=8
Day 7	1020.0 (434.2) n=11	808.4 (901.2) n=13
Day 14	569.2 (254.1) n=6	1284.6 (1687.4) n=10
Mean (SD) presented.		

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Supplemental Figure Legends

Figure E1: Screening and recruitment data for each clinical site.

Figure E2: Post infusion parameters

Mean (SD) for Temperature (A), FiO_2 (B), PEEP (C), Oxygen Saturation (D), Plateau Pressure (E), PaO_2 (F), $PaCO_2$ (G), Heart Rate (H), Systolic Blood Pressure (I) and Diastolic Blood Pressure (J) following administration of the study drug are presented. Repeated measures ANOVA demonstrated plateau pressure differed significantly over time ($p=0.01$). No other differences over time or between treatment groups were significant ($p<0.05$).

Figure E3: Pulmonary and non-pulmonary organ function

PaO_2/FiO_2 (PF) ratio (A), Respiratory compliance (B), Driving pressure (C), and SOFA score (D) at day 0, 4, 7, and 14. Mean (SD) are presented.

Figure E4: Clinical laboratory measurements.

White Blood Cells, WBC (A), and C-Reactive Protein, CRP (B), over time up to day 14. Mean (SD) are presented. Between group comparison by repeated measures ANOVA demonstrated no difference between the ORBCEL-C and placebo group over time from day 0 to day 14 (CRP $p=0.70$, WBC $p=0.85$).

Figure E5: Clinical laboratory measurements of coagulation.

Prothrombmin Time, PT (A), Activated Partial Thromboplastin Time, APTT (B), and Fibrinogen (C) over time up to day 14. Mean (SD) are presented.

Figure E6: Heatmap highlighting the magnitude of expression of DEGs and the overlap between the treatment groups.

Figure E7: Transcriptomic analysis – Deconvolution of immune cell populations

Boxplots depicting the expression of immune cell populations at baseline (D0), day 4 (D4) and day 7 (D7) in ORBCEL-C (pink) and placebo (lemon) groups, generated using the deconvolution package *Immunostates*.

