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Journal Pre-proof

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Bone Marrow Mesenchymal Stem Cell Derived Extracellular Vesicle Infusion for the Treatment of Respiratory Failure from COVID-19: A Randomized Placebo Controlled Dosing Clinical Trial

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ABSTRACT

Background: Bone marrow mesenchymal stem cell (BM-MSC)-derived extracellular vesicles (EVs), ExoFlo™, convey the immunomodulatory and regenerative properties of intact BM-MSC. This study aimed to determine the safety and efficacy of ExoFlo as treatment for moderate-to-severe Acute Respiratory Distress Syndrome (ARDS) in patients with severe COVID-19.

Research Question: Does two doses of ExoFlo safely reduce mortality in COVID-19 associated moderate to severe ARDS as compared to placebo?

Study Design and Methods: A prospective phase 2 multicenter, double-blind, randomized, placebo-controlled dosing trial was conducted at five sites across the US with infusions of placebo, 10 mL of ExoFlo, or 15 mL of ExoFlo on Day 1 and 4. Patients (102) with COVID-19 associated moderate-to-severe ARDS were enrolled and randomized. Adverse events were documented throughout. The primary outcome measure was all-cause 60-day mortality rate. Secondary outcomes included time to death (overall mortality), the incidence of treatment emergent serious adverse events, proportion of discharged patients at 7, 30, and 60 days, time to hospital discharge, and ventilation free days.

Results: No treatment-related adverse events were reported. Mortality (60-day) in the Intention-to-Treat (ITT) population was reduced in ExoFlo-15 compared to Placebo (not significant, Chi-square p=0.1343). For the post-hoc subgroup analyses, 60-day mortality was decreased in ExoFlo-15 compared to Placebo (Relative Risk=0.385; 95% confidence interval [CI]=0.159,0.931; p=0.0340; N=50). In ExoFlo-15 a Relative Risk of 0.423 (CI=0.173,1.032; p=0.0588; N=24) was determined for participants aged 18-65 with moderate to severe ARDS. Ventilation-free days (VFDs) improved in ExoFlo-15 (p=0.0455; N=50) for all participants aged 18-65.

Interpretation: ExoFlo (15 mL dose) is safe in patients with severe or critical COVID-19 respiratory failure. In participants aged 18 to 65, the risk reduction in 60-day mortality was further improved from all aged subjects in the ITT population after two doses of 15 mL of ExoFlo as compared to placebo.

TRIAL REGISTRATION ClinicalTrials.gov Identifier: NCT04493242.

KEY WORDS: Extracellular vesicle, bone marrow mesenchymal stem cell, COVID-19, safety, efficacy

ABBREVIATIONS: ARDS, Acute respiratory distress syndrome; BM-MSC, Bone marrow mesenchymal stem cell; CGMP, Current Good Manufacturing Practice; CMC, Chemistry, Manufactuing and Controls; EV, Extracellular vesicle; PEEP, Positive end expiratory pressure; SAE, Serious adverse event; SOFA, Sequential organ failure assessment; TEAE, Treatment emergent adverse event

Optimal management of acute respiratory distress syndrome (ARDS) morbidity remains critical. ARDS develops in 33%-42% of hospitalized patients with COVID-19 and in 61-81% of patients admitted to the intensive care unit (ICU). COVID-19 ARDS patients demonstrate similar pathologic changes of diffuse alveolar damage as classic ARDS.[1,2] Pooled mortality estimates of ARDS cases in COVID-19 patients showed similar mortality to non-COVID-19 ARDS patients.[3]

Bone marrow mesenchymal stem cells (BM-MSC) show promise for the treatment of ARDS. The phase I START trial monitored outcomes for 60 days following a single IV administration to patients with moderate-to-severe ARDS; no SAEs were observed following infusion of allogeneic BM-MSC.[4] Transplantation of healthy donor BM-MSC into patients with COVID-19 pulmonary disease improved functional outcomes without any observed adverse effects, and serum level changes in TNF-α and IL-10 suggest BM-MSC may inhibit cytokine storm.[5] MSCs from other tissue sources also exhibit efficacy.[6] Yet, the challenges of cryodamage, fresh product distribution, cell product heterogeneity, immunogenicity, thrombotic events, and scalability make BM-MSC technology impractical for global delivery.[4,7,8]

ExoFlo™ is an extracellular vesicle (EV) product manufactured per CGMP regulations from a single donor BM-MSC culture that conveys the immunomodulatory and regenerative properties of BM-MSC without cellular therapy limitations.[9-12] Extensive characterization of ExoFlo EVs reveals an absence of immunogenic surface epitopes that would cause acute immune reactions. The BM-MSC used to manufacture ExoFlo are fully characterized to meet the ISCT definition of possessing trilineage differentiation capability (bone, adipose and cartilage), and to be positive for the surface markers CD90 and CD166 but negative for CD45. The cells are evaluated by, and have a master file on record, with the FDA that includes information about the chemistry, manufacturing and controls (CMC) requirements for an approved Phase II IND clinical study. ExoFlo's efficacy and safety potential was evidenced by an investigator-initiated safety study treating COVID-19-associated ARDS patients.[13] These findings combine with the acellular nature, homogeneity, and scalability of ExoFlo to increase its potential as a practical therapeutic for respiratory failure from COVID-19.[13,14]

To further evaluate the safety and efficacy of ExoFlo for the treatment of hospitalized patients with respiratory failure from severe or critical COVID-19 a randomized, controlled trial, Extracellular Vesicle Infusion Treatment for COVID-19

(EXIT COVID-19), was conducted. We hypothesized ExoFlo would be safe in the treatment of severe and critical COVID-19 patients and compared the safety and efficacy of two doses of ExoFlo to Placebo.

Methods

Study design and participants

A prospective, multi-center, phase 2, randomized, double-blind, placebo-controlled trial was conducted. Enrollment for EXIT COVID-19 began September 24, 2020 and completed May 22, 2021. Five clinical trial sites in the United States actively participated in patient recruitment and enrollment. Patients with severe or critical COVID-19 as defined by a SpO_2 <94% on room air at sea level, partial pressure of arterial oxygen to fraction of inspired oxygen (PaO_2/FiO_2) <300 mm Hg and a respiratory rate >30 breaths/min, or lung infiltrates >50% were included (see e-Table 1, Inclusion Criteria).

The trial protocol was approved by the institutional review board (IRB) at each site (or a centralized IRB as applicable) and overseen by a data and safety monitoring board (DSMB) that was fully independent of both study sponsor and director. Written informed consent (or consent by other IRB-approved process) was obtained from each patient or patient's legally authorized representative if the patient was unable to provide consent.

Randomization and masking

See Figure 1 for CONSORT diagram of patient screening and enrollment, and e-Appendix 1 for full description of the clinical trial protocol. Patients (102) were randomized 1:1:1 by the clinical trial sites to 15 mL ExoFlo, 10 mL ExoFlo, or Placebo arms on Day 1. ExoFlo is colorless when thawed so only treatment masking was required to maintain blinding. Unblinded pharmacists prepared interventions that were delivered to the blinded nursing staff who delivered the infusion. Pharmacists are trained on blinding principles, sign a Delegation of Authority Log, and do not intermingle with practitioners or patients and their family.

Procedures

Each lot of ExoFlo meets stringent release specifications, including proteomic, mRNA and miRNA characterization. Additionally, the size and quantity of EVs and the presence of an exosome specific tetraspanin profile

for CD9, CD63 and CD81 are confirmed. Identity assays are combined with validated potency assays to demonstrate the mechanism of action is functional.

Dosing of ExoFlo was calculated based on (1) the 24-patient preliminary COVID-19 ExoFlo pilot study;[13] (2) the phase I START trial using IV administration of BM-MSC for ARDS, which demonstrated safety at up to 5 million cell/Kg and a ceiling dose of 10 million cell/Kg;[7] (3) observation of approximately 2,000 extracellular vesicles secreted per cell; and (4) lab analysis indicating 60-80 billion EV/mL. Extrapolation from the START trial MSC ceiling dose indicates an IV ExoFlo ceiling dose of 17.5 mL/70 Kg adult, and 15 mL and 10 mL of IV ExoFlo were determined as reasonable high and low dosing arms providing 1.2 and 0.9 trillion EV particles per dose, respectively.

All enrolled patients received a 100 mL intravenous infusion over 60 minutes on Day 1. Treatment arms were: 100 mL normal saline (NS, Placebo), 10 mL ExoFlo mixed with 90 mL NS (ExoFlo-10), and 15 mL ExoFlo with 85 mL NS (ExoFlo-15). A repeat of the same study treatment occurred on Day 4 if the patient had not recovered (SpO_2 ≥93% on room air or PaO_2/FiO_2 ≥300 mmHg). All patients were followed for 60 days, or until hospital discharge or death. Regardless of the allocated treatment arm, patients were offered standard supportive care according to hospital guidelines. There was no major numerical difference between ExoFlo-15 and placebo arms for 3 types of prior and concomitant medications (Remdesivir, Plasma, Dexamethasone), and >75% and 100% of both groups received prior and concomitant glucocorticoids, respectively, per recent guidelines.[15] No statistical testing was provided due to a small sample size per arm and no pre-defined limits for the test of equivalence. Although all means and percentages between ExFlo 15ml and Placebo arms in e-Table 2 were not significant (P>0.1) by a superiority test, we could not make a conclusive statement on equivalence between ExoFlo 15ml and placebo arms. Other experimental treatment or off-label use of marketed medications were prohibited. Patients were assessed daily from Day 1 to Day 60 during hospitalization. All serious adverse events (SAEs) and grade 3 or 4 adverse events (AEs) representing increased severity from Day 1, and any grade 2 or higher suspected drug-related hypersensitivity reactions were recorded.

Outcomes

The primary endpoint was improvement in the mortality rate within 60 days from randomization. Secondary endpoints included time to death, 2) incidence of treatment-emergent serious adverse events, 3) proportion of discharged patients, 4) time to hospital discharge at 7, 30, and 60 days from randomization, and 5) ventilation free days. Exploratory outcome measurements included viremia, serum acute phase reactants, immune cell subset counts, Sequential Organ Failure Assessment (SOFA) scores, and Quality of Life (EQ-5D-5L) scores.

Statistical analysis

Calculation of the sample size was based upon 60-day binomial mortality rates of 32% for ExoFlo-15 referring to the Expanded Access preliminary data and publication of 43% for placebo.[16] Sixty-eight patients in the ITT analysis set generated approximately 38% power based on a type I error rate of 0.2 (80% CI) to reject the null hypothesis with the underlying assumption of 60-day mortality rates.

The study was designed to assess safety at two doses of ExoFlo towards nominating a safe and effective dose of ExoFlo for the treatment of respiratory failure from COVID-19, and to understand trends in morbidity and mortality for future phase 3 hypotheses and study design. Analysis of the primary outcome of 60-day binominal mortality rate was planned and tested by a Chi-square test as a primary method. Predefined subgroup analyses were performed in patients who met criteria for moderate to severe ARDS and/or post-hoc subgroup of aged ≥18 to <65 to investigate primary and secondary endpoints in this disease-specific cohort.

Role of the funding source

The study was conducted in accordance with ethical principles as denoted in the International Council for Harmonization (ICH) E6 requirements. The role of the funding source and sponsor of the trial was protocol development including study design, analysis and interpretation of the data, writing the manuscript, and decision for submission of the manuscript.

Results

Trial participants

Thirty-four subjects were randomized per treatment arm. There were no significant demographic or clinical differences

in the treatment arms based on age, gender, race, body mass index (BMI), respiratory rate, intubation prior to enrollment, time from first diagnosis of COVID-19 to time of first treatment dose, total SOFA score, PaO_2/FiO_2 ratio, and prior therapy for COVID-19 (e-Table 2).

Subjects in the three arms were comparable with respect to the number of doses received, reason for not receiving the second infusion, and completion of all 60 days of the study. Of the patients who received two doses, 27 of 34 subjects (79.4%) randomized to ExoFlo-15, 29 of 34 subjects (85.3%) to ExoFlo-10 and 27 of 34 subjects (79.4%) to Placebo.

Safety

The Safety Analysis Set (Table 1) consisted of all 68 enrolled subjects who received any dose of ExoFlo. No AEs or SAEs caused a pause in patient recruitment or clinical trial discontinuation. No infusion reaction or AEs were observed in any cohort within the first 72 hours. No AEs were attributed by the investigators to administration of ExoFlo, and there was no apparent difference across the three study arms of the percentage of subjects with AEs or the distribution of types of AE.

AEs included worsening hypoxic respiratory failure requiring intubation (N=4), expiration (N=4), acute renal failure (N=3), and pulmonary embolism (N=1). All events occurred more than 72 hours following treatment and were evaluated by an independent DSMB to be reasonably attributable to COVID-19 disease progression or a temporally correlated provoking stimulus. Both Treatment-Emergent Adverse Events (TEAEs) and serious TEAEs of grade 3 or 4 occurred with comparable frequency between ExoFlo-15 and placebo, as did TEAEs of any grade. The frequency of serious TEAEs of any grade in ExoFlo-15 was less than that of Placebo and ExoFlo-10. The only treatment related TEAE (grade 2 hypotension) occurred in the Placebo arm. No serious treatment related TEAEs occurred in any of the three arms. TEAEs that led to death occurred in 47.1% of the subjects in Placebo, 38.2% in ExoFlo-10, and 29.4% in ExoFlo-15. For all clinical laboratory parameters, the mean values for the three groups were comparable at baseline and there were no apparent major differences across the three groups in changes from baseline (not shown).

Efficacy

Intention-to-Treat (ITT) Population Analysis

The overall mortality rate among all subjects was 61%. The 60-day mortality was numerically lower in the ITT ExoFlo groups compared with the Placebo group (Table 2). Although alpha significance level of 0.2 was suboptimal and may not indicate true statistical significance, the study rejected the null hypothesis for the primary endpoint (p=0.1343) for ExoFlo-15. For all other analyses including ExoFlo-10 and secondary endpoints, subgroup analyses were not pre-defined with a properly adjusted type I error rate, and p-values were calculated for a descriptive purpose only. No multiplicity adjustment applies to subgroup analyses.

The overall mortality (Kaplan-Meier (KM), Figure 2) was improved at all timepoints for ExoFlo-15 compared with ExoFlo-10, which was superior at all timepoints than Placebo. The overall mortality comparison between Placebo and ExoFlo-15 was measured by the KM curves and a hazard ratio with 95% Confidence Intervals (CI) using a Cox regression model and tested using a log-rank test. No arm reached median overall mortality with a 60-day follow-up. Although statistical significance was not achieved for the log-rank test (p=0.1820) or the hazard ratio (HR=0.59; 95% CI=[0.27, 1.30]), the KM curves suggest an increasing reduction in the mortality risk over time in ExoFlo-15 compared to placebo. The relative difference in mortality rates across the three groups increased with time from randomization; ExoFlo-15 was 3% better than Placebo at Day 15, 9% better at Day 30, and 18% better at Day 60. Similar trends, although of lesser magnitude, were observed in ExoFlo-10 vs Placebo. Mortality rates for ExoFlo-15 and ExoFlo-10 diverged by 60 days, and the mortality rate for ExoFlo-10 at 60 days was similar to that of Placebo.

The percentage of subjects discharged was highest for ExoFlo-15 (58.8%), followed by ExoFlo-10 (52.9%), and Placebo (50.0%). The median time to hospital discharge was estimated to be 22 days for ExoFlo-15, 29 days for ExoFlo-10 and not reached by Placebo when evaluated with KM curve. The KM curves suggested a decreasing time to discharge from Placebo to ExoFlo-10 to ExoFlo-15, although statistical significance was not achieved for the log-rank test (p=0.5554) or the recovery ratio (HR=1.21; 95% CI=[0.63, 2.31]) as estimated by a Cox regression model when comparing time to hospital discharge between ExoFlo-15 and Placebo.

In the ITT population, ventilation-free days (VFDs) were highest for ExoFlo-15 (Mean (SD) = 41.3 (25.8)) and similar for

ExoFlo-10 (Mean (SD) = 32.0 (26.2)) and Placebo (Mean (SD) = 33.9 (28.1)). The difference in VFDs between ExoFlo-15 and Placebo failed to reach statistical significance (p=0.303, Wilcoxon rank sum test), but encouraging trends in several endpoints emerged in analyses of subpopulations of the ITT population that were not pre-defined (e-Tables 3-5).

Subpopulations

Important post-hoc sub analyses were in the patients aged 18-65 with respiratory failure or moderate to severe ARDS. Those with respiratory failure had a 60-day mortality of 50% in the Placebo and 19.2% in the ExoFlo-15, representing absolute risk reduction of 30.8% and Relative Risk of 0.385 (95%CI=0.159,0.931, p=0.0340, Table 3). For this age group who met modified Berlin criteria for moderate to severe ARDS, the 60-day mortality was 72.7% in the Placebo and 30.8% in ExoFlo-15, yielding Absolute Risk reduction of 41.9% and a Relative Risk of 0.423 (95%CI=0.173,1.032, p=0.0588), indicating a trend towards improvement (Table 3).

For the 18-65 year age group the number of VFDs in ExoFlo-15 (47.6 days) was improved (p=0.0455, Wilcoxon rank-sum test) compared to Placebo (30.3 days, e-Table 4). A dose response effect trend was observed for VFDs in both Moderate and Severe ARDS in this age group (e-Table 5): Moderate ARDS – 47.0 (ExoFlo-15), 25.3 (ExoFlo-10), 13.3 (Placebo); Severe ARDS – 34.6 (ExoFlo-15), 26.5 (ExoFlo-10), 19.6 Placebo).