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Figure E1

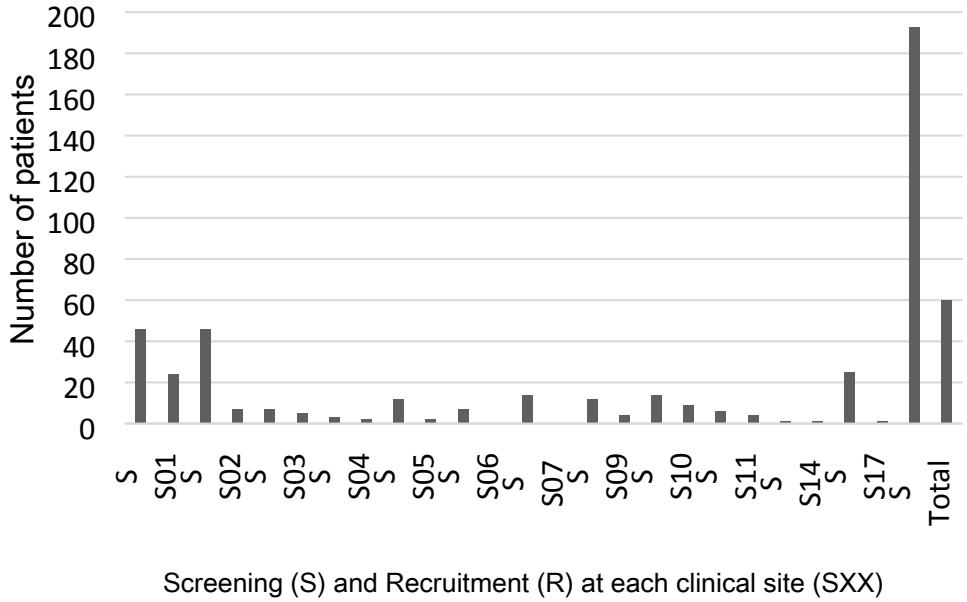

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Figure E2

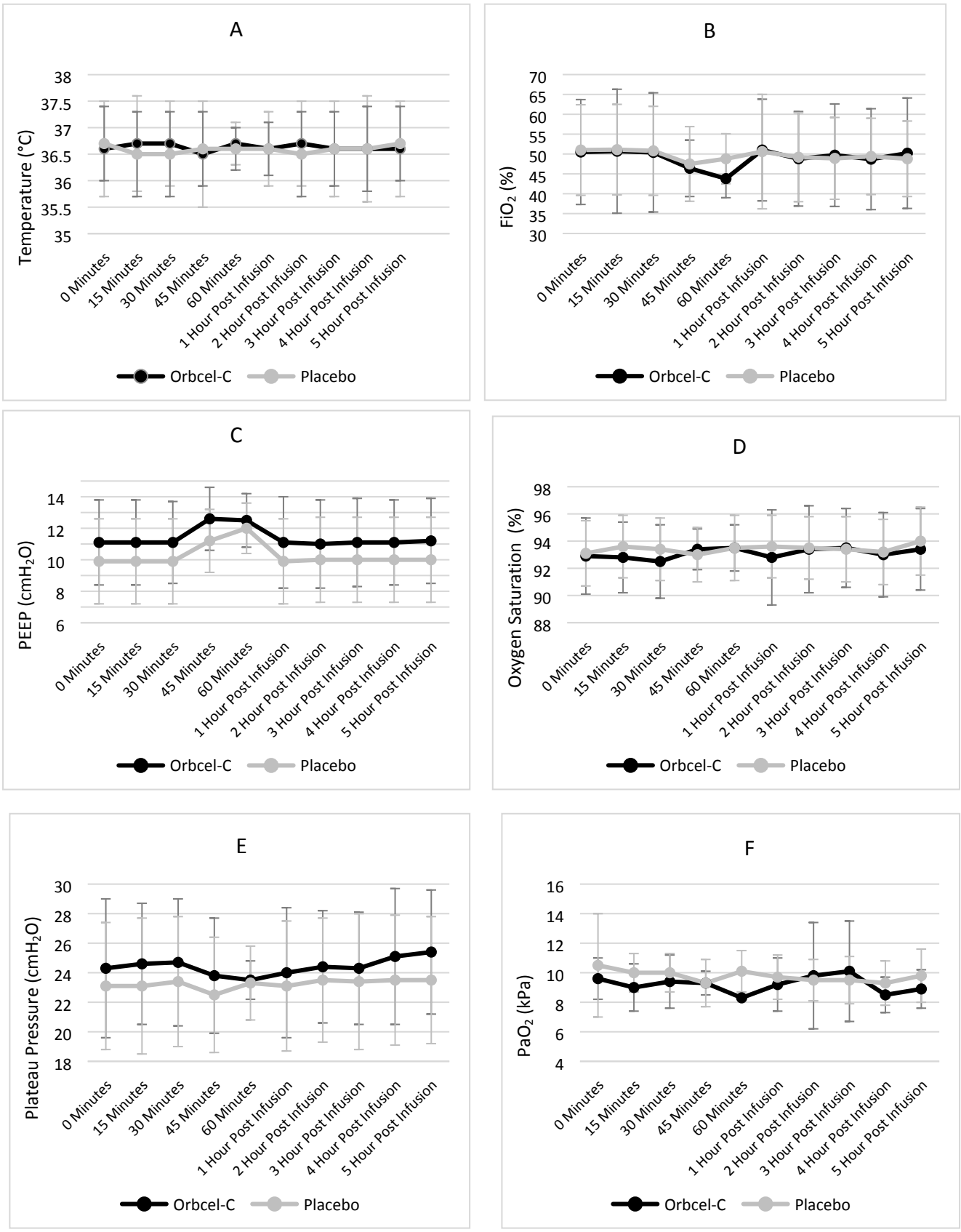

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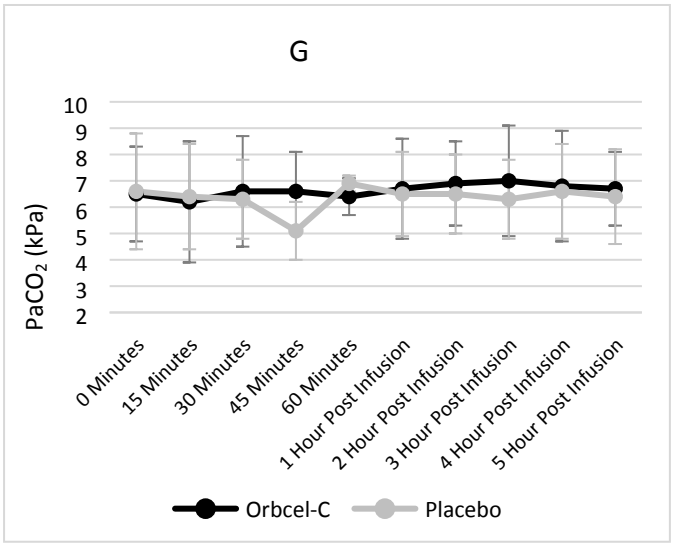
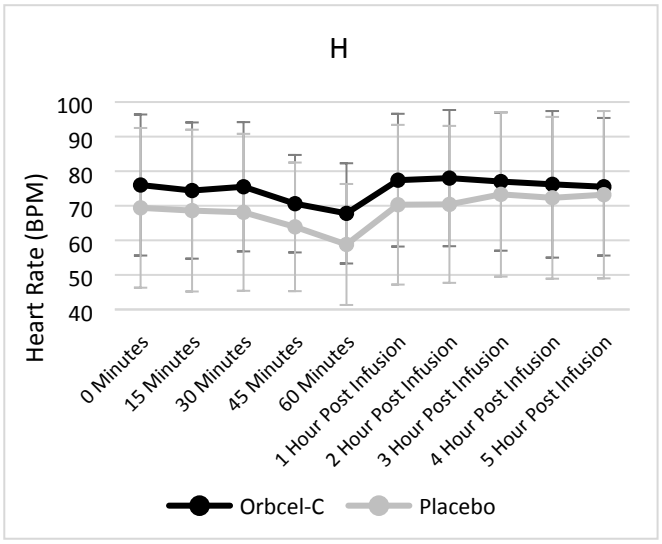
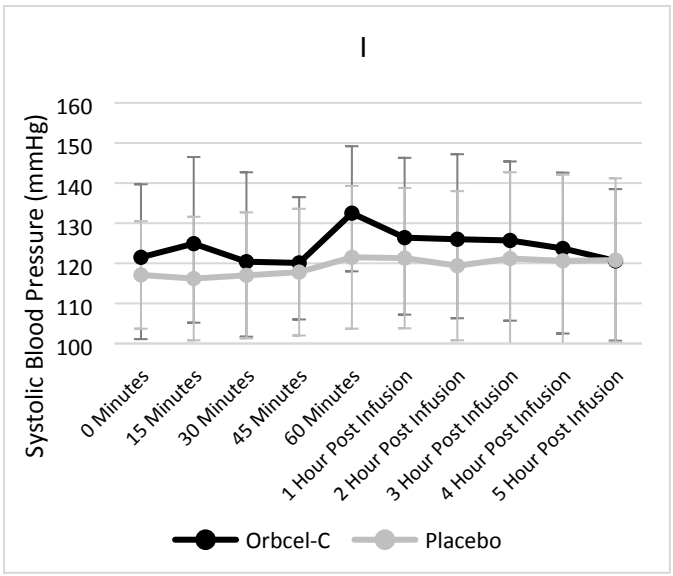
Figure E2 (continued)