Discussion

This prospective, double-blind, randomized, placebo-controlled phase 2 trial is the first trial to show BM-MSC EVs are safe and exhibit potential for efficacy based on post-hoc subgroup analyses in the treatment of severe or critical COVID-19. A critical finding of this study was the safety profile of ExoFlo. There was a lack of adverse or serious adverse events related to ExoFlo at either 10 mL or 15 mL treatment doses. Given the severity of illness in this patient population, the overwhelming safety profile is highly encouraging for regulatory path in severely impaired COVID-19 patients. Fortunately, there were no differences in the safety profile at either dose despite the difference in efficacy trends observed between ExoFlo-10 compared to ExoFlo-15, and no adverse events were related to investigational product. The rate of TEAEs and SAEs of any severity grade did not increase beyond Placebo with either dose of ExoFlo. The number of

patients in treatment arms who died were lower with treatment relative to Placebo. In fact, overall mortality trended lowest in ExoFlo-15 and improved with increasing time from randomization. This safety profile is superior to the known side effects attributed to dexamethasone, remdesivir and IL-6 antagonists.[17,18]

All-cause 60-day mortality in the ITT population was 29.4% with ExoFlo-15 and 47.1% with placebo. Although not statistically significant, our findings are consistent with the findings of the initial investigator-initiated trial and expanded access program (NCT04657458) wherein two treatments of ExoFlo-15 resulted in a mortality reduction among patients hospitalized with severe or critical COVID-19. Additional secondary endpoints here supporting the benefit of ExoFlo-15 included confirmation of overall mortality by the KM curves, shorter time to hospital discharge, increased ventilation free days, and biomarker trends. Dose-response trends were observed in the ITT population for 60-day mortality rate, overall mortality (KM), median time to discharge (KM) and VFDs. In the age 18-65 patient subgroup with moderate or severe ARDS, the VFDs showed a dose-response trend with ExoFlo-15 > ExoFlo-10 > Placebo. Although these metrics did not reach statistical significance these results will inform the subsequent Phase 3 trial design. While this study was not adequately powered for a mortality benefit between treatment arms, a larger mortality risk reduction was identified in subjects aged 18-65 experiencing respiratory failure due to COVID-19, and a similar trend toward risk reduction was seen in this age group with moderate to severe ARDS.

In the subgroup of patients aged 18-65 who met modified Berlin criteria for moderate to severe ARDS, mortality was 30.8% for ExoFlo-15 (N=11) as compared to 72.7% (N=13) in the placebo group, demonstrating a 60-day mortality absolute risk reduction of 41.9% and a Relative Risk of 0.423 (95%CI=0.173,1.032). Some of this difference may be due to significant co-morbidities in the aging population, age as the known independent prognostic factor that affects the >65 age group overwhelmingly to decrease treatment effects on mortality, or small sample size and a Type I error. Another reason for such a mortality benefit in the ARDS cohort is that ExoFlo may have a more substantial impact in patients both nearing intubation and those intubated at the time of treatment, demonstrating the value of ExoFlo in a critically ill patient population. Importantly, this suggests that ExoFlo could be beneficial in pre-ARDS patients. Larger sample sizes are needed to confirm a significant difference in mortality, as the treatment arm size was too small to adequately power this question.

While this is the first completed prospective, randomized, placebo controlled trial of an extracellular vesicle (EV) product for the treatment of respiratory failure from COVID-19, several other randomized clinical trials have been conducted with anti-viral and immunomodulatory therapeutics for the treatment of COVID-19.[15,19-29] Those with documented effects on mortality include remdesivir (anti-viral), and dexamethasone and IL-6 antagonists (immunomodulatory). The trial herein is the first to show an EV product with potential mortality benefit, that, in phase 3, may be superior to the aforementioned clinical trial results.

While weaknesses of our study include that insufficient power was proposed for the primary endpoint and indications of efficacy may arise from the small sample size and post-hoc subgroup analyses, preliminary efficacy inferences may be drawn from trends in the endpoint data to guide generation of hypotheses for the future phase 3 trial design. Suggestions of efficacy were observed, particularly in subjects receiving the higher dose of ExoFlo; overall mortality, VFDs, and days to discharge all trended in favor of the higher dose of ExoFlo versus placebo. In addition, these trends seemed to be improved in a younger patient cohort with ARDS.

Interpretation

Based on preliminary results demonstrated from ExoFlo, the FDA issued a regenerative medicine advanced therapeutic (RMAT) designation and also authorized proceeding with a phase 3 clinical trial that is currently underway to confirm the results described herein. Given the limited approved therapeutics with proven mortality benefit, expedient results of our phase 3 will be critical to the ongoing treatment of ARDS patients. Evidence of significant efficacy against respiratory failure from COVID-19 disease by ExoFlo would represent a significant advancement in efforts to reduce morbidity and mortality caused by SARS-CoV-2.

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TAKE HOME POINTS:

Study Question: Does two doses of ExoFlo safely reduce mortality in severe COVID-19 moderate to severe ARDS as compared to placebo?

Results: No AEs or SAEs were related to investigational product. For participants aged 18-65 with respiratory failure, 60-day mortality was significantly decreased in ExoFlo-15 compared to Placebo (Relative Risk or 0.385, 95%CI=0.159,0.931, p=0.0340).

Interpretation: Two doses of ExoFlo safely and significantly reduces mortality in patients aged 18-65 with respiratory failure due to critical or severe COVID-19.

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Table 1. Overall Summary of Safety Events (Safety Analysis Set)

Safety Parameter	ExoFlo-15 (N=34)	ExoFlo-10 (N=34)	Placebo (N=34)	ExoFlo Total (N=68)
Any TEAEs [1]				
Any Grade (%)	24 (70.6)	26 (76.5)	23 (67.6)	50 (73.5)
Grade 3 or 4 (%)	5 (14.7)	9 (26.5)	5 (14.7)	14 (20.6)
Serious TEAEs [1]				
Any Grade (%)	10 (29.4)	18 (52.9)	16 (47.1)	28 (41.2)
Grade 3 or 4 (%)	3 (8.8)	7 (20.6)	3 (8.8)	10 (14.7)
Study Treatment-Related TEAEs (%)	0	0	1 (2.9)	0
Study Treatment-Related Serious TEAEs (%)	0	0	0	0
TEAEs That Led to Dose Interruption (%)	1 (2.9)	0	0	1 (1.5)
TEAEs That Led to Missing Dose or Discontinued the Treatment Early (%)	0	0	1 (2.9)	0
TEAEs That Led to Death (%)	10 (29.4)	13 (38.2)	16 (47.1)	23 (33.8)

TEAE = Treatment-Emergent Adverse Events, are defined as any adverse event that started between the first dose date and 30 days post the last dose date, inclusively.

[1] Toxicity grades of adverse events are evaluated based on criteria of NCI-CTCAE v5.0. Each subject is counted once to the worst grade at subject-level.

Note: Related = Possibly Related, or Probably Related.

Table 2. Summary of Efficacy (ITT Analysis Set)

Study Endpoints	Statistics	ExoFlo-15 (N=34)	ExoFlo-10 (N=34)	Placebo (N=34)
Subjects Who Discharged Within 60 Days	n (%)	20 (58.8)	18 (52.9)	17 (50.0)
Subjects Who Discharged Within 30 Days	n (%)	19 (55.9)	17 (50.0)	17 (50.0)
Subjects Who Discharged Within 7 Days	n (%)	11 (32.4)	9 (26.5)	11 (32.4)
Median Time to Discharge (KM) [1]	n	34	34	34
	Median	22.0 days	29.0 days	NR
	95% CI	(6.0, NE)	(9.0, NE)	(7.0, NE)
Subjects Who Died Within 30 Days	n (%)	9 (26.5)	10 (29.4)	12 (35.3)
Subjects Who Died Within 60 Days	n (%)	10 (29.4)	14 (41.2)	16 (47.1)
	80% CI	(19.1, 41.6)	(29.6, 53.6)	(35.0, 59.4)
	95% CI	(15.1, 47.5)	(24.6, 59.3)	(29.8, 64.9)
ExoFlo-15 vs Placebo	P-value [2]	0.1343		
Median Time to Death (KM)	Median	NR	NR	NR
Mortality Rate at 15 Days (KM)	%	21.2	22.2	24.2
Mortality Rate at 30 Days (KM)	%	27.3	32.3	36.3
Mortality Rate at 60 Days (KM)	%	30.4	46.6	48.4
	95% CI	(17.7, 49.2)	(30.6, 65.8)	(33.1, 66.4)
P/F Ratio Increase from Baseline to Day 7 (mmHg) [3]	n	17	18	18
	Mean (SD)	55.5 (86.37)	42.9 (53.39)	48.9 (78.38)
	95% CI	(18.9, 92.1)	(21.0, 64.8)	(16.8, 81.0)
	Min, Max	0, 311	0, 176	0, 303.16
[#]Ventilation-Free Days (within 60 Days)	n	34	34	34
	Mean (SD)	41.3 (25.78)	32.0 (26.23)	33.9 (28.06)
	95% CI	(33.8, 48.7)	(24.4, 39.6)	(25.8, 42.1)
	Min, Max	0, 61	0, 61	0, 61
ExoFlo-15 vs Placebo	P-value [4]	0.3030		

KM = Kaplan Meier method, NE = Not Evaluable, NR = Not Reached

[1] Subjects who died or discontinued from the study due to a reason other than discharge before reaching 60 days (Day 61) are censored at Day 61.

[2] Chi-square test for 60-day mortality rates. P-value is displayed for a descriptive purpose.

[3] P/F ratio: All treated subjects with baseline and at least one P/F ratio measured at Day 4 or 7. For missing Day 7 data, 380 mmHg was assigned for discharged patients, and no change (0) was assigned to patients with negative change from the baseline or died before Day 7.

[#]Ventilation-free days: days when patients are not on mechanical ventilation within 60 days of follow-up.

[4] Wilcoxon rank-sum test. P-value is displayed for a descriptive purpose.

80% CI and 95% CI of Subjects Who Died Within 60 Days are calculated using exact (Clopper-Pearson) method; 95% CI of P/F Ratio Increase and Ventilation-Free Days are calculated using the student's T distribution.

Table 3. 60-Day Mortality Rate for Patients Aged 18-65 with Respiratory Failure or Moderate to Severe ARDS

Indication	Placebo n/N (%)	ExoFlo 15 mL n/N (%)	Absolute Risk Reduction	Relative Risk (RR) (95% CI)	N	P-value for RR
Respiratory Failure due to Severe or Critical COVID-19, Age 18-65	12/24 (50.0)	5/26 (19.2)	30.8%	0.385 (0.159, 0.931)	50	0.0340
Moderate to Severe ARDS Subgroup (CPAP, BiPAP, MV), All Ages	11/17 (64.7)	6/16 (37.5)	27.2%	0.580 (0.281, 1.195)	33	0.1394
Moderate to Severe ARDS Subgroup (CPAP, BiPAP, MV), Age 18-65	8/11 (72.7)	4/13 (30.8)	41.9%	0.423 (0.173, 1.032)	24	0.0588
Absolute Risk Reduction = (60-Day Mortality Placebo) – (60-Day Mortality ExoFlo) Relative Risk = 60-Day mortality rate in ExoFlo / 60-day mortality rate in Placebo Moderate to Severe ARDS defined per the modified Berlin definition where moderate ARDS is to be 100 mmHg < P/F ratio ≤ 200 mmHg, and severe ARDS is to be P/F ratio ≤ 100 mmHg.						

Figure Legends:

Figure 1. CONSORT diagram for study enrollment, allocation of treatment arm and follow-up.

Figure 2. Mortality (ITT population).

Time to death was compared between the IP 15 mL and Placebo using a log-rank test. Median time to death was estimated by the KM method. The hazard ratio of IP 15 mL to Placebo was estimated using a Cox regression model with a 95% Confidence Interval (CI).

Figure 1. CONSORT diagram for study enrollment, allocation of treatment arm and follow-up.

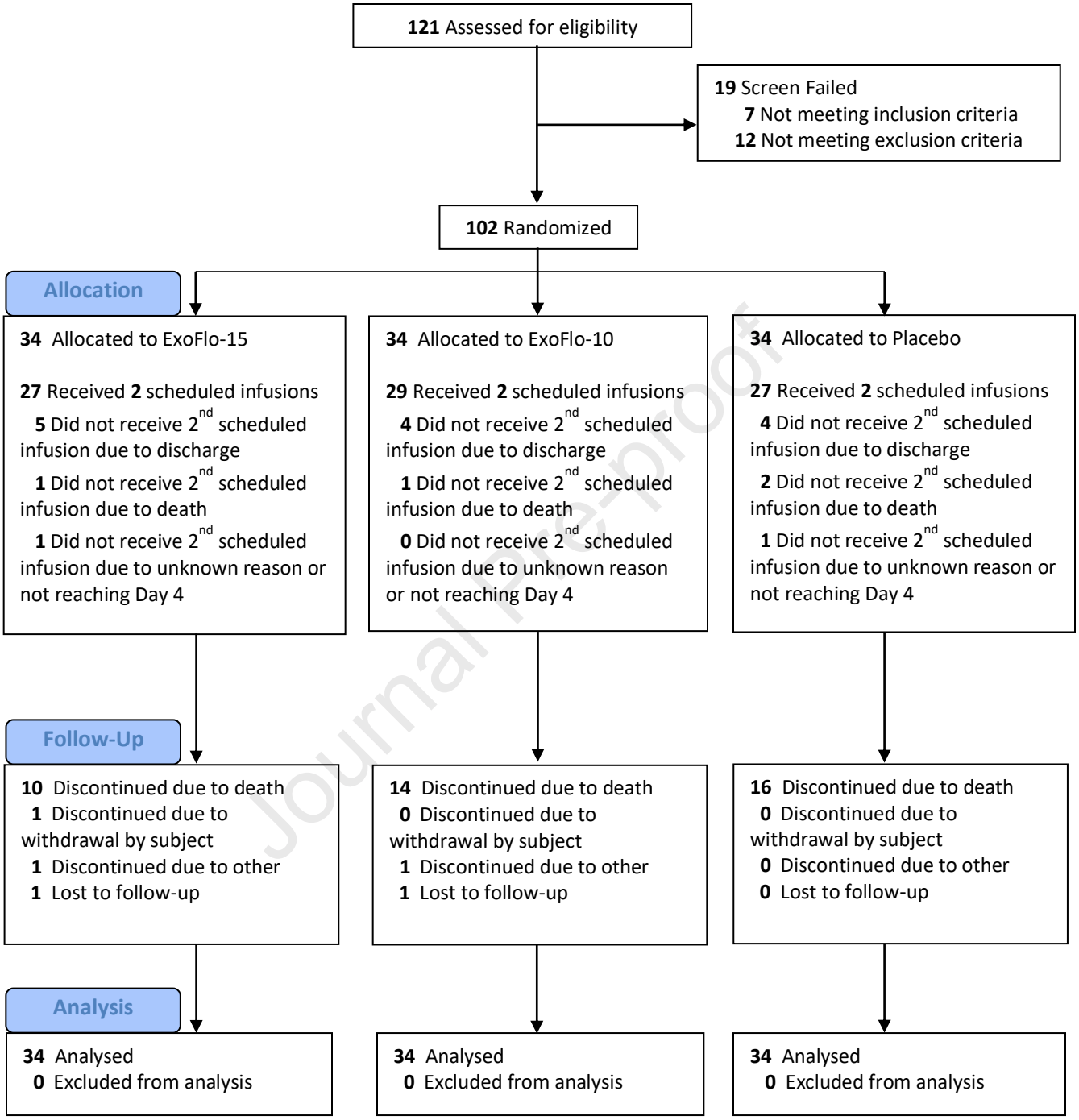
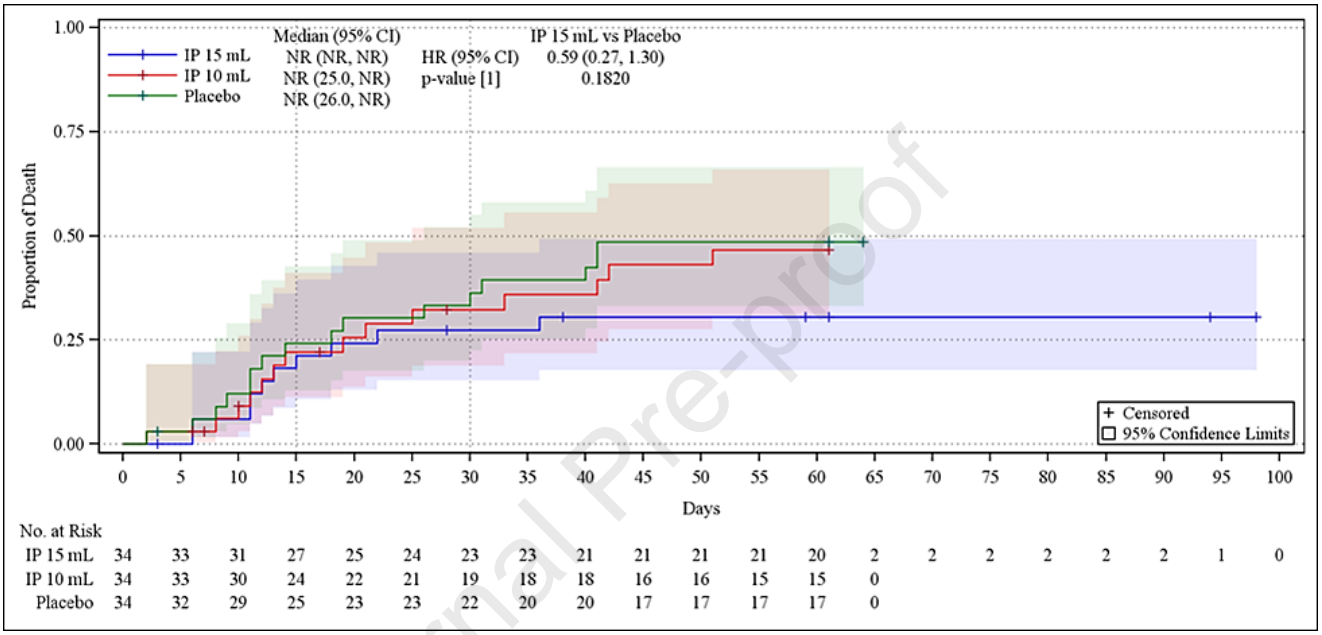


Figure 2. Mortality (ITT population).

Time to death was compared between the IP 15 mL and Placebo using a log-rank test. Median time to death was estimated by the KM method. The hazard ratio of IP 15 mL to Placebo was estimated using a Cox regression model with a 95% Confidence Interval (CI).



NR = Not Reached

Time to Death is the interval in days from randomization to subject's death. The interval is censored to study discontinuation or completion if the subject is alive.

[1] p-value is from the log-rank test.

Conflict of interest: CMO at Direct Biologics, Vikram Sengupta: prior CMO at Direct Biologics, Sascha Sengupta: prior associate CMO at Direct Biologics, John Ransom: PhD at Direct Biologics, Sam Suzuki: statistician at Direct Biologics

REQUEST FOR REGENERATIVE MEDICINE ADVANCED THERAPY (RMAT) DESIGNATION

Investigational New Drug (IND) Number: 21669

Study Investigational Product: Bone Marrow Mesenchymal Stem Cell (bmMSC)-Derived Extracellular Vesicles (via Intravenous Administration)

Date & Format: 8 February 2021| Cover Letter for Protocol Amendment

Based on the following criteria from Section 3033 of the 21st Century Cures Act, the study investigational product (IP), ExoFlo™, meets eligibility for regenerative medicine advanced therapy (RMAT) designation:

A. As a biologic product derived from human bone marrow mesenchymal stem cells, ExoFlo is an extracellular vesicle isolate product (EVIP) and a regenerative medicine therapy.

B. ExoFlo is intended to treat, modify and reverse COVID-19 associated moderate-to-severe Acute Respiratory Distress Syndrome (ARDS), which is a life-threatening disease.

C. Preliminary clinical evidence indicates that ExoFlo has the potential to address unmet medical needs for such disease or condition.

1. UNMET NEED: A systematic prospective observational analysis published in *The Lancet* in November 2020 suggests that the lung injury in COVID-19 associated ARDS is similar to that of classic ARDS. The overall 28-day mortality was high (36%). Furthermore, the latest statistics demonstrate that the current U.S. healthcare system is unable to accommodate all patients who should be admitted with COVID-19 associated ARDS: As of January 13th, 2021, 95% of intensive care hospital beds are occupied nationwide. More than a third of Americans live in areas where hospitals are running critically short of intensive care beds. Intensive care units are already at full capacity in states like California and Texas—a scenario which will only worsen in the upcoming months. According to the CDC, the number of new deaths in the next 4 weeks will increase with the total number of deaths estimated around 479,000 to 514,000 by February 20th, 2021—the primary cause of death is COVID-19 associated ARDS.

2. CLINICAL EVIDENCE FOR ADDRESSING UNMET NEED: In an open-label investigator-initiated study in April 2020, 17 out of 24 patients with COVID-19 moderate-to-severe ARDS were able to reverse their profound hypoxia following a single infusion of the investigational product; patients were discharged from the hospital with median time to discharge of 5.6 days (whereas median to recovery for Remdesivir is 10 days). The mortality rate of 17% was lower compared to retrospective institutional control of approximately 30% for the same period. To date, 42 patients have been randomized in EXIT COVID-19, the Phase II double blinded, placebo-controlled, randomized controlled trial and 7 patients have received the investigational product through single patient Expanded Access for Compassionate Use without any adverse reactions and 5 out of 7 patients were able to recover.

3. THERAPEUTIC SPECIFICITY FOR UNMET NEED: Potential therapeutic mechanisms of the investigational Product may be specific for COVID-19 associated ARDS and include the following:

   - The reduction of inflammation as demonstrated by decrease in acute phase reactants (CRP, Ferritin, D-dimer) and the reconstitution of adaptive immunity as demonstrated by increase in CD4+, CD8+ lymphocytes.
   - *In Vitro* study show ExoFlo has moderate capacity (approximately 39.9%) to directly inhibit SARS-CoV-2 activity.
   - *In Vivo* acute lung injury murine study showed that following treatment with ExoFlo, cytokine storm activities were reduced as demonstrated by the inhibition of GM-CSF, M-CSF, as well as CXCL-9.
   - Independent molecular characterization identified 27 miRNA as potential inhibitors of the ACE-2 related protein network; 7 miRNA as inhibitors of serine protease 2 (TMPRSS2)—a transmembrane protease that is required for Spike (S) protein priming and SARS-CoV-2 entry into a host cell; 5 miRNA as inhibitors of IL-6 and 2 miRNA that target tumor necrosis factor alpha (TNF-a)—consistent with the clinical

observation and *in vivo* observation that ExoFlo may down-regulate the cytokine storm.

Bone Marrow Mesenchymal Stem Cell Derived Extracellular Vesicles Infusion Treatment for COVID-19 Associated Acute Respiratory Distress Syndrome (ARDS): A Phase II Clinical Trial

Protocol Number:	DB-EF-PHASEII-001
Investigational New Drug (IND) Number:	21669
Study Phase:	II
Study Type:	Double-Blinded, Placebo-controlled, Randomized Controlled Trial
Study Investigational Product:	Bone Marrow Mesenchymal Stem Cell (bmMSC)-Derived Extracellular Vesicles (via Intravenous Administration)
Administrative Amendment: **Date & Document Version:**	6 8 February 2021 | 7.0
Administrative Amendment: **Date & Document Version:**	5 31 December 2020 | 6.0
Administrative Amendment: **Date & Document Version:**	4 2 November 2020 | 5.0
Administrative Amendment: **Date & Document Version:**	3 7 October 2020 | 4.0
Administrative Amendment: **Date & Document Version**	2 31 August 2020 | 3.0
Administrative Amendment: **Date & Document Version**	1 7 August 2020 | 2.0
Original Protocol: **Date & Document Version**	- 24 July 2020 | 1.0
Sponsor:	Direct Biologics, LLC 13492 Research Blvd, Ste 120-758 Austin, TX 78750

CONFIDENTIAL

This protocol is provided to you as an Investigator, potential Investigator, or consultant for review by you, your staff, and Ethics Committee/Institutional Review Board (EC/IRB). The information contained in this document is regarded as

confidential and, except to the extent necessary to obtain informed consent, may not be disclosed to another party unless such disclosure is required by law or regulations. Persons to whom the information is disclosed must be informed that the information is confidential and may not be further disclosed by them.

PROTOCOL AUTHORIZATION

_____

Signature of Direct Biologics Chief Medical Officer
Vik Sengupta, MD
Chief Medical Officer
Direct Biologics, LLC

_____

Printed Name of Direct Biologics Chief Medical Officer

_____

Date Signed (mm/dd/yy)

_____

Signature of Direct Biologics Chief Scientific Officer
Timothy Moseley, PhD
Chief Scientific Officer
Direct Biologics, LLC

_____

Printed Name of Direct Biologics Chief Scientific Officer

_____

Date Signed (mm/dd/yy)

INVESTIGATOR'S AGREEMENT

Title: Bone Marrow Mesenchymal Stem Cell Derived Extracellular Vesicles Infusion Treatment for COVID-19 Associated ARDS: A Phase II Clinical Trial

Protocol Number: DB-EF-PHASEII-001

Signature of Investigator	dd/mm/yy
Printed Name of Investigator and Title	
Site Number(s):	
By my signature, I agree to supervise and oversee the conduct of this study and to ensure its conduct is in compliance with the protocol, informed consent, IRB/EC procedures, instructions from Direct Biologics representatives, the Declaration of Helsinki, International Conference on Harmonisation (ICH) Good Clinical Practices (GCP) guidelines, and the applicable parts of the United States (US) Code of Federal Regulations (CFR) and local regulations governing the conduct of clinical studies.	

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LIST OF IN-TEXT TABLES

STATEMENT OF COMPLIANCE

The trial will be conducted in accordance with ICH GCP and applicable United States CFR. The Principal Investigator (PI) will assure that no deviation from, or changes to the protocol will take place without prior agreement from the IND, funding agency and documented approval from the central IRB, except where necessary to eliminate an immediate hazard(s) to the trial participants. All personnel involved in the conduct of this study have completed Human Subjects Protection and ICH GCP Training. The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the central IRB for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the central IRB before the changes are implemented to the study. All changes to the consent form will be central IRB approved; a determination will be made regarding whether a new consent needs to be obtained from participants who provided consent, using a previously approved consent form.

LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

ABG	Arterial Blood Gas
ACE2	Angiotensin-converting Enzyme 2
ACLS	Advanced Cardiac Life Saving
AE	Adverse Events
AESI	Adverse Event of Special Interest
ALI	Acute Lung Injury
ANC	Absolute Neutrophil Count
ANOVA	Analysis of Variance
ARB	Angiotensin Receptor Blocker
BMI	Body Mass Index
bmMSCs	Bone marrow mesenchymal stem cells
BMP	Basic Metabolic Profile
CBC	Complete Blood Count
CBER	Center for Biologics Evaluation and Research
CD	Cluster of Differentiation, i.e., CD4+ T cell
CFR	Code of Federal Regulations
cGMP	Current Good Manufacturing Practice
COVID-19	Coronavirus Disease 2019
CRO	Contract Research Organization
CRP	C-Reactive Protein
CRF	Case Report Form
CT	Computerized Tomography
CXR	Chest X-ray
DNA	Deoxyribonucleic Acid
DOB	Date of Birth
DSMB	Data Safety Monitoring Board
EC	Ethics Committee
ECG or EKG	Electrocardiogram
ECMO	Extracorporeal Membrane Oxygenation
eCRF	Electronic Case Report Form
EMR	Electronic Medical Record
EQ-5D-5L	EuroQol-5D, a widely validated metric for quality of life; the 5 five dimensions (5D) include mobility, self-care, usual activities, pain/discomfort, and anxiety/depression

EV	Extracellular Vesicles
EVIP	Extracellular Vesicle Isolate Product
FM	Face mask
FDA	Food and Drug Administration
FiO_2	Fraction of Inspired Oxygen
GvHD	Graft versus Host Disease
HF O_2	High Flow Oxygen
HFOV	High Frequency Oscillatory Ventilation
HIV	Human Immunodeficiency Virus
HR	Heart Rate
ICH GCP	International Conference on Harmonisation Good Clinical Practice
IgM	Immunoglobulin M
IL	Interleukin, i.e., IL-6
IND	Investigational New Drug
IP	Investigational Product
IRB	Institutional Review Board
IRT	Interactive Response Technology
IV	Intravenous
LFTs	Liver Function Tests
LPM	Liters Per Minute
MAP	Mean Arterial Pressure
MAR	Medical Administration Record
microRNA or miRNA	Microcoding Ribonucleic Acid
MOP	Manual of Procedures
MRN	Medical Record Number
mRNA	Messenger RNA
MV	Mechanical Ventilation
NIH	National Institutes of Health
NK cells	Natural Killer cells
NRB	Nonrebreather
PaO_2/FiO_2	Partial Pressure of Arterial Oxygen to Fraction of Inspired Oxygen Ratio
PCR	Polymerase Chain Reaction
PEEP	Positive End Expiratory Pressure
PI	Principal Investigator
PT/INR	Prothrombin Time / International Normalized Ratio

PTSD	Posttraumatic Stress Disorder
PTT	Partial Thromboplastin Time
QC	Quality Control
QOL	Quality of Life
RCT	Randomized Control Trial
RNA	Ribonucleic Acid
RR	Respiratory Rate
RT-PCR	Reverse Transcriptase Polymerase Chain Reaction
SAE	Serious Adverse Event
SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus 2
SOFA score	Sequential Organ Failure Assessment score
SpO_2	Peripheral Capillary Oxygen Saturation, commonly also referred to as Oxygen Saturation
SUSAR	Suspected Unexpected Serious Adverse Event
T Cells	Thymus Cells, also known as T Lymphocytes
TNF	Tumor Necrosis Factor, i.e., TNF-α
UP	Unanticipated Problems
VFD	Ventilator-free Day

1. PROTOCOL SUMMARY

1.1 Synopsis

Title: Bone Marrow Mesenchymal Stem Cell Derived Extracellular Vesicles Infusion Treatment for COVID-19 Associated Acute Respiratory Distress Syndrome (ARDS): A Phase II Clinical Trial

Study Description: ExoFlo™ Infusion Treatment for COVID-19 Associated ARDS (EXIT COVID-19), a multicenter, double-blinded, placebo-controlled, randomized control trial to evaluate the efficacy and safety in COVID-19 associated moderate to severe ARDS.

Objectives: To evaluate the safety and efficacy of intravenous (IV) administration of bone marrow mesenchymal stem cell derived extracellular vesicles (EVs), ExoFlo, versus placebo as treatment for COVID-19 associated moderate-to-severe Acute Respiratory Distress Syndrome (ARDS).

Endpoints: Primary Endpoint:

1) Improvement in partial pressure of arterial oxygen to fraction of inspired oxygen (PaO_2/FiO_2) ratio from pre-infusion baseline (Day 0) to Day 7. PaO_2 may be calculated from arterial blood gas (ABG) or imputed from the SpO_2 daily (See Appendix 11.1).

Note: Day 0 is designated as Day of Screening and Day 1 as Day of the First Study Intervention. Patients may be screened and treated within the same 24 hours—in this scenario, Day 1 will be synonymous with Day 0 & the pre-infusion value used will be from Day 1.

Secondary Endpoints:

2) Time to recovery as defined by the number of days from the first study treatment until return of oxygenation saturation (SpO2) ≥ 93% on room air (or PaO2/FiO2 ≥ 300 mmHg). If patient has chronic lung disease, recovery is defined as pre-COVID-19 SpO2 and O2 support.
3) Incidence of serious adverse events.
4) All-cause mortality.

Exploratory Endpoints:

5) Viremia: qualitative serum severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) ribonucleic acid (RNA) load on Days 0 or 1, 15, 29, and 61.
6) Acute phase reactants: C-reactive protein (CRP), D-dimer, Ferritin, interleukin-6 (IL-6), tumor necrosis factor alpha (TNF-α) on Days 1, 4, 7, 10, 15, 29. CRP, D-dimer, & Ferritin also on Day 0.

7) Immune cell counts: Absolute neutrophil count (ANC); CD3+, CD4+, CD8+ thymus cells also known as T lymphocytes (T cells); natural killer (NK) cells on Days 1, 4, 7, 10, 15, 29.
8) Sequential Organ Failure Assessment (SOFA) Score on Days 1, 15, 29 for patients who are still hospitalized.
9) Quality of life (QOL) assessment for patients who are discharged. EQ-5D-5L on Days 29 and 61. See Appendix 11.2 for sample EQ-5D-5L, which includes dimensions of mobility, self-care, usual activities, pain/discomfort, and anxiety/depression.

Study Population: Up to 120 adult male and female patients between 18 and 85 years of age hospitalized with COVID-19 associated ARDS.

Phase: Phase II

Site Number: 2-15

Description of Intervention: Patients will be randomized to one of the following:

Treatment Arm 1: PLACEBO Normal saline 100 mL

Treatment Arm 2: IP (Exoflo) 10ml dose in Normal saline 90 mL, which is approximately 800 billion EVs (Lot# P-441-1901-E5, P-441-2004-C5).