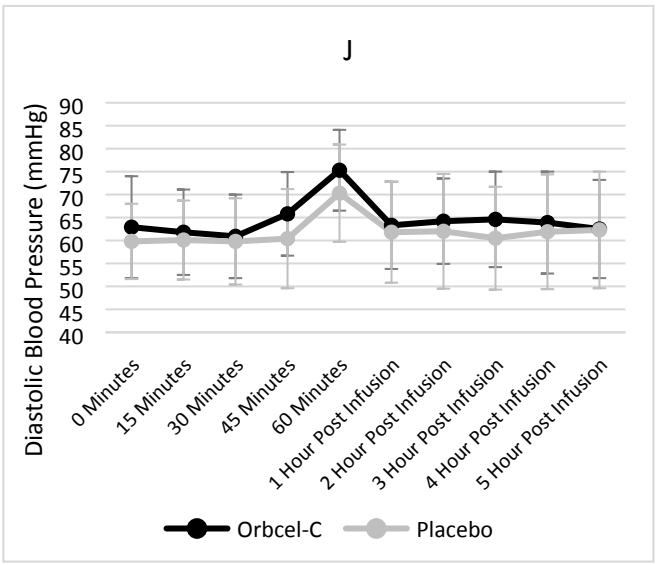


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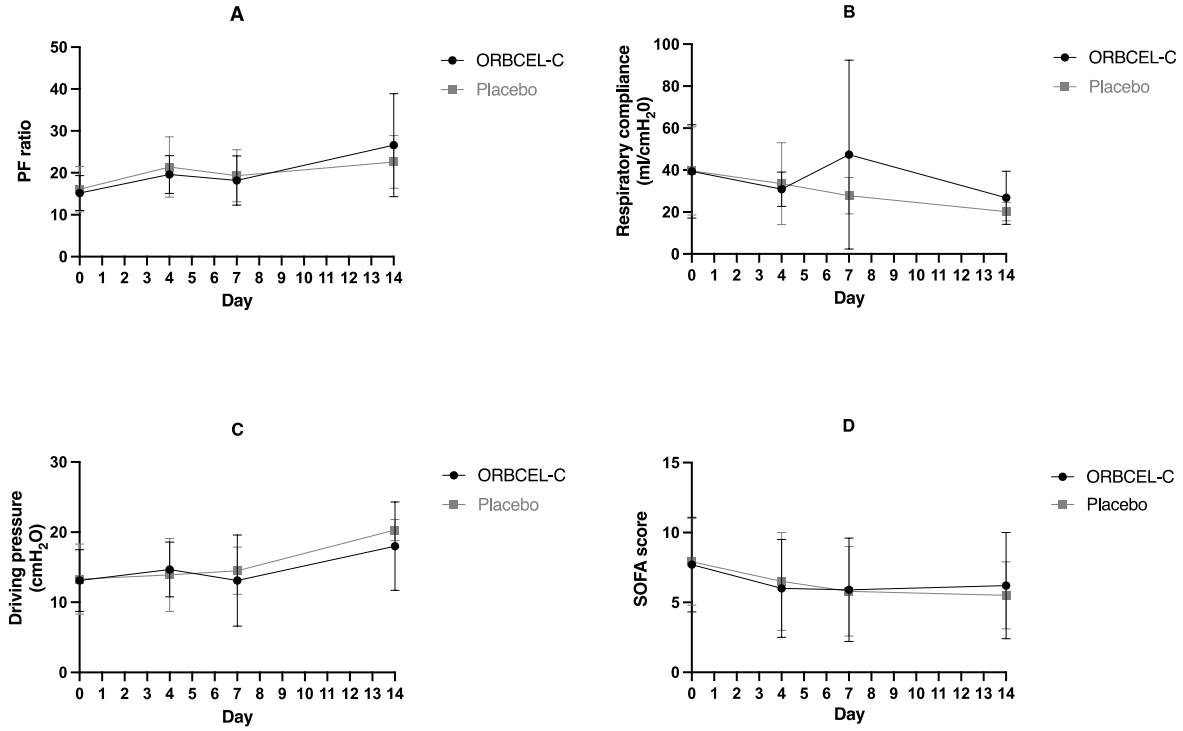
Figure E3