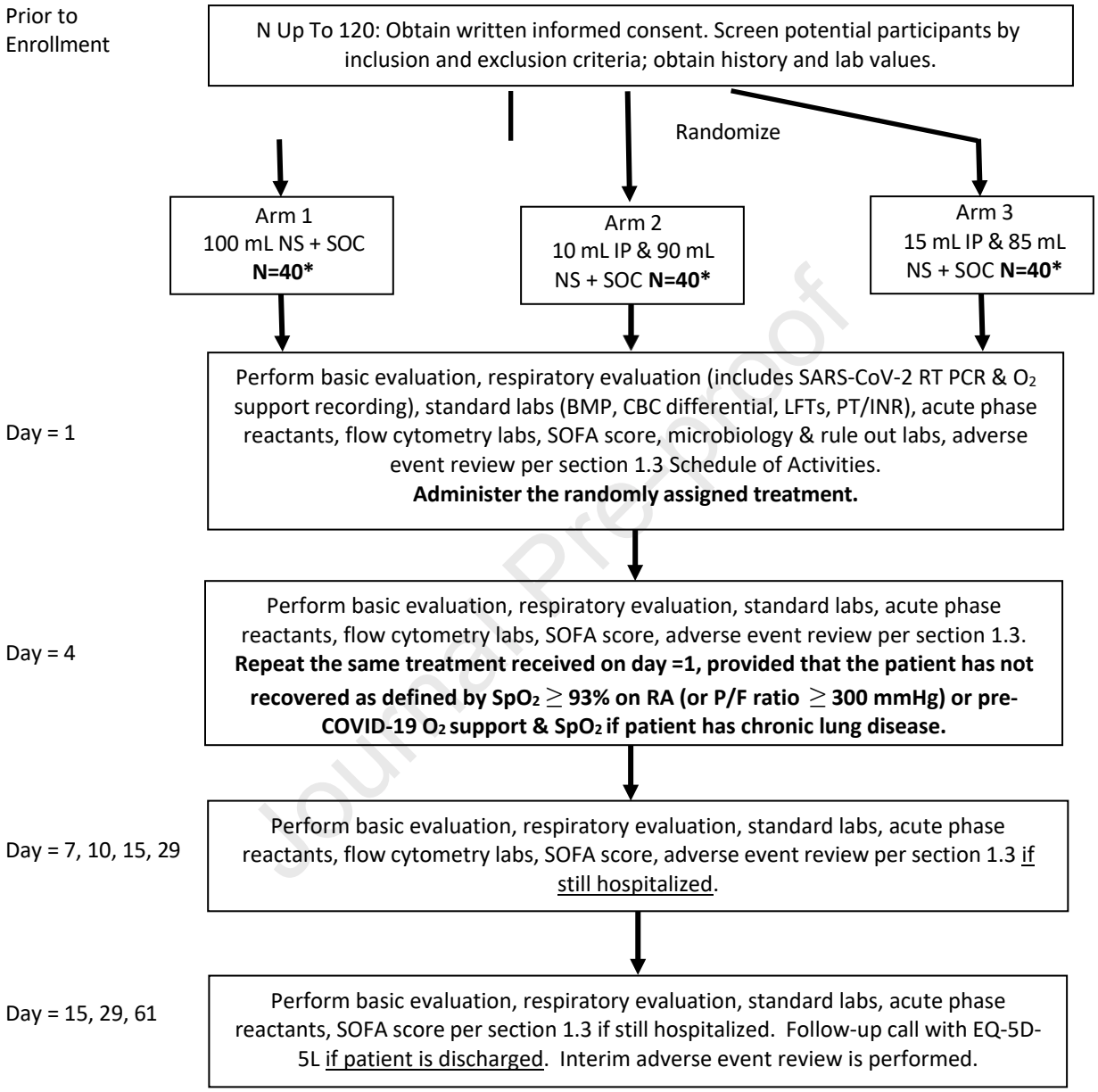
Treatment Arm 3: IP (Exoflo) 15ml dose in Normal saline 85 mL, which is approximately 1.2 trillion EVs (Lot# P-441-1901-E5, P-441-2004-C5).

The intervention will be infused over 60 minutes on Day 1 and repeated on Day 4 provided that the patient has not recovered as defined by SpO2 ≥ 93% on RA or PaO2/FiO2 ≥ 300 mmHg. If patient has chronic lung disease, recovery is defined as baseline SpO2 and O2 support.

Allocation of Intervention: Patients will be randomized 1:1:1 to each of the three treatment arms initially. The Interactive Response Technology will notify the DSMB such that a safety and interim analysis can be held following day 7 of the 60th patient randomized.

Duration: Each patient will be enrolled in the study for an estimated 60 days.

1.2 Schema



Abbreviations: AE=adverse event; BMP=basic metabolic profile; CBC=complete blood count; EQ-5D-5L=quality of life assessment; IP=investigational product; IV=intravenous; LFT-liver function test; NK cells=natural killer cells; NS=normal saline; P/F ratio_= partial pressure of arterial oxygen to fraction of inspired oxygen ratio; PT/INR= prothrombin time/international normalized ratio; RNA=ribonucleic acid; SOC=standard of care; SOFA=sequential organ failure assessment score; SARS-CoV-2=severe acute respiratory syndrome coronavirus 2.

1.3 Schedule of Activities*

NOTE: If eligibility criteria are met, patients may receive the first study intervention on the same day as screening, such that Day 1 is synonymous with Day 0 and the pre-infusion value from Day 1 may be used in place of the value from Day 0. See table footnotes A-J for answers to other common site questions.

Table 1. Schedule of Activities.

	Screen	Day						
PROCEDURES	**0 or 1**	**1**	**4**	**7**	**10**	**15**	**29**	**61**
Visit	***0***	***1***	***2***	***3***	***4***	***5***	***6***	***7***
BASIC EVALUATION								
Informed consent	X							
Inclusion/Exclusion	X	X						
Demographics	X							
Medical history	X							
Days of Illness Before Admission	X[A]							
Concomitant Meds	X	X	X[B]	X[B]	X[B]	X[B]	X[B]	X[B]
Vital Signs[C]	X	X	X[B]	X[B]	X[B]	X[B]	X[B]	X[B]
Height & Weight[I]	X[I]							
Physical examination	X	X	X[B]	X[B]	X[B]	X[B]	X[B]	X[B]
Glasgow Coma Score	X	X	X[B]	X[B]	X[B]	X[B]	X[B]	X[B]
Pregnancy Test[D]	X[D]	X[D]						
EKG[I]	X[I]	PRN AS INDICATED; N/A AFTER D/C						
Randomization		X						
Administer IV Study Intervention		X	X[E]					
RESPIRATORY EVAL								
SARS-CoV-2 RT-PCR[F]	X	X[J]				X[B]	X[B]	X[B]
Record Prone Posn (Y/N, Freq)	X	X[J]	X[B]	X[B]	X[B]	X[B]	X[B]	X[B]
PaO2/FiO2 ratio	X	CALCULATE DAILY UNTIL D/C; SEE *SECTION 11.1*						
Record O2 Support	X	DAILY UNTIL D/C; NOTE NC (LPM), FM (LPM), NRB, BiPAP (FiO2), HFNC O2 (FiO2), MV (FiO2, PEEP), HFOV (FiO2).						
CXR or CT chest	X	PRN AS INDICATED; N/A AFTER D/C						

STANDARD LABS								
BMP	X	X[J]	X[B]	X[B]	X[B]	X[B]	X[B]	X[B]
CBC with differential	X	X[J]	X[B]	X[B]	X[B]	X[B]	X[B]	X[B]
LFTS (including Bilirubin)	X	X[J]	X[B]	X[B]	X[B]	X[B]	X[B]	X[B]
PT/INR	X	X[J]	X[B]	X[B]	X[B]	X[B]	X[B]	X[B]
PTT	X	X[J]	X[B]	X[B]	X[B]	X[B]	X[B]	X[B]
ACUTE-PHASE REACTANTS								
CRP	X	X[J]	X[B]	X[B]	X[B]	X[B]	X[B]	
D-dimer	X	X[J]	X[B]	X[B]	X[B]	X[B]	X[B]	
Ferritin	X	X[J]	X[B]	X[B]	X[B]	X[B]	X[B]	
Il-6		X[J]	X[B]	X[B]	X[B]	X[B]	X[B]	
TNF-α		X[J]	X[B]	X[B]	X[B]	X[B]	X[B]	
FLOW CYTOMETRY								
T-lymphocyte panel (stain $CD3^+$, $CD4^+$, $CD8^+$)		X[J]	X[B]	X[B]	X[B]	X[B]	X[B]	
NK cells count ($CD3^-$ $CD56^+$ subset of lymphocyte gate)		X[J]	X[B]	X[B]	X[B]	X[B]	X[B]	
METRIC								
SOFA Score		X[J]				X[G]	X[G]	
EQ-5D-5L (Call Survey)							X[H]	X[H]
MICROBIOLOGY								
Urinalysis[I]	X[I]	PRN AS INDICATED; N/A AFTER D/C						
Urine culture[I]	X[I]	PRN AS INDICATED; N/A AFTER D/C						
Blood culture x 2[I]	X[I]	PRN AS INDICATED; N/A AFTER D/C						
Sputum culture[I]	X[I]	PRN AS INDICATED; N/A AFTER D/C						
RULE OUT TESTS								
Mycoplasma IgM[I]	X[I]							
QuantiFERON Gold[I]	X[I]							
Legionella Ag[I]	X[I]							
***Strep. Pneumoniae* Ag**[I]	X[I]							
Influenza A/B PCR[I]	X[I]							
Adverse Events Review	X	DAILY UNTIL D/C; CALL F/U ON DAY 15, 29, 61						

Abbreviations: Ag=antigen; ANC=absolute neutrophil count; BiPAP=bilevel positive airway pressure; BMP=basic metabolic profile; CRP=C-reactive protein; CBC=complete blood count; char=characteristics; CT=computed tomography; CXR=chest x-ray; D/C=discharge; EKG=electrocardiogram; EQ-5D-5L=5-dimentional quality of life assessment; FiO2=fraction of inspired oxygen; FM=face mask; Freq=frequency; HF NC O2=high flow nasal cannula oxygen support; HFOV=high frequency oscillatory ventilation; IgM=immunoglobulin M; IP=investigational product; IV=intravenous; LFT-liver function test; LPM=liters per minute; Meds=medications; MV=mechanical ventilation; N/A=not applicable; NC=nasal cannula; NK cells=natural killer cells; NRB=nonrebreather; PCR=polymerase chain reaction; PEEP=positive end expiratory pressure; POS=positioning; PT/INR= prothrombin time/international normalized ratio; PTT=partial prothrombin time; Quant=quantitative; RT-PCR=reverse transcriptase polymerase chain reaction; SARS-CoV-2=severe acute respiratory syndrome coronavirus 2; SOFA=sequential organ failure assessment score; T cells=thymus cells also known as T lymphocytes; TNF-α=tumor necrosis factor alpha.

A: The number of COVID-19 symptomatic days prior to the current admission is recorded along with the number of days from admission to first study administration.

B: Patients will be monitored for 72 hours as inpatients following each study intervention. Recovery will be defined in the study as SpO_2 reaching ≥ 93% on RA (or P/F ≥ 300 mm Hg) or pre-COVID-19 SpO_2 or O_2 support if patient has chronic lung disease.

- Following the first treatment on Day 1, the patient may be discharged on Day 4 or later.
- Following the second treatment on Day 4, the patient may be discharged on Day 7 or later.
- If a patient received the first but not the second treatment, he/she may be discharged prior to Day 7.
- Following patient's discharge, no labs will be drawn. Follow-up via phone-call only on Days 29 and 61.

C: During the infusion, measurements +/- 2 minutes of specified times for q5min vitals and +/- 5 minutes of specified times for q15min vitals are considered permissible; following the infusion, vital sign measurements are permissible +/-15 minutes of the specified times. Temperature is required only 5 minutes prior and q15min during the course of the infusion.

D: Serum pregnancy test (in women of childbearing potential) will be obtained on Day 0. Urine dipstick obtained on Day 1 at 4 hours prior to the first study treatment if >24 hours passed since the serum pregnancy test.

E: Not all patients who recovered will receive a repeat study intervention. Patients who recovered by Day 4 do not receive a repeat study intervention.

F: Qualitative SARS-CoV-2 RT PCR will be used for Day 0 or 1 (prior to the first dose), 15, 29, 61. If there is a shortage of tests, documentation of positive test within 14 days prior to admission suffices for Day 0 or 1.

G: SOFA Score will be administered only if patient is still hospitalized.

H: ED-5D-5L score will be administered over the phone only if patient is discharged. Note: This is not a meaningful measure for patients who are still hospitalized due to common use of IV sedatives or PO antipsychotics for delirium. A baseline prior to study infusion is not obtained because the patient is hypoxic, and the hospital staff needs to prioritize diagnostics and treatment rather than time-consuming quality of life metric.

I: The Screen column refers to tests obtained prior to the first study treatment, usually obtained on Day 0, i.e., 24 hours prior to the first study treatment. However, height & weight, EKG, microbiology, and rule out tests do not have to be repeated on Day 0 if obtained once following the current hospital admission.

J: All lab tests including SARS-CoV-2 viremia, standard tests, acute phase reactants, flow cytometry will be obtained around 1-2 hours prior to the first study treatment on Day 1. On Day 1, SOFA score will be calculated on the morning values prior to the study treatment.

2. INTRODUCTION

2.1 Study Rationale

The objective of this clinical trial is to evaluate the safety and efficacy of ExoFlo, a bone-marrow mesenchymal stem cell (bmMSC) derived extracellular vesicles product, via IV infusion, for the treatment for ARDS in patients with severe COVID-19. Containing a panoply of chemokines, messenger RNA (mRNA), and noncoding RNA (microRNA or miRNA) secreted from bmMSCs, extracellular vesicles are the essential paracrine mediators of bmMSC function—which is not necessarily engraftment or differentiation at the target tissue, but rather cell-to-cell communication.[1,2] Extracellular vesicles retain the potent anti-inflammatory effects of bmMSCs, but are acellular and nonimmunogenic, containing no nucleus or deoxyribonucleic acid (DNA).[3,4] And at 1/1000 the size of an MSC, extracellular vesicles pass easily through capillaries, potentially rendering safer IV dosing and redosing compared to allogeneic stem cells.

Treatment with bmMSCs has already shown promise in the treatment of COVID-19 related ARDS, other sepsis-based ARDS, and other hyperinflammatory disease states.[4-11] These findings taken together with the role of extracellular vesicles as the primary therapeutic mediators of bmMSC function, and the ongoing absence of any proven therapy for COVID-19, prompted our physician-investigators to conduct the first clinical trial worldwide on therapeutic use of bmMSC-derived extracellular vesicles — in an open label phase I study, 24 patients with moderate to severe ARDS in setting of severe COVID-19 were enrolled at the height of the pandemic and received a single IV 15 mL dose of IP.[12] All safety endpoints were met with no immediate infusion-related adverse reactions in addition to no treatment-attributable adverse events (AEs). Furthermore, following a single infusion of IP, 17 out of 24 patients demonstrated a profound reversal of their initial hypoxia, correlating with rapid reduction in supplemental oxygen and median time to recovery of 5.6 days.[12]

Exploratory endpoints in our preliminary clinical trial revealed statistically significant improvements in acute phase reactants, absolute neutrophil counts (ANC), and T-lymphocyte subsets following treatment with the IP—suggesting that the therapeutic mechanisms of action for

ARDS may be related to the reduction of inflammation and the reconstitution of adaptive immunity.[12] Independent molecular characterization of the IP have since indicated other promising therapeutic actions, which may be specific for COVID-19 associated ARDS. Notably, we have identified many highly expressed and unique chemokines in IP including 27 miRNA, which are potential inhibitors of the ACE-2 related protein network; 7 miRNA, which are inhibitors of serine protease 2 (TMPRSS2)—a transmembrane protease that is required for Spike (S) protein priming and SARS-CoV-2 entry into a host cell; 5 miRNA that inhibit IL-6, a regulator of the acute phase response that can lead to the severe systemic inflammatory response known as "cytokine storm" and activation of the coagulation cascade when present at an excessive level; and 2 miRNA that target tumor necrosis factor alpha (TNF-α), a proinflammatory cytokine, that also plays a prominent role in mediating cytokine storm.

As the COVID-19 pandemic has continued, with substantial concern for resurgence in the US, these findings evince the need for further and more rigorous study of extracellular vesicle therapy in the way of a double-blinded, randomized controlled trial (RCT). However, to date, there has been no randomized study on the safety and efficacy of bone-marrow derived extracellular vesicles for the treatment of severe COVID-19 nor for the treatment of ARDS.

This study should be of national and global interest as timely treatment with MSC extracellular vesicles can potentially turn the tide of the devastating disease process of COVID-19, not to mention its catastrophic and evolving economic consequences. Thus far, purported treatments for COVID-19 associated viral pneumonia and ARDS, involving single-target agents such as hydroxychloroquine and ritonavir/lopinavir, have shown mixed results.[13-17] A phase III study of remdesivir reached statistical significance with median time to recovery (11 vs 17 days in treatment vs placebo) but not reduction in mortality rate.[18] Our preliminary clinical study using the IP as treatment for COVID-19 showed a superior median time to recovery of 5.6 days and was performed in a population of patients with baseline demographics and clinical characteristics, which portended poorer outcomes. Eighty-three percent (83%) of the patients had type II diabetes; all patients had pre-enrollment PaO_2/FiO_2 ratio consistent with moderate (46%) or severe ARDS (54%).[12]

Due to the potent immunomodulatory properties, the IP may have the therapeutic potential of reducing mortality rate in COVID-19 related ARDS as well as other sepsis-associated ARDS, especially when used as part of an early goal directed therapy. To date, there is still no disease-altering treatment for ARDS that has been shown to reduce mortality rate.[19-23] The lack of significant therapeutic advancement has been acknowledged by National Institutes of Health (NIH) ARDS Clinical Trials Network, which has since declared the necessity of prioritizing prevention and early treatment of ARDS.[24,25] If the success of early goal directed therapy in sepsis is any indication, recognizing ARDS as a continuum rather than fixating on the positive pressure mechanical ventilation (MV) criteria of the Berlin definition may be the essential first step in finally changing mortality outcomes in patients with ARDS.[24,26] Thus, one distinguishing feature of this study will be the inclusion of non-intubated patients with ARDS as defined by all other criteria of the 2012 Berlin definition—a purposeful identification, which has been adopted in other key studies in critical care, in order to identify at risk patients and facilitate timely treatment.[27-34]

2.2 Background

2.2.1 The Status Quo in Healthcare

Since December 2019, COVID-19, the disease caused by the SARS-CoV-2 strain of the coronavirus, has expanded into a global pandemic.[35-38] Due in part to its population density, New York City and its immediate vicinity, became the initial epicenter of COVID-19 in the US.[39,40] Common symptoms of COVID-19 include fever, non-productive cough, nasal congestion, and fatigue. A significant portion of the population may not exhibit pronounced symptoms during the first 10-20 days of infection, while symptomatic patients may present to a healthcare facility with reduced oxygen saturation (SpO_2), ABG revealing hypoxia, and chest X-ray revealing bilateral infiltrates.[37,41,42] The remaining labs may be significant for leukocytosis, lymphopenia, elevated acute phase reactants, and biomarkers of end-organ damage such as renal and myocardial injury.[43,44] In patients with underlying health conditions, an estimated 30% of the infected population will require hospitalization—once admitted, roughly half of this population will require MV in an intensive care unit. Early observations cites mortality rates as high as 88-94% following

intubation, indicating MV as a significant predictor of mortality at rates which exceeded even those observed in other-sepsis associated ARDS.[37-40,45] Early observations also cite surprisingly high mortality rates of 68% and higher in patients requiring noninvasive oxygen support, which indicates the importance of timely intervention.[40,46-48]

Due to the explosion of cases in the first wave, concerns regarding resource limitations, and emerging understanding of how best to treat COVID-19, major urban hospitals have developed increasing thresholds for hospital admission as well as MV.[49,50] Prior to the pandemic, patients presenting with acute onset of fever, shortness of breath, and hypoxia, meeting criteria for moderate to severe ARDS, would typically be intubated. However, these patients are now first maintained with noninvasive supplemental O_2 and other optimization measures like intermittent proning, with endotracheal intubation delayed for as long as possible. This group of patients holds significant interest for this study, as an appropriately timed intervention could substantially reduce progression to complete hypoxic respiratory failure requiring MV, a critical event associated with high morbidity, mortality, and healthcare expenditure.

The paradigm shifts in the treatment algorithm for COVID-19 induced respiratory failure has evolved in response both due to the perceived shortage in resources in addition to the increasing controversy surrounding the current understanding of COVID-19 pulmonary disease.[51] There is a growing consensus among physicians that COVID-19 pulmonary disease is poorly understood, and MV may pose more harm than benefit.

2.2.2 Rationale for Exploring New Treatment

Common practices for COVID-19 thus far included: (1) empiric antibiotics for presumptive bacterial coinfection, (2) initiation of potential disease altering medications such as antivirals, (3) daily intermittent proning of both intubated and non-intubated patients, (4) addition of steroids in patient who met criteria for moderate to severe ARDS, and (5) the addition of empiric anticoagulation in hypercoagulable patients.[46,52,53] Strictly speaking, while all therapies for COVID-19 are considered experimental therapies, many practices have already been adopted by hospitals systems across the US as a basic regimen for severe COVID-19.

Trials for experimental single target agents, including antivirals, antibiotics, and biologics, like remdesivir, hydroxychloroquine, and tocilizumab, respectively, have yielded mixed outcomes with some associated with significant mortality and morbidity.[13,14,16-18] For example, remdesivir has been associated with significant hepatotoxicity that requires discontinuation in some patients and Actemra® (tocilizumab) has been associated with significant thrombocytopenia, gastrointestinal perforation, and leukopenia.[16,54] Other options for prevention and treatment include vaccination and convalescent plasma—both of which may pose the risk of antibody- dependent enhancement—a cascade of events whereby non-neutralizing antibodies bind to the newly infecting virus and enhance the viral invasion of host cells.[55-58] Vaccination and convalescent plasma also notably require relatively stable viral epitopes for efficacy—this is a problematic requirement for treatment efficacy since much like the RNA virus HIV, SARS-CoV-2 directly suppresses host T-cell function.[59,60] Clinically, this has been borne out with frequent presentations of multi-organ failure secondary to immunodeficiency even in previously healthy individuals; use of antiretroviral may show initial efficacy but only transiently.

The Food and Drug Administration (FDA) has yet to approve for official use a COVID-19 potent medication that can account for the mutability of SARS-CoV-2 coronavirus as well as enhance both innate and adaptive immunity.[61-64] The mortality rates for critically ill patients with COVID-19 range 50-94% in some hospitals, vastly exceeding the well-studied mortality rate of 27-45% reported for mild, moderate, and severe ARDS of the past.[23,39,40] Clearly, the continued exploration of treatments for severe COVID-19 is greatly needed.

2.2.3 COVID-19: Pathophysiology

The pathophysiology of COVID-19 has been studied with intense interest while the exact mechanism remains elusive. The prevailing theory is that the virus gains entry into host cells through cross-linking of the SARS-CoV-2 spike protein (S protein) with angiotensin-converting enzyme 2 (ACE2) receptor on host cells, thereby leading to the fusion of the viral and host cell membrane.[65] Following entry into the host cell, the viral RNA is translated into protein in the host cell's cytoplasm and then assembled into more viral copies (viral envelope containing genomic

RNA) with subsequent secretion and further invasion of host cells.[66] The normal role of ACE2 is the inactivation of angiotensin II. Early studies show that ACE2 is downregulated in response to SARS-CoV2, rendering it unavailable to play its physiologic role, and thereby leading to the accumulation of Angiotensin II. In addition to acting as a profound vasoconstrictor, Angiotensin II, also elicits increased synthesis and elaboration of pro-inflammatory, pro-fibrotic, pro-thrombotic, and pro-oxidative factors, thereby causing local injury to the tissue.[67] Especially considering its ubiquitous expression in lung, heart and kidney tissues, the accumulation of Angiotensin II may be central to the hyper-inflammatory state, referred to as cytokine storm.[63,64] This state observed in some cases of severe COVID-19 is believed to be amplified and sustained by two evolving parallel processes: (1) the activation of macrophages and other antigen presenting cells (APC), which then alert lymphocytes to the presence of the virus; (2) viral RNA replication within host cells, which activates synthesis of proinflammatory factors.[68] Both pathways lead to immune dysregulation and hyperinflammation. Indeed, immunological studies from Wuhan, China, revealed lymphopenia in patients with severe cases of COVID-19, specifically among CD3+, CD4+ and CD8+ thymus cells also known as T lymphocytes (T cells), as well as regulatory T cells, the depletion of which likely facilitates ongoing immune evasion by the virus.[43]

Following findings of high mortality among intubated patients with COVID-19, many intensivists have expressed doubt that the initial presentations were entirely consistent with classic ARDS. These reservations originated from the observation that hypoxemia in a subset of patients with severe COVID-19 is often out of proportion to their relatively normal work of breathing and lung compliance.[51] Some physician researchers hypothesize that this uncoupling of hypoxemia and lung mechanics may be due to polymorphisms of SARS-CoV-2 pathogenicity that causes distinct phenotypes. Alternatively, some have suggested that distinct phenotypes may be separated into early versus stages.

Ultimately, irrespective of the presenting phenotype, hospitalized patients with persistent COVID-19 associated ARDS develop lung-mechanics and pathology similar to other sepsis- associated ARDS. These patients accumulate inflammatory infiltrates in the lung parenchyma and airspaces, oxidative stress from high FiO_2 and mechanical microtrauma from positive pressure

MV—all factors leading to diffuse alveolar damage, which is the most common histologic pattern identified in patients with ARDS.[69] Pathologic examinations of patients with COVID-19 associated ARDS in Wuhan, China reveal alveolar epithelial injury, reactive hyperplasia of type II pneumocytes, hyaline membrane formation, and fibroblastic plugs in the air-spaces, all of which are indicative of how COVID-19 pulmonary disease can rapidly devolve into a fatal state of minimal gas exchange.[70]

2.2.4 A Potential Treatment for COVID-19 Associated ARDS

As a regenerative medical therapy, bone-marrow derived extracellular vesicles can potentially shift the tide of the devastating disease process caused by COVID-19. Extracellular vesicles are naturally occurring vesicles produced by most eukaryotic cells and are the primary mode of intercellular paracrine signaling.[2,3,71-73] Exosomes, one of the primary types of extracellular vesicles, are typically 30-150 nanometers in diameter and approximately 1/1000th size of a cell. These signals are not cells and contain no nucleus or DNA. The population of extracellular vesicles secreted by bone marrow-derived mesenchymal stem cells have been studied extensively in preclinical studies of inflammation and are notable for their ability to downregulate inflammation and upregulate repair.[1-3,74-81]

IV administration of bmMSCs-derived exosomes has already shown safety and potential efficacy in investigational studies in patients with ARDS. In 2015, the phase I START trial enrolled 9 patients with moderate-to-severe ARDS and monitored outcomes for 60 days following a single dose of IV administration; no SAEs were observed in the 6 hours following the infusion nor in the weeks following the one dose infusion of allogeneic bmMSCs, including up to 10 million MSCs/kg.[7] In a recent pivotal study in Wuhan, China in March 2020, transplantation of bmMSCs from healthy donors into seven patients with COVID-19 pulmonary disease improved functional outcomes without any observed adverse effects including infusion-related or allergic reactions within two hours after treatment nor delayed hypersensitivity or secondary infections.[5] Within 2 days of the treatment, 6 out of 7 patients recovered; within 3 days, 3 out of 7 patients were discharged from the hospital. Exploratory endpoints were significant for decreased pro-

inflammatory cytokine TNF-α and for increased anti-inflammatory IL-10—suggesting that one therapeutic value of bmMSCs in patients with COVID-19 may be cytokine storm inhibition.[5]

Given that the therapeutic properties of bmMSCs is primarily due to mediation of cell-to-cell communication via secretion of soluble factors and extracellular vesicles, rather than actual engraftment and differentiation into target tissue, the treatment potential of extracellular vesicles purified from bmMSCs hold particular clinical interest during the COVID-19 pandemic. Containing a panoply of chemokines, mRNA, microRNA, exosomes and other extracellular vesicles promote the synthesis of regenerative, tissue protective, antimicrobial, and anti-inflammatory proteins and silence the expression of pro-inflammatory genes that fuel the cytokine storm.[1] These signals also regulate immune dysregulation at an epigenetic level by influencing histone methylation and acetylation. And since extracellular vesicles are the primary therapeutic effector of MSCs, the question arises as to why US physicians would accept the risks of allogeneic stem cell transplant, such as graft versus host disease (GvHD), when the same therapeutic effects can be derived from a significantly more standardized product and administered at much lower risk to the patient.

The IP is a bone marrow derived extracellular vesicle product notable for its high production standards, purity, and potency. Extensive characterization of the extracellular vesicles contained in the IP has revealed an absence of immunogenic surface epitopes that make it highly unlikely to cause acute immune reactions, and there are no known product reactions or reported adverse reactions to date. The IP exerts a powerful anti-inflammatory effect as well as promotes regeneration of tissues damaged by inflammation. Each mL of IP, from the lots to be used in this study (P-441-1901-E5 and P-441-2004-C5), contains approximately 80 billion extracellular vesicles, and each mL is comprised of over 2000 different anti-inflammatory cytokines, anti-oxidative stress, pro-regeneration, tumor suppressor, and antimicrobial agents--the potential for changing the course of ARDS in patients with COVID-19 is therefore compelling (see Table 2 for a sample overview).

Table 2: Classes of Factors Contained in the IP for this Study

Class	Factor	Description	Function
Angiogenesis & Wound Healing	uPAR	CD87	Wound and Tissue Healing
	VEGF	Vascular endothelial growth	New blood vessel formation
	Thrombomodulin	CD141, thrombin cofactor	Anti-clotting factor, wound remodeling
	CD97	G protein-coupled receptor	Promotes angiogenesis attracts endothelial
Chemotaxis, Cell Migration	IGFBP2	Insulin growth factor binding	Regulates IGF levels, Supports TIMPs and
	TSLP	Thymic Stromal Lymphoprotein	T-Cell Differentiation and Recruitment
	NCAM	Neuronal Cell Adhesion	Cell Adhesion to Neurons, NK Cells
	NUP85	Nucleoporin85	Monocyte migration; mRNA transport &
Immune Modulation	MIF	Macrophage inhibitory Factor	Regulates macrophages-Anti-inflammatory
	TNF-α RI	Tumor necrosis factor- α receptor	Negative regulation of TNF-a- Anti-
	IL1-R6	Interleukin 1 Receptor 6	Cytokine, chemokine and antimicrobial
	PF4	Platelet factor 4	Antimicrobial activity
Tumor suppressor	IGFBP-4	Insulin growth factor binding	Anti-tumorigenic In vivo & in vitro; binds
	bIG-H3	TGFB Induced protein	ECM protein induced by TGFB to inhibit
	Serpin F1	Secreted multifunctional protein	Anti-tumorogenic, anti-angiogenic,
	DKK3	Dickkopf-related protein 3	Wnt signaling, tumor suppressor
ECM Development & Remodeling	Cathepsin B	Catabolic Protease	Collagen matrix Remodeling
	TIMP-1	Collagenase Inhibitor	Regulates Collagen Remodeling
	TIMP-2	Collagenase Inhibitor	Regulates Collagen Remodeling
	FAP-A	Fibroblast activation protein,	Regulation ECM integrity, collagen content
Regenerative	Semaphorin 6c	Signal regulator of tissue	Nervous system development, cartilage r-
	IGF2	Insulin-like Growth Factor 2	Reproductive and brain tissue regeneration
	FGF-16	Fibrolast Growth Factor 16	Cardiomyocte proliferation, heart tissue

Molecular characterization of over 2000 specific chemokines, mRNA, and microRNA in the IP reveal the therapeutic potential for ARDS and COVID-19 due to their associations with increasing bacterial clearance, restoring lung protein permeability, increasing alveolar fluid clearance, stabilizing the capillary alveolar barrier, and reprogramming immune cells toward anti-inflammatory phenotype. This observation is supported by multiple preclinical studies on the role of MSC exosomes in acute lung injury and ARDS models revealing that (1) these signals can suppress the secretion of TNF-α and other pro-inflammatory cytokines, (2) promote the secretion of anti-inflammatory cytokines, including IL-10, (3) repair human lung microvascular endothelial cells by increasing expression of Ang-1, (4) induction of anti-inflammatory M2 phenotypes in macrophages, (5) promote regulatory T cell proliferation by increasing expression of TGFβ1 and

reducing circulating levels of IL-6, and (6) enhance alveolar and lung edema fluid clearance.[2,74,77,80]

Clinically, the safety and efficacy potential of the IP has already been explored in our preliminary open-label single-arm prospective study. During April 2020, at the height of the pandemic, 24 patients with severe COVID-19 received a single dose IV IP for moderate to severe ARDS.[12] Following a single dose of IV IP, 17 out of 24 patients showed profound improvement in oxygenation, as measured by the PaO_2/FiO_2 ratio, within 48-72 hours. The median time to discharge was 5.6 days. Exploratory endpoints were significant were reduced acute phase reactants include CRP, D-dimer, and Ferritin in addition to reduced neutrophil count and improved CD3+, CD4+, and CD8+ T cells.

In addition to the availability of preliminary clinical safety and efficacy data, the IP (bone marrow-derived extracellular vesicles) manufactured by Direct Biologics, should be considered for use due the following reasons pertaining to safety, efficacy, reproducibility, and overall quality.

(1) The IP has undergone current good manufacturing processes (cGMP): the IP is a bmMSC derived extracellular vesicle product produced under cGMP standards. The bmMSCs and media both have master files on record at the FDA. The IP meets donor and manufacturing safety profiles, has lot-specific tissue traceability and a comprehensive labeling including detailed instructions for use.
(2) Safety profile in clinical use: There are no documented AEs associated with the use of this product. Our preliminary Institutional Review Board (IRB) approved clinical study showed no adverse effects within 24 hours of IV administration and no suspected unexpected serious adverse reaction (SUSAR) or treatment-attributable SAE within 14 days following IV administration of the IP to 24 patients with severe COVID-19.
(3) No allogeneic DNA exposure: In contrast to MSC transplantation treatment with extracellular vesicles does not involve the introduction of foreign DNA, thereby eliminating the complexities and variability involved in human-to-human MSC transplantation.

(4) Size advantage: It has been demonstrated that due to their size, nearly 100% of MSCs administered by an IV route become lodged in and occlude pulmonary capillaries. In contrast, extracellular vesicles (30-150 nanometers) are roughly 1000x smaller than MSCs and easily pass through capillaries, thereby eliminating the risk of pulmonary vascular occlusion in the setting of COVID-19 pulmonary disease. An additional benefit conferred by the smaller size of these vesicles, as in the case of the IP, they can be delivered in highly concentrated doses, is that multiple doses can be given without increasing the risk for vascular occlusion.

(5) Extensive characterization: Extracellular vesicles derived from bmMSCs are the most extensively studied in the peer-reviewed literature and have been shown to be safer and possess more favorable signaling factors from a clinical standpoint. In addition, the molecular characterization of the IP is readily available.

(6) Lack of immunogenicity: Extracellular vesicles are not known to express surface epitopes that would be recognized by the immune system as foreign and can be easily redosed for efficacy whereas a cellular biologic has a much higher risk of eliciting an immune reaction, GvHD, or other reactions which could be amplified with redosing. Additionally, the use of xeno-free medium in the IP's manufacturing process further reduces the likelihood of an adverse immune response. To date, there are no known reactions to infused or injected IP.

(7) Standardization & scalability: As in the case of the IP, which is produced under rigorous conditions described below, extracellular vesicle products can be produced with a high level of quality and consistency that most closely approximates a pharmaceutical grade product. In contrast, perinatal exosomes have a high level of batch-to-batch variability and contamination rate related to the use of multiple donors, the absence of external FDA validation processes, the use of immunogenic bovine serum in cellular cultivation processes, and the heterogeneity of exosomes obtained from a source where maternal and fetal exosomes are inevitably co-mingled.