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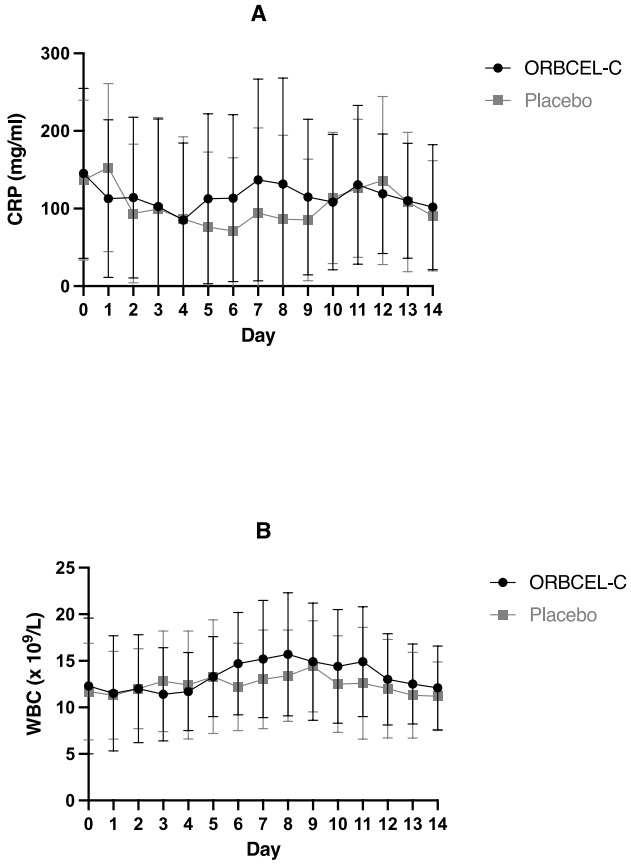