(8) Product stability and ease of storage: While extracellular vesicles require storage ≤ -40°C, short-term storage up to 14 days at 2-8°C has been shown not to degrade the particle distribution size of the IP, indicating its stability. Storage of these vesicles does not require

preservatives nor a full facility, which is necessary, for example, for the storage of live cells in the context of bone marrow or stem cell transplantation.

(9) Scalability: the IP, the bone marrow derived extracellular vesicles product used in this study has been uniformly tested for quality. These signals originate from a single human donor and are abundantly available due to proprietary production technology. In contrast, bmMSCs and perinatal exosomes must be harvested from different human donors after a meticulous vetting process, posing challenges to uniform quality control, and immediate availability. Furthermore, the use of extracellular vesicles precludes the need for a transplant facility to store living cells, which increases the number of facilities that can administer therapy.

(10) Lack of carcinogenicity: bmMSCs and their signals have been extensively characterized without evidence of oncogene expression. The safety and standardization of bone marrow derived extracellular vesicles is superior compared to MSCs in addition to amniotic exosomes, also known as amniosomes, which is a tissue product of inherently high variability when considering each lot is harvested from a different donor.

2.3 Risk/Benefit Assessment

2.3.1 Known Potential Risks

There are no known immediate or long-range potential risks based on preclinical studies of bone marrow derived extracellular vesicles. Specifically regarding ExoFlo, there are no known reported immediate or long-range potential risks following intra-articular treatment for osteoarthritis based on a preliminary clinical study. There are also no known immediate risks (≤72 hours) based on our preliminary open-label safety study on 24 patients with severe COVID-19 following a single 15 mL infusion of IP.[12] The patients were followed for 14 days following treatment. There were no immediate infusion-related adverse reactions within 4 hours or SAEs which were attributable to the IP following independent Data Safety Monitoring Board (DSMB) review. Currently, there are no known long-range potential risks of IV administration of the IP as treatment for severe COVID-19.

2.3.2 Known Potential Efficacy

Based our preliminary safety study, which was also revealing for potential efficacy of the IP, known potential immediate benefit (≤72 hours) of the IP as IV treatment for severe COVID-19 is improved oxygenation, as demonstrated by improved PaO_2/FiO_2 ratio and de-escalation of oxygen support requirement within 48-72 hours following a single IV infusion; reduced inflammation, as demonstrated by reduction of D-dimer, Ferritin, and CRP; reconstitution of adaptive immunity, as demonstrated by improved neutrophilia and lymphopenia.[12]

One major potential long-range benefit (>72 hours) of the IP as IV treatment for severe COVID-19 is improved survival. Seventy-one percent (71%) of the patients (17/24) recovered and were discharged from the hospital within a mean of 5.6 days. Estimated 45-55% of the patients with severe COVID-19 were discharged from the same hospital in the same month following standard of care only, suggesting treatment with the IP was associated with increased likelihood of recovery. Other potential long-range benefits include decreased incidence of intubation among non-intubated patients, in addition to shorter duration of hospitalization.

2.3.3 Assessment of Potential Risks and Benefits

Given that the safety and efficacy of IV administration of the IP for severe COVID-19 has yet to be proven in an appropriately powered randomized clinical trial (RCT), one primary feature of this investigational study to maximize the potential benefit to risk ratio is that patients will only be enrolled only if they are clinically deteriorating despite receiving standard of care for COVID-19 associated moderate-to-severe ARDS and no established alternative treatment is available. The rationale of the necessity of exposing participants to potential risks is that they are manifesting clinical deterioration from severe COVID-19, which is a life-threatening disease, despite the best available and established clinical treatments. Other features of the study design that maximize the potential benefit to risk ratio is the daily evaluation of AEs, use of stopping rules, and a staggering protocol.

3. OBJECTIVES AND ENDPOINTS

Table 3: Study Objectives, Endpoints, and Justification for Endpoints

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
PRIMARY		
The primary objective is to evaluate efficacy of IP as treatment for COVID-19 associated moderate to severe ARDS* compared to placebo.	Improvement in Partial pressure of arterial oxygen to fraction of inspired oxygen (PaO_2/FiO_2) from pre-infusion (Day 0) baseline to Day 7.	There is a high variation in physician practices and hospital practices, which affect chosen mode of O_2 support and rate of intubation & ventilator weaning. Focusing on dichotomous variables of intubated/non-intubated or ordinal scales of oxygen support overinflates the relative measure of true hypoxia. For example, FiO_2 of a nonrebreather (NRB) can reach 0.8-0.9 while high flow oxygen (HF O_2) is set at FiO_2 of 0.8-1.0—defining HF O_2 as an entire class above NRB is problematic. Therefore, even when imputing PaO_2 from SpO_2 (see Appendix 11.1), PaO_2/FiO_2 ratio is still a comparatively more meaningful as a standardized measure of oxygenation.
SECONDARY		
The secondary objective is to evaluate the safety and efficacy of IP as treatment for COVID-19 associated moderate to severe ARDS* compared to placebo.	Time to recovery as defined by the number of days from the first study treatment until return of oxygenation saturation (SpO2) ≥ 93% on room air (or PaO2/FiO2 ≥ 300 mmHg). Incidence of Serious Adverse Events (SAE) All-cause mortality rate.	Patients who received IP + SOC are more likely to recover faster compared to patients who received Placebo + SOC. Recovery is defined as days from the first study treatment until return of oxygen saturation (SpO_2) ≥ 93% on room air or to pre-COVID-19 baseline SpO_2 if patient has chronic lung disease. Recovery may also be defined as PaO_2/FiO_2 ratio ≥ 300 mmHg. All adverse events must be reviewed and evaluated by an independent Data Safety Monitoring Board (DSMB). All-cause mortality is included in most studies of ARDS and COVID-19.
EXPLORATORY		
The explorative objectives are to evaluate whether IV treatment with the IP is associated with significant surrogate markers compared to placebo.	Qualitative SARS-CoV-2 RNA level on days = 0 or 1 (prior to first dose), 15, 29, 61 following the 1st infusion of IP. CRP, D-dimer, Ferritin, IL-6, TNF-α; ANC; CD3+, CD4+, CD8+ T cells; NK cells on days = 1, 4, 7, 10, 15, 29 and 61. SOFA Score on days = 1, 15, 29.	Progression of severe COVID-19 is associated with detectable viral load, rising levels of acute phase reactants, worsening neutrophilia, depletion of lymphocytes, and decreased functioning of natural killer cells (NK cells) in addition to concurrent multiorgan failure. Preliminary study on the IP shows acute phase reactants, neutrophilia and lymphocytopenia improved significantly within 48-72 hours of the IP. The secondary endpoints will help determine whether the treatment mechanism of action is via reduction of COVID-19 viremia, reduction of inflammation, and/or enhancement of both adaptive and innate immunity. SOFA score is a common mortality prediction score used in sepsis research.[84] Individual scores can be useful as a

Table 3: Study Objectives, Endpoints, and Justification for Endpoints

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
		measure of organ dysfunction as pulmonary, cardiovascular, hematologic, hepatic, renal, and neurologic systems are all affected.
	EQ-5D-5L among patients discharged from the hospital on days = 29, 61	EQ-5D-5L is the widely used metric used in cost-effective analysis.[82] Post-Intensive Care Syndrome among survivors is underrecognized; prominent features include cognitive dysfunction, posttraumatic stress disorder (PTSD)-like symptoms, and muscle weakness.[85]

Abbreviations: ANC=absolute neutrophil count; BMP=basic metabolic profile; CRP=C-reactive protein; CBC=complete blood count; CT=computed tomography; CXR=chest x-ray; EKG=electrocardiogram; EQ-5D-5L=5-dimentional quality of life assessment; FiO2=fraction of inspired oxygen; HFOV=high frequency oscillatory ventilation; IgM=immunoglobulin M; IP=investigational product; IV=intravenous; LFT-liver function test; LPM=liters per minute; NK cells=natural killer cells; PCR=polymerase chain reaction; PEEP=positive end expiratory pressure; PT/INR= prothrombin time/international normalized ratio; PTT=partial prothrombin time; RT-PCR=reverse transcriptase polymerase chain reaction; SARS-CoV-2=severe acute respiratory syndrome coronavirus 2; SOFA=sequential organ failure assessment score; $Sp0_2$=peripheral capillary oxygen saturation; T cells=thymus cells also known as T lymphocytes.

4. STUDY DESIGN

4.1 Overall Design

Phase:	Phase II
Description:	Multicenter, placebo-controlled, dose-ranging, double-blinded, RCT.
Intervention Model:	All eligible participants will receive standard of care for severe COVID-19 while being randomized to one of the following three treatment arms—in each arm, a 60-minute study intervention is administered on Day 1. If the patient has not recovered by Day 4, the study intervention is repeated on Day 4.
# Research Sites:	2-15; competitive enrollment will be used.
Study Intervention:	(1) Placebo or IV 100 mL of NS, (2) IV 10 mL of IP mixed with 90 mL of NS, (3) IV 15 mL of IP mixed with 85 mL of NS. (Lots# P-441-1901-E5, P-441-2004-C5).
Allocation & Stratification:	An Interactive Response Technology (IRT) system will be used to electronically randomize subjects in a blinded fashion. Participants will be randomized to three treatment arms following stratification by research site.
Masking Description:	Following randomized allocation of participants to each of the 3 treatment arms, the hospital pharmacists, who are provided the study intervention identity by the IRT, will independently prepare the appropriate infusion of (1) 100 mL of IV normal saline, (2) 10 mL of IP mixed with 90 mL of normal saline, or (3) 15 mL of IP mixed with 85 mL of normal saline for each patient.

4.2 Scientific Rationale for Study Design

The rationale for choosing a double blinded, placebo controlled, RCT is that this is the gold standard for proving safety and efficacy of the IP compared to standard of care. Randomized block design further minimizes selection bias. In this case, institutions may vary in terms of clinician preferences, hospital resources, patient demographics, other characteristics (such as poor versus

good outpatient follow-up), and severity of illness, in addition to overall effectiveness of ICU care. Intubation status may be better stronger predictor of clinical deterioration rather than P/F ratio. Adopting stratified randomization allows the randomization to be performed within each stratum so that the balance between the treatment arms is as close to equal as possible.

4.3 Justification for Dose

4.3.1 Rationale for Dosing:

Dosing of the IP was calculated based on (1) the 24-patient preliminary COVID-19 clinical case series with the IP; (2) the START trial, which is a phase I trial of IV administration of bone-marrow derived stromal cells as treatment for ARDS (NCT01775774), which demonstrated safety of using IV doses of 1 million cell/Kg, 5 million cell/Kg, in addition to a ceiling dose of 10 million cell/Kg; (3) observation of approximately 2,000 extracellular vesicles secreted per stromal cell; and (4) lab analysis of the IP (Lots# P-441-1901-E5 and P-441-2004-C5), which showed that each mL contained approximately 80 billion extracellular vesicles. For an adult of 70 Kg, extrapolation from the START trial MSC ceiling dose would yield an IV IP ceiling dose of 17.5 mL. Given a range of body mass indexes (BMIs) that is typically on the higher end (BMI ≥30) when analyzing hospitalized patients with severe COVID-19, 15 mL of IV IP was determined as a reasonable starting dose for one treatment arm while 10 mL of IV IP was determined as a reasonable lower end dose for a second treatment arm.

** Note: only IP lots # 441-1905-E5 and* P-441-2004-C5 *will be used in the clinical study.*

4.3.2 Rationale for Dosing in Special Populations:

Pediatric population: The safety and efficacy of the IP for adults with COVID-19 have not been assessed yet via RCT. Current use of the IP for children with severe COVID-19 is not under consideration.

Pregnancy: The safety and efficacy of the IP for adults with COVID-19 have not been assessed yet via RCT. Current use of the IP for pregnant patients is not under consideration. Of note, in the proof-of-concept study, the IP was infused safely in one post-partum patient. Further investigation, including any risk to the fetus, is required before use of the IP as treatment for severe COVID-19 in pregnant patients.

Renal impairment: It is currently not known if dosage adjustment is needed in patients with renal impairment. Preclinical studies of pharmacokinetics of exosomes reveal that clearance is via the reticuloendothelial system, specifically the mononuclear phagocyte system (i.e., monocytes and macrophages) in the liver and the spleen, rather than renal clearance, suggesting that dose adjustment in this study for renal impairment is most likely unnecessary.[86, 87]

Hepatic impairment: It is not currently known if dosage adjustment is needed in patients with hepatic impairment, especially if clearance of the IP can also be accomplished by monocytes and macrophages localized to the lungs, spleen, and lymph nodes. Clearance of the IP likely also depends on the total dose as well as the biodistribution to sites of injury.[88]

4.3.3 Rationale for Redosing:

Pharmacokinetics studies of the IP in patients with severe COVID-19 are ill advised as it would require not only fluorescent tagging of surface proteins of the IP, but also serial MRI analysis of the biodistribution of immunofluorescence. Sending a patient with severe COVID-19 for serial MRI scans is neither practical nor safe, especially considering the progressing hypoxemia in many of these patients. Therefore, our previous proof-of-concept study focused on exploratory endpoints in order to observe a time-dependent effect—in this study, 23 out of 24 patients showed a favorable trend in biomarkers within 48-72 hours, with the peak of the favorable biomarkers and PaO_2/FiO_2 occurring closer to 72 hours following the first infusion of the IP. Seventeen (17) out of the 23 initial responders had a sustained response following a single dose of the IP whereas the remaining 6 appeared to have "lost the effect" after 72-96 hours, suggesting that a second dose at day = 4 may have been helpful in improving clinical outcomes. Therefore, our RCT study will involve a second dose on day 4 for patients who have not recovered when the second dose is due, where recovery is defined by return of oxygenation saturation (SpO2) ≥ 93% on room air (or PaO2/FiO2 ≥ 300 mmHg). If patient has chronic lung disease, recovery is defined as pre-COVID-19 SpO2 and O2 support.

4.4 End of Study Definition

A subject is considered to have completed the study if he or she has completed all the phases of the study shown the Schedule of Activities (Section 1.3) or if the subject has expired.

5. STUDY POPULATION

5.1 Inclusion Criteria

Eligibility for study enrollment includes meeting all of the following criteria:

1. Provision of signed and dated informed consent form (either by the individual or by the individual's healthcare proxy).
2. Stated willingness to comply with all study procedures and availability for the duration of the study
3. Male or female aged 18-85.
4. COVID-19 positive as defined by positive Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) SARS-CoV-2.
5. Moderate to severe ARDS as defined by modified Berlin definition, * which includes timing within 1 week of known clinical insult or new or worsening respiratory symptoms; bilateral opacities not fully explained by effusions, or lung collapse; respiratory failure not fully explained by cardiac failure or fluid overload; $PaO_2/FiO_2 \leq 200$ mm Hg.
6. Hypoxia requiring noninvasive oxygen support such as Nasal Cannula (NC), Nonrebreather (NRB), Bilevel Positive Airway Pressure (BIPAP), Continuous Positive Airway Pressure (CPAP), high flow nasal cannula oxygen (HFNC O2) or mechanical ventilation (MV) despite initiating standard of care.
7. If the candidate is either a male or female of reproductive potential, he or she must agree to use of double barrier method of highly effective birth control contraception such as condoms with oral contraceptive pill or choose to remain abstinent if already practicing abstinence during the screening period. The required duration of usage of double barrier method OR maintenance of abstinence must include the time from the beginning of the screening period until 90 days following the last dose of the study treatment.

*Modified Berlin definition used in this study is the full Berlin definition, albeit without the PEEP specification, which implies mechanical ventilation. See last paragraph of study rationale for reasoning (Section 2.1).

**To ensure flexible adaptation of products approved by the FDA for the treatment of severe COVID-19, standard of care is defined as the NIH Current COVID-19 Treatment Guidelines.[53]

5.2 Exclusion Criteria

Exclusion from study enrollment includes meeting one or more of the following criteria:

1. Vulnerable populations such as pregnant patients, children, individuals with severe physical or mental disabilities who cannot provide meaningful consent.
2. Active malignancy requiring treatment within the last five years.
3. Major physical trauma in the last 5 days, including motor vehicle accidents, assaults, mechanical falls with sequalae of significant bleeding or craniofacial bruising, and surgeries.
4. Active tuberculosis or cystic fibrosis.
5. Severe chronic respiratory disease including chronic obstructive pulmonary disease or pulmonary fibrosis requiring home oxygen > 5L/min.
6. Use of extracorporeal membrane oxygenation (ECMO) during the current hospitalization.
7. Pre-existing pulmonary hypertension.
8. Severe pre-existing hepatic impairment (presence of cirrhosis, liver function tests (LFTs) ≥ 6x baseline, INR ≥ 2.0).
9. Pre-existing Chronic Kidney Disease (CKD) stage IIIb or End Stage Renal Disease (ESRD) prior to onset of COVID-19 (stage I, II, and IIIa are acceptable)
10. Irreversible coagulopathy (e.g., frequently occluded vascular access despite anticoagulation, precipitous platelet drops concurrent with end-organ damage suggesting consumptive process) or irreversible bleeding disorder (e.g., frequent bleeding from vascular access, endotracheal tubes, and foley).
11. Pneumonia clearly attributable to a non-COVID-19 related process, including aspiration pneumonia or pneumonia that is exclusively bacterial, or originating from a diagnosed alternative virus (e.g., influenza).
12. Patients who are not full code.
13. Endotracheal intubation duration ≤ 24 hours.
14. Moribund—expected survival < 24 hours.
15. Severe metabolic disturbances on presentation (e.g., ketoacidosis, pH < 7.3)

5.3 Screen Failures

Screen failures are defined as participants who consent to participate in the clinical trial but are not subsequently randomized to the study intervention or entered in the study.