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Figure E5

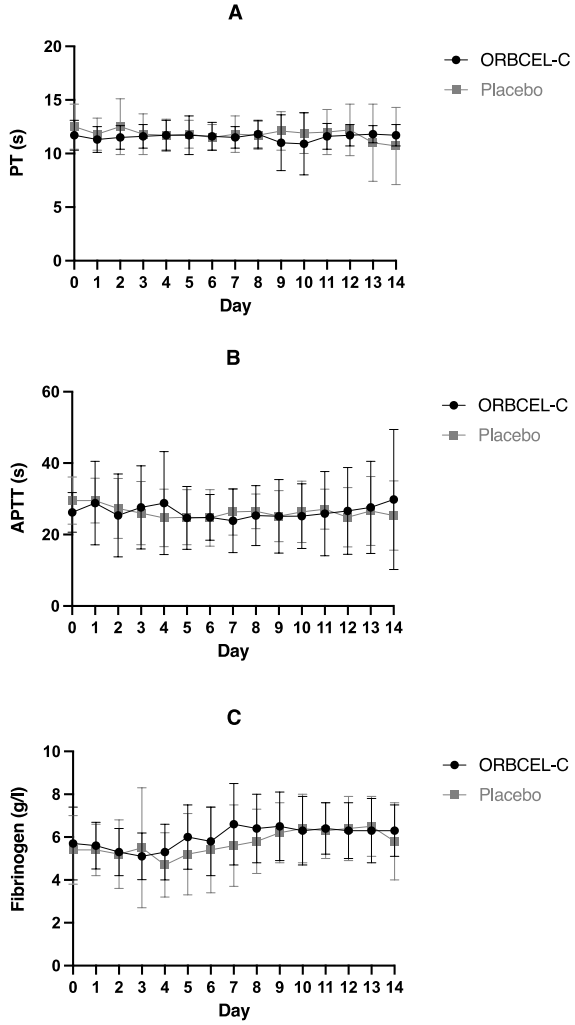

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Figure E6