5.4 Strategies for Recruitment and Retention

1. Anticipated number of research sites in the US = 2-15 inpatient hospital settings.
2. Potential participants will be identified in the emergency room, floor, stepdown, and intensive care units and approached by one of the study investigators or resident physicians who have had protocol training on the inclusion and exclusion criteria.
3. Recruitment strategy is primarily based on direct discussion with the patient and/or healthcare proxy.
4. The recruiting physician's ability to establish transparency and direct communication with the participant or healthcare proxy is one of the major factors in improving recruitment and retaining participants, particularly historically under-represented populations.
5. Retention can also be improved via telephone and email reminder of follow-up calls at Days 15 and 29 and obtaining multiple contact numbers for the participant prior to hospital discharge.

6. STUDY INTERVENTION

6.1 Study Intervention(s) Administration

6.1.1 Study Intervention Description

A central randomization service will randomize each participant to one of the following:

Treatment Arm 1: PLACEBO: 100 mL of 0.9% sodium chloride IV on Days 1 and 4.

Treatment Arm 2: IP (ExoFlo) 10 ml in 90 mL of 0.9% sodium chloride IV on Days 1 and 4.

Treatment Arm 3: IP (ExoFlo) 15 ml in 85 mL of 0.9% sodium chloride IV on Days 1 and 4.

6.1.2 Dosing and Administration

The study intervention will be administered intravenously over 60 minutes on Days 1 and 4.

6.1.3 Monitoring of study intervention

During the intervention, the patient will be on continuous 3-lead cardiac monitoring and continuous pulse oximetry measuring heart rate (HR) and SpO_2 with mean arterial pressure (MAP) obtained via either intermittent noninvasive blood pressure (NIBP) or continuously via an arterial line. Baseline vitals, including HR, MAP, respiratory rate (RR), and SpO_2 will be obtained and recorded 5 minutes prior to the infusion of IP/Placebo and every 5 minutes for the first 15 minutes of the infusion and every 15 minutes thereafter for the remainder of the 60-minute total infusion of IP and every hour thereafter for a total of 4 hours following the infusion, and every 3 hours thereafter for the first 24 hours. The temperature will be obtained and recorded 5 minutes prior to the infusion of the IP/Placebo and repeated every 15 minutes over the course of the infusion. During the infusion, measurements +/- 2 minutes of specified times for q5min vitals and +/- 5 minutes of specified times for q15min vitals are considered permissible; following the infusion, vital sign measurements are permissible +/-15 minutes of the specified times.

6.1.4 Detailed Treatment Plan in the Event of an Infusion Reaction

If the patient demonstrates signs and symptoms including but not limited to hypotension, tachycardia, fever or temperature increase ≥1 degree Celsius (1°C), chills, nausea, shortness of breath, or urticaria, stop the infusion.

A. Respiratory distress is a sign of significant clinical instability, consistent with severe infusion reactions such as anaphylaxis or septic infusion reaction from bacterial contamination. Direct a staff member to call for the hospital code team. Be ready to start Advanced Cardiac Life Support (ACLS).

B. If appropriate, ACLS should be implemented as per standard procedures.

C. If the patient is responsive, remain vigilant and focus on airway, breathing, and circulation. Cycle the NIBP every 1-5 minutes if the patient does not have an arterial line.

1. RESPIRATORY: If there is respiratory distress in a non-intubated patient, ask another staff member to call for the anesthesiologist on-call. Make sure that the patient is at least on a NRB. If the patient is hypoventilating with stridor, assist ventilation with AmbuBag connected to oxygen and ask respiratory technician to set-up noninvasive positive pressure ventilation (NIPPV). If the patient shows rapid clinical deterioration and demonstrates complete respiratory failure, ask the anesthesiologist to help with endotracheal intubation.

2. If anaphylaxis is suspected, administer IM Epinephrine into the lateral thigh 1:10000 IM. May add IV Hydrocortisone 4 mg/kg and Albuterol nebulizer. When patients have severe bronchospasms, rescue maneuvers may be inadequate and clinical scenario reassessment is always needed.

3. CIRCULATION: If the patient is hypotensive, bolus IV normal saline 250-500 mL and can consider IM Adrenaline to be administered into the lateral thigh.

D. The nurse or other responsible provider should notify the Principal Investigator (PI) immediately. Tryptase level will be sent to evaluate for possible anaphylactic reaction per institutional standard of care.

E. Patient should be on continuous EKG and pulse oximetry. If there is no arterial line, the NIBP should be cycled every 5 minutes.

F. Patients may be given acetaminophen 1000 mg for temperature and diphenhydramine 25 mg oral or IV for urticaria.

If the patient does not demonstrate signs and symptoms of a severe transfusion reaction and only has minor symptoms such as temperature elevation less than 1°C and urticaria, the study physician should remain vigilant as infusion reactions can be delayed. The above treatment plan for an infusion reaction is a guide that can be followed to the greatest extent possible; however, it is

understood that hemodynamic fluctuations and other clinical events may necessitate deviation from this standard regimen. Doses and times as detailed in the current protocol are suggested guidelines to be followed by the physician and team caring for the subject. The actual doses and times administered are at the discretion of the physician, based on the clinical status of the subject and will not be considered protocol deviations if not given exactly as described in the protocol.

6.2 Preparation/Handling/Storage/Accountability

6.2.1 Acquisition and accountability

After written informed consent is completed by the participant or the participant's healthcare proxy, the co-investigator will contact the central randomization service for randomized allocation to the study intervention on Day 1. The same randomization code will be provided for the study intervention and used for ordering of the study intervention—the same identifier will be used for Days 1 and 4. Once the study intervention order is received, the hospital pharmacist will call the Interactive Response Technology (IRT), reporting the randomization code and receiving in return the unblinded allocation. The unblinded hospital pharmacist will prepare the study intervention, labeling with the randomization code and without the study identity. The blinded study intervention will be then be delivered via the hospital transport system to the participant's nurse, who will perform a series of safety checks including directly confirmation with the hospital site principal investigator prior to the intravenous administration.

6.2.2 Formulation, Appearance, Packaging, and Labeling

The IP, the formulation of extracellular vesicles isolated from human donor bone marrow using proprietary technology from Direct Biologics LLC and processed under cGMP standards, is currently available in glass vials containing 1 mL, 2 mL or 5 mL frozen liquid product. The IP is aseptically processed. The sterile biologic product is sealed inside the glass vial. Both the glass vial and product box are labeled with the manufacturer's lot number, product volume, and expiration date (month/day/year). The IP product box includes instructions for use, tissue traceability postcard (pre-addressed & postage paid) and 6 chart stickers. The product box is sealed with Direct Biologics, LLC, logo sticker and labeled with the manufacturer's lot number, the manufacturer's catalog number, product volume, and expiration date (month/day/year); in addition

to the contact information for Direct Biologics, LLC, the external packaging exhibits the widely used symbols for single use only, limited to use by a physician, temperature limits (≤ -40°C) to which the product can be safely stored prior to immediate use. At room temperature, the IP thaws within 5 minutes; once properly thawed, The IP is a clear liquid that is visually identical to normal saline, which is used as placebo in this study.

6.2.3 Product Storage and Stability

The IP is stored ≤ -40°C in ultra-low temperature freezers for up to 5 years at designated Direct Biologics, LLC, storage facilities. It can be stored between -20°C and -40°C for up to 6 months or at room temperature for 6 hours. The product must be stored while it is still frozen and once thawed, the IP is NOT recommended for sterilization or refreezing for future use. The research site directors and hospital pharmacists are instructed to refuse the product and inform Direct Biologics, LLC, if the dry ice has sublimated. The product boxes will be kept sealed and stored within the pharmacy medication freezer between - 20°C and - 40°C no more than 6 months prior to administration within 2-3 weeks of shipping. The IP manual may be referred to for further details.

6.2.4 Study Treatment Preparation

The pharmacist will check the expiration date, discrepancies in the label information, intact seal on the product box, and intact seal on the glass vial. The pharmacist will use standard practices for handling and disposal of human tissue. While the outer packaging (product box and HDPE [plastic] vial card) is not sterile, the biologic product inside the glass vial is considered sterile. The hospital pharmacist will let the IP thaw completely before drawing the IP out of the vial with a sterile needle into a syringe. For treatment arm 2 study intervention, 10 mL of 0.9% sodium chloride will be withdrawn sterilely from a 100 mL 0.9% sodium chloride and discarded; IP from two 5 mL glass vials will be withdrawn (10 mL total) and sterilely injected into and mixed with 90 mL of 0.9% sodium chloride. For treatment arm 3 study intervention, 15 mL of 0.9% sodium chloride will be withdrawn sterilely from a 100 mL 0.9% sodium chloride and wasted; IP from three 5 mL glass vials will be withdrawn (15 mL total) and sterilely injected into and mixed with 85 mL of 0.9% sodium chloride. For placebo, 100 ml of 0.9% sodium chloride will be used.

After receiving the study treatment, the participant's nurse will perform the following checks:

(1) Appropriate venous access,

(2) The patient's name, DOB, MRN

(3) The study intervention randomization code on the sticker label matching the randomization code on the IP order,

(4) Expiration time for use has not passed,

(5) Direct verbal confirmation with the attending physician in case there are any significant that would change study eligibility.

Then the study treatment will be administered intravenously over 60 minutes on an IV pump.

6.3 Measures to Minimize Bias: Randomization and Blinding

This research study involves blinding of both participant and research investigators as well as randomized and concealed allocation of each participant into one of the three treatment arms by Interactive Response Technology (IRT). Stratified block randomization will be used to minimize bias and equally distribute the confounding variable between the treatment groups following stratification by research site. Randomization code will be provided by IRT. The hospital pharmacists will not be blinded to the intervention as they must prepare the correct allocated study intervention; therefore, the hospital pharmacists must be provided random treatment assignment code directly from the IRT and are not allowed to reveal the study intervention identity until the study is completed and the database is locked.

Planned Unblinding will occur after the data is fully collected, the source is verified, and the database is locked. Planned early unblinding due to the IP's superior efficacy may occur following day = 7 for the final patient randomized. Organizational model for planned early and final unblinding are included in this protocol.

Unplanned Unblinding: Emergency unblinding may be required to protect the participant's safety if knowing the participant's treatment assignment would affect immediate medical management. For emergency unblinding, the investigator may call:

Phone: 1-888-ASK-BIO2 Option 2, Then Option 1 (Email: support@bioclinica.com)

The investigator must then inform both the sponsor and the DSMB of the emergency unblinding.

Early unblinding of a participant's treatment assignment may occur for non-urgent reasons. Contact the medical monitor for any non-urgent unblinding. The non-urgent cases will be reviewed by the DSMB on a case-by-case basis and the decision to unblind will be rendered on a need-to-know basis with the fewest number of people informed as possible. In the event of non-urgent early unblinding of participant for medical/safety reasons, a case report form (CRF) capturing the self-reported and subjective data must be entered into the study database and the event of unblinding is reported to both the sponsor and the DSMB.

All SAEs will be reported to the safety CRO and the data will be reviewed by the DSMB periodically. Intentional and unintentional breaking of the blind, such as accidental reveal of the study intervention by the hospital pharmacist both directly or in the patient chart, will be reported to and recorded by the DSMB, who will determine the necessity of also notifying the PI and/or the study sponsor. Inadvertent blinding by laboratory measures is reduced by the lack of standard direct or indirect detection assay for the therapeutic intervention.

6.4 Staggering Protocol

BATCH 1: Nine patients will be enrolled across sites*. There will be a delay of 24 hours prior to the next enrollment batch following the redosing of the randomly assigned study treatment to allow for assessment of any redosing-related adverse reaction.

BATCH 2: Approximately fifty-one additional patients will be enrolled across sites—the IRT is alerted specifically following Day 7 for the 60th patient randomized overall into the study. There will be a delay of 24 hours.

BATCH 3: The remainder of the patients will be enrolled across sites*.

**Patients are not necessarily enrolled on the same day within the same batch.*

6.5 Study Intervention Compliance

The site principal investigator is responsible for ensuring all study staff are appropriately trained:

- Residents and medical attendings on obtaining informed consent if delegated to do so.
- Unblinded pharmacist on the proper preparation of the study infusions.
- Nursing staff on safety checks and correct monitoring during and after the study infusions.

The staff will be trained on the protocol, IP manual, and the IRT system. The site PI will be responsible for either in-person or electronic daily rounds with the clinical team—evaluating for (1) any immediate infusion related adverse reaction (< 4 hours), (2) SAE, (3) any other meaningful events or observations, and (4) any questions or delinquencies with not adhering to the schedule of activities.

6.6 Concomitant Medications

Concomitant medications are permitted if they are considered standard of care according to the updated NIH guidelines for COVID-19, which may be accessed via the following link:

https://files.covid19treatmentguidelines.nih.gov/guidelines/covid19treatmentguidelines.pdf

With regard to concomitant medications taken during the study, we will conduct analyses to compare usage between treatment groups and to assess the potential effects of their use on the primary endpoint. Concomitant use of investigational agents for COVID-19 which have not been established as standard of care will need to be discontinued for 24 hours prior to receiving the first dose of the study administration.

6.7 Rescue Medications for Infusion Reaction

Although no targeted “rescue medication” or antidote exists for the therapeutic intervention, the study site may administer generic rescue medications when indicated that will be obtained locally and will consist of standard management for infusion reactions, including but not limited to: (1) Fever or suspected hypersensitivity reaction: Acetaminophen 1000 mg PO or IV for fever or temperature increase ≥ 1°C.

(2) Suspected hypersensitivity reaction: diphenhydramine 25 to 50 mg PO or IV for acute reaction. The date and time of rescue medication administration as well as the name and dosage regimen of the rescue medication must be recorded in the case report form.

7. STUDY INTERVENTION & PARTICIPANT DISCONTINUATION

7.1 Discontinuation of Study Intervention:

Discontinuation or pausing of the study intervention may occur for many reasons, including physiologic changes during the study intervention suggesting potential infusion reaction, unfavorable physiologic changes within 24 hours following the study intervention, stopping rules, voluntary patient withdrawal. Unique and unanticipated scenarios will be evaluated by the investigators and the DSMB on a case-by-case basis.

7.1.1 Clinical Criteria* for Slowing or Suspension of Study Intervention:

The following criteria is included for guidance rather than as strict clinical algorithms. The guiding principle is that the clinical team should be assessing for signs and symptoms consistent with an immunologic reaction following initiation of a study intervention:

A) Criteria* for slowing product administration (by reducing flow rate by at least 50%) include itchiness, elevation in temperature > 1°C, significant changes in heart rate and MAP consistent with significant vasodilation possibly leading to distributive shock.

B) Criteria* for suspending product administration include criteria for slowing product administration plus additional symptoms of acute infusion; rigors, nausea, abdominal pain, chills, worsening dyspnea or hypoxia, hypotension, tachycardia, facial swelling, generalized swelling, profuse bleeding from vascular access sites, significant changes in heart rate and MAP consistent with distributive shock.

7.1.2 Clinical Criteria for Not Receiving the Second Study Intervention:

Patient should not receive the second study intervention if the oxygenation improves to a level of recovery, as defined by $SpO_2 \geq 93\%$ on room air (or P/F ratio ≥ 300 mmHg) or pre-COVID-19 baseline O_2 support for ≥ 4 hours. Patient should not receive the next study intervention if patient has developed SUSAR to the investigational product within 4 hours of intravenous administration.

7.1.3 Stopping Rules:

Sequential stopping rules for safety will be adopted by the DSMB. Because SAEs are expected in the critically ill population of study, the safety data will be examined intermittently for potential disproportionate incidence of SAEs in one treatment arm versus another. Based on the initial first clinical study of ExoFlo in COVID-19 subjects, mortality rate was reported to be 16% (4 of 24 patients) and there were no reports of serious adverse events attributable to the investigational product. The DSMB will be conducting interval review of cumulative unblinded data to determine if excessive risks/adverse events occur in the study and if stopping rules should be applied in accordance with the DSMB charter. In addition, the DSMB will continuously receive SAE reports for information.

In each data review cycle, the DSMB biostatistician will compare the patient-level incidence of Grade 3 adverse events of special interests and of Grade 4 adverse events of special interests within 72 hours of the study administration between the treatment group (i.e., the two active arms pooled) and the control group. The study will be stopped if either of the following criteria is met: (A) The difference in incidence of Grade 3 related adverse events of special interests within 72 hours of the study administration in the treatment group as compared to the control group is statistically significantly greater than 0.30. (B) The difference in incidence of related Grade 4 adverse events of special interests within 72 hours of the study administration as compared to the control group is statistically significantly greater than 0.20. Tests for statistical significance will be performed using one-sided Wald tests conducted at the 0.10 level of significance. If the stopping rules are met, enrollment and treatment will be paused by the DSMB. The DSMB will convene and review at the intervals based on the data available with one planned safety and interim analysis following Day 7 after the 60th patient (50% of N) randomized overall into the trial.

<u>Adverse Events of Special Interests:</u>

<u>Grade 5 events</u>

Any deaths (Grade 5 events) unless it is unequivocally not due to treatment.