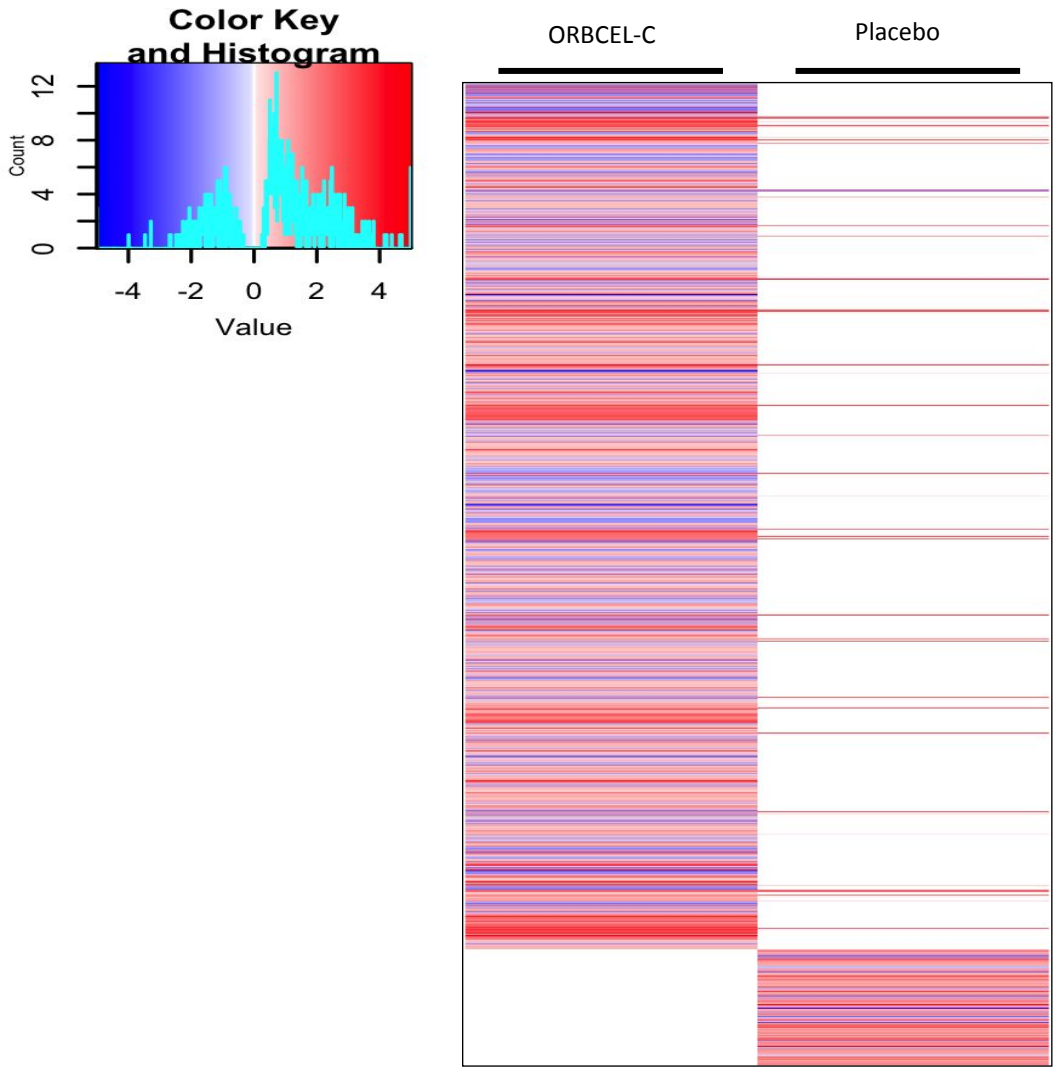

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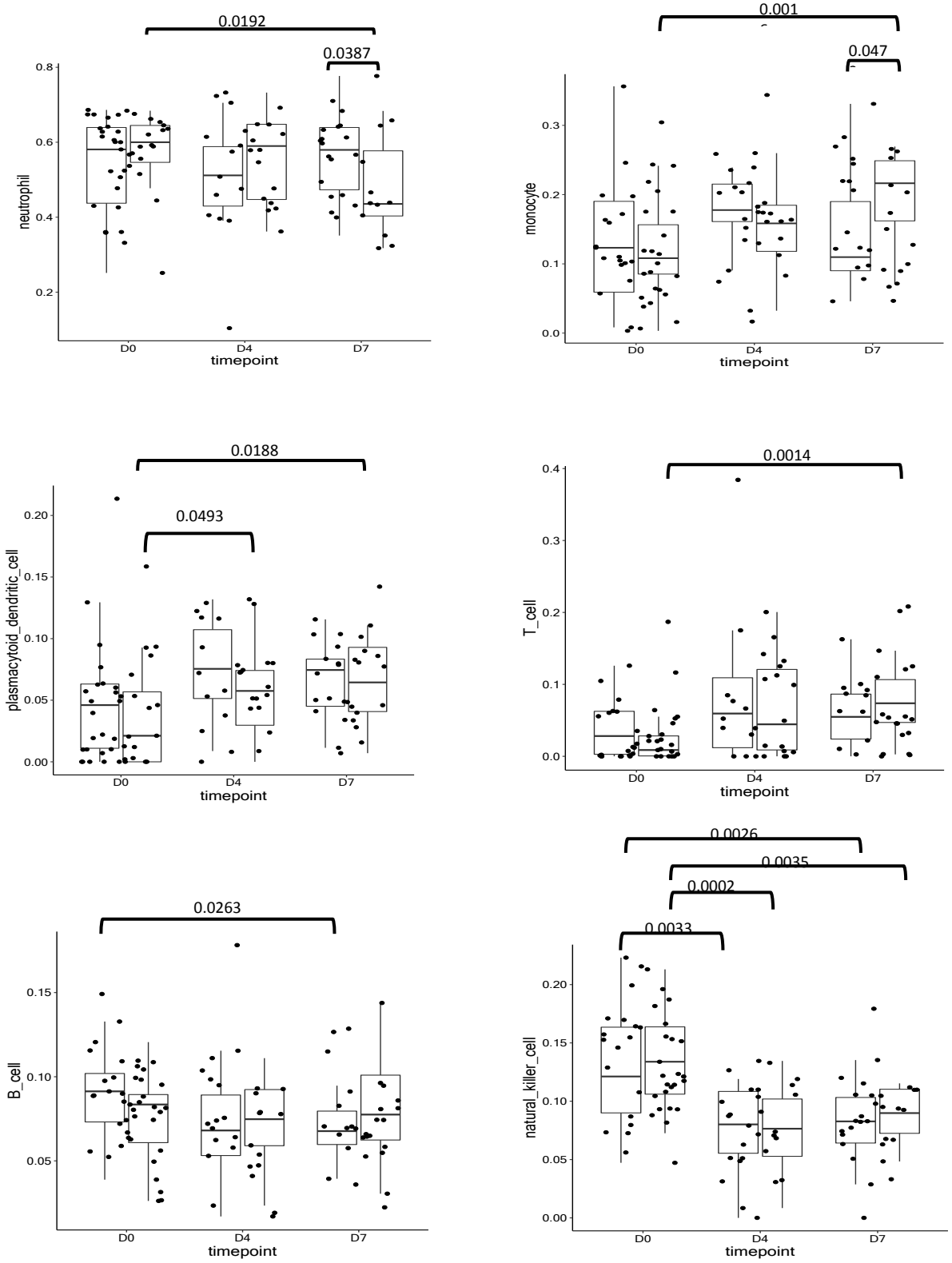
Figure E7



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