Grade 4 events

Generally, life-threatening; urgent intervention required for the following events:

- Allergic reaction- urgent intervention required
- Anaphylaxis/Anaphylactic reaction
- Alanine aminotransferase/ or alkaline phosphatase/ or aspartate aminotransferase increase ≥ 20.0 x ULN if baseline was normal or ≥ 20 times baseline if baseline was abnormal)
- Creatinine increased ≥ 6.0 x ULN
- UOP decreased: anuria ≤ 240 ml in 24 hours
- ARDS—intubation or urgent intervention required
- Hypoxia—airway compromise, urgent intubation or trach required
- Hypotension- life threatening; urgent intervention required
- Thromboembolic event—hemodynamic or neurologic instability requiring urgent intervention
- Vasculitis—evidence of peripheral or visceral ischemia; urgent intervention indicated

Grade 3 events

Allergic reaction—bronchospasm; intravenous medications indicated Anaphylaxis—symptomatic bronchospasms with or without urticaria

Alanine aminotransferase or alkaline phosphatase or aspartate aminotransferase increase – > 5.0 – 20.0 x ULN if baseline was normal or > 5 up to 20 times baseline if baseline was abnormal

Creatinine increased: > 3.0 x baseline or > 3.0-6.0 x ULN

UOP decreased—oliguria < 80 ml in 8 hour

Hypotension—medical intervention indicated but not immediately life threatening

Thromboembolic event—medical intervention indicated but not immediately life threatening

Vasculitis—severe symptoms; medical intervention, i.e. steroids, indicated but not immediately life threatening

7.1.4 Discontinuation of the Study Intervention:

Discontinuation of the study intervention does not mean discontinuation from the study. In this modified intention-to-treat analysis, all participants who were randomized and received any portion of the study intervention will be followed according to the schedule of activities and will be followed for primary, secondary, and exploratory end points until day 61. A participant who was randomized but then opted out either due to personal choice or did not receive any study intervention will still be followed for primary and secondary endpoints. A dedicated CRF will be used for all instances in which a study intervention is discontinued; the CRF will record the date and the specific underlying reasons for discontinuation/withdrawal.

7.2 Participant Discontinuation/Withdrawal from the Study

Participants are free to withdraw from the study at any time upon request. An investigator may discontinue or withdraw a participant from the study for the following reasons:

- Pregnancy
- If the participant meets an exclusion criterion (either newly developed or not previously recognized) that precludes further study participation.
- If any clinical AE, laboratory abnormality, or other medical condition or situation occurs such that continued participation in the study would not be in the best interest of the participant.
- Participant unable to receive the study intervention for 7 days following enrollment due to clinical instability.

The reason for participant discontinuation or withdrawal from the study will be recorded on the CRF. Subjects who sign the informed consent form and are randomized but do not receive the study intervention may be refilled. Subjects who sign the informed consent form and are randomized and receive any portion of the study intervention, and subsequently withdraw, or are withdrawn or discontinued from the study, will not be refilled.

7.3 Lost to Follow-Up

A participant will not be lost to follow-up if still hospitalized at Days 29 and 61. However, once the participant is discharged, he or she will be considered lost to follow-up if he or she fails to

respond to at least three telephone attempts on day 29 and 61 and at least three telephone attempts on day 29 and 61. The following actions must be taken if a participant fails to return the follow-up call:

- Before a participant is deemed lost to follow-up, the investigator or designee will make every effort to regain contact with the participant (where possible, at least 3 telephone calls and, if necessary, a certified letter to the participant's last known mailing address or local equivalent methods). These contact attempts should be documented in the participant's medical record or study file.
- Should the participant continue to be unreachable, he or she will be considered to have withdrawn from the study with a primary reason of lost to follow-up.

8. STUDY ASSESSMENTS AND PROCEDURES

8.1 Study Procedures By Visit

SCREENING VISIT (Day 0 | Can be Day 1)

Basic Evaluation: Patient's age, gender, race/ethnicity, height and weight (which will be used to calculate BMI, should be obtained following hospital admission but does not have to be repeated to be Days 0 or 1), medical history, concomitant medications, days symptomatic of COVID-19 prior to admission, date of admission (which will be used to calculate number of days from admission to the first study treatment), date of intubation if the patient is currently intubated with an endotracheal tube (which will be used to calculate number of days of intubation—NOTE, patient cannot be intubated more than > 24 hours for enrollment), physical examination, serum pregnancy test for women of childbearing potential. EKG should be obtained following hospital admission but does not have to be repeated to be either Day 0 or 1.

Respiratory Evaluation: Qualitative SARS-CoV-2 RT PCR, prone positioning (if used and if so, the frequency—both ventilated and nonventilated patients may be intermittently "proning" per hospital site protocol), PaO2/FiO2 (see appendix 11.1 for imputation if no ABG or arterial line—please note that the use of ABG or insertion of arterial line is dependent on clinical need only and should not be performed in the sole interest of the clinical trial), radiographic imaging of the chest (CXR or CT chest), and oxygen support (i.e. RA vs NC vs FM vs NRB vs BIPAP or CPAP vs HFNC O2 vs MV; FiO2 setting should be recorded for BIPAP, CPAP, HFNC, and MV; L/min of O2 should be recorded for NC and FM; PEEP should be recorded for MV, BIPAP, or CPAP.

Labs: BMP, CBC with differential, LFTs, PT/INR, PTT; CRP, D-dimer, Ferritin will be collected. Interleukin-6 and tumor necrosis factor alpha do not have to be collected prior to enrollment.

Microbiology: Urinalysis, urine culture, blood culture (2 sets), and sputum culture should be collected following current hospital admission—they do not have to be repeated to be on Day 0 or 1.

Rule out tests: Mycoplasma IgM, QuantiFERON Gold, Legionella Antigen, Strep. Pneumoniae Antigen, Influenza A/B PCR should be obtained following current hospital admission—they do not have to be repeated to be on Day 0 or 1.

Adverse event review: Review of adverse events accumulated over the course of hospitalization thus far should be performed prior to study enrollment.

Study Evaluation and Consent: Participant is considered for the study if patient meets eligibility criteria. Informed consent is obtained if patient or proxy agrees following description of the study and the informed consent process. The informed consent form is collected, and the patient is considered enrolled.

VISIT DAY 1

Study Re-Evaluation and Randomization & IV Study infusion: Participant's eligibility criteria will be re-evaluated to make sure there are no significant interim changes that would disqualify him or her from the study. Women of childbearing potential should have a urine dipstick obtained 4 hours prior to the first dose of study treatment if it has been more than 24 hours since the serum pregnancy test was collected. If patient remains as an eligible participant for the trial, the study investigator calls the Interactive Response Technology. The hospital pharmacist(s) will be notified of the study infusion order and can call the Interactive Response Technology with the randomization code in order to receive the study infusion identity and prepare the infusion appropriately. The study infusion is labeled with the randomization code but not with the infusion identity; the nurse administers the study infusion over the course of 60 minutes. Monitoring is already specified in section 6.1.3.

Basic Evaluation, **Respiratory Evaluation**, **Standard Labs**, **Acute Phase Reactants**, **Metrics**, **Microbiology (if indicated)** are to be performed according to the Schedule of Activities (Section 1.3).

Flow Cytometry Labs: T-lymphocyte panel ($CD3^+$, $CD4^+$, $CD8^+$); NK cell count (defined as $CD3^-$ and $CD56^+$ subset of a light scatter characterization of lymphocytes. These labs should be drawn within 4-6 hours prior to the first study infusion.

Adverse Event Review: Interim adverse events should be reviewed and recorded. SAE(s) if any are continuously monitored and reported within 24 hours of knowledge.

VISIT DAY 4

Study Re-Evaluation & IV Study Infusion: Participant's eligibility criteria will be re-evaluated primarily for placement on ECMO and change in code status—these are the only two interim changes that would disqualify him or her from the second study administration aside from: Patient should not receive the second study infusion if there was a SUSAR within 4 hours following the first dose. Patient also should not receive the second study infusion if he or she has $SpO_2 \geq 93\%$ on RA or returned to baseline SpO_2 and O_2 requirement if patient has chronic lung disease. Otherwise, the patient may receive the second study infusion, which is the same identity and dosing as the first study infusion, also administered over 60 minutes.

Basic Evaluation, **Respiratory Evaluation**, **Standard Labs**, **Acute Phase Reactants**, **Metrics**, **Microbiology (if indicated)** are to be performed according to Schedule of Activities.

Flow cytometry labs: T-lymphocyte panel ($CD3^+$, $CD4^+$, $CD8^+$); NK cell count (defined as $CD3^-$ and $CD56^+$ subset of a light scatter characterization of lymphocytes. These labs should be drawn within 4-6 hours prior to the study infusion.

Adverse event review: Interim adverse events should be reviewed and recorded. SAE(s) if any are continuously monitored and reported within 24 hours of knowledge.

VISIT DAY 7, 10, 15, 29, 61

Basic Evaluation, **Respiratory Evaluation**, **Standard Labs**, **Acute Phase Reactants**, **Metrics**, **Microbiology (if indicated)** are to be performed according to Schedule of Activities. Regarding metrics, SOFA score (Days 1, 15, and 29) will only be tabulated on inpatients and the nonvisual portion of EQ-5D-5L will be collected over the phone on Days 29 and 61 on outpatients.

Flow cytometry labs: T-lymphocyte panel ($CD3^+$, $CD4^+$, $CD8^+$); NK cell count (defined as $CD3^-$ and $CD56^+$ subset of a light scatter characterization of lymphocytes). These labs should be drawn within 4-6 hours prior to the study infusion and performed according to Schedule of activities Section 1.3.

Adverse event review: Any adverse events /serious adverse event should be reviewed and recorded per Schedule of activities and requirements for reporting SAEs.

8.2 Efficacy Assessments

Evaluation of the patient's charts will include baseline demographics including age, gender, race/ethnicity, BMI. There will be detailed review of the medical comorbidities, admission documentation, noting days of illness prior to admission, in addition to past medical history and presenting symptoms, concomitant medications and therapies, daily progress note, and the most recent progress note in the chart, noting both the subjective interval history and critical events in addition to the highlights of the vitals and labs. The day and time of the study intervention administration will be verified via the MAR; from this day and time, it will be calculated how many days following the first treatment the patient was hospitalized prior to treatment and/or how many the days the patient was intubated prior to the treatment. The date and time of other events such as patient expiration, patient's intubation or extubation, patient's recovery as defined by SpO_2 $\geq$ 93% on room air for longer than 4 hours, and hospital discharge will be tabulated. Improvement in P/F ratio from pre-infusion baseline (Day 0) to Day 7 will be analyzed as a primary endpoint in addition to time to recovery, incidence of SAE, and all-cause will be analyzed as secondary endpoints. The exploratory endpoints will be trended according to the Schedule of Activities in Section 1.3.

8.3 Safety and Data Safety Monitoring Board

Stopping rules are established in this study to determine if the serious or severe adverse events have met a pre-defined statistical parameter. If stopping rules are met, the enrollment will halt for the DSMB to review and adjudicate the cause of the AE and to determine if study should be modified for continuation.

8.4 Adverse Events and Serious Adverse Events

8.4.1 Definition of Adverse Events

Any medical condition that is present at the time that the participant is screened will be considered as baseline medical history and not reported as an AE. However, if the study participant's condition deteriorates at any time during the study after signing the informed consent, it will be recorded as an AE.

An AE is any untoward medical occurrence in a subject or clinical investigation subject administered a pharmaceutical product and which does not necessarily have to have a causal relationship with the intervention. An AE can therefore be:

- Any unfavorable and unintended sign, symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not considered related to the product
- Any new disease or exacerbation of a pre-existing disease (a worsening in the nature, frequency, or severity of a medical condition)
- Recurrence of medical conditions that are not present at baseline
- Any changes in laboratory value or other clinical tests (e.g., AST, ALT, CPK) that are associated with symptoms, or that lead to a change in study treatment or additional concomitant treatment, or that result in discontinuation from the study drug

8.4.2 Definition of Serious Adverse Events

An AE or suspected adverse reaction is considered "serious" if, in the view of either the investigator or sponsor, it results in any of the following outcomes: death, a life-threatening AE, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or a congenital anomaly/birth defect. Important medical events that may not result in death, may be life-threatening, or require hospitalization, may be considered serious when, based upon appropriate medical judgment, they jeopardize the participant, possibly requiring medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

A serious adverse event (SAE) is any untoward medical occurrence that meets one of the following criteria:

- Fatal (results in the outcome death)

- Life-threatening*
- Requires hospitalization or prolongation of existing hospitalization. (Hospitalizations for elective medical or surgical procedures or treatments planned before the signing of informed consent in the study are not SAEs by this criterion).
- Results in persistent or significant disability/incapacity or substantial disruption of the subject's ability to conduct normal life functions
- Is a congenital anomaly/birth defect in a child or fetus of a subject who has been exposed to the molecule or study treatment regimen before conception or during pregnancy.
- Is medically significant meaning the AE did not meet any of the above criteria but could have jeopardized the subject and might have required medical or surgical intervention to prevent one of the outcomes listed above or involves suspected transmission via a medicinal product of an infectious agent.

*Note: The term "life-threatening" in the definition of "serious" refers to an event in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.

8.4.3 Follow-Up Reporting

As further information regarding the SAE becomes available, follow-up information should be provided and emailed to the Sponsor (or designee), including any records during hospitalization and tests performed that were necessary to evaluate the SAE.

All SAEs that have not resolved by the end of study, or that have not resolved upon discontinuation of the subject's participation in the study, must be followed until any of the following occurs:

- The event resolves
- The event stabilizes
- The event returns to baseline, if baseline value/status is available

- The event can be attributed to agents other than the study drug or to factors unrelated to study conduct

It becomes unlikely that any additional information can be obtained (subject or health care practitioner refusal to provide additional information, lost to follow-up after demonstration of due diligence with follow-up efforts).

8.4.4 Definition of Adverse Events of Special Interest (AESI)

As per Council for International Organizations of Medical Sciences (CIOMS) VI, an adverse event of special interest (serious or non-serious) is one of scientific and medical concern specific to the sponsor's product or program, for which ongoing monitoring and rapid communication by the investigator to the sponsor may be appropriate. Such events may require further investigation in order to characterize and understand them. Depending on the nature of the event, rapid communication by the trial sponsor to other parties may also be needed. In the case of ExoFlo, there are presently no anticipated AESIs. However, as with AEs, possible AESIs will be reviewed and assessed individually and in aggregate on a continuous basis to identify and track any emerging safety signals. Suspicion for AESIs could be triggered by non-serious events that may be prodromes of serious medical conditions and will be evaluated and reported on a case-by-case basis. See Stopping rule Section 7.1.3.

8.4.5 Classification of Severity of Adverse Events and Relationship to Study Treatment

All clinical Aes encountered during the study will be reported on the AE page of the CRF. Intensity of Aes will be graded based on the CTCAE, Version 5.0 and reported in detail as indicated on the CRF. For any Aes not found in the CTCAE, the following descriptions of intensity grading can be used:

Mild – Events require minimal or no treatment and do not interfere with the participant's daily activities.

Moderate – Events result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.

Severe – Events interrupt a participant's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually potentially life-threatening or incapacitating.

All Aes must have their relationship to study intervention assessed by the clinician who examines and evaluates the participant based on temporal relationship and his/her clinical judgment. The degree of certainty about causality will be graded using the categories below.

Definitely Related – There is clear evidence to suggest a causal relationship, and other possible contributing factors can be ruled out. The clinical event, including an abnormal laboratory test result, occurs in a plausible time relationship to study intervention administration and cannot be explained by concurrent disease or other drugs or chemicals. The response to withdrawal of the study intervention should be clinically plausible. The event must be pharmacologically or phenomenologically definitive.

Probably Related – There is evidence to suggest a causal relationship, and the influence of other factors is unlikely. The clinical event, including an abnormal laboratory test result, occurs within a reasonable time after administration of the study intervention, is unlikely to be attributed to concurrent disease or other drugs or chemicals, and follows a clinically reasonable response on withdrawal.

Possibly Related – There is some evidence to suggest a causal relationship (e.g., the event occurred within a reasonable time after administration of the trial medication). However, other factors may have contributed to the event (e.g., the participant's clinical condition, other concomitant events). Although an AE may rate only as "possibly related" soon after discovery, it can be flagged as requiring more information and later be upgraded to "probably related" or "definitely related", as appropriate.

Unlikely to be related – A clinical event, including an abnormal laboratory test result, whose temporal relationship to study intervention administration makes a causal relationship improbable (e.g., the event did not occur within a reasonable time after administration of the study intervention) and in which other drugs or chemicals or underlying disease provides plausible explanations (e.g., the participant's clinical condition, other concomitant treatments).

Not Related – The AE is completely independent of study intervention administration, and/or evidence exists that the event is definitely related to another etiology. There must be an alternative, definitive etiology documented by the clinician.

8.4.6 Expected Adverse Reactions

Expected adverse reactions are Aes that are known to occur for the study intervention being studied. Expectedness is assessed based on the awareness of Aes previously observed, not on the basis of what might be anticipated from the properties of the study intervention. Thus far based on the preliminary data, there is no expected adverse reaction from the IP. All treatment emergent adverse event would be considered unexpected for regulatory reporting purpose.

8.4.7 Time Period and Frequency for Event Assessment and Follow-up

The occurrence of an AE or SAE may come to the attention of study personnel during study visits and interviews of a study participant presenting for medical care, or upon review by a study monitor. All Aes including local and systemic reactions not meeting the criteria for SAEs will be captured on the appropriate CRF. Information to be collected includes event description, time of onset, clinician's assessment of severity, relationship to study product (assessed only by those with the training and authority to make a diagnosis), and time of resolution/stabilization of the event. All Aes occurring while on study must be documented appropriately regardless of relationship. All Aes will be followed to adequate resolution. Any medical condition that is present at the time that the participant is screened will be considered as baseline and not reported as an AE. However, if the study participant's condition deteriorates at any time during the study, it will be recorded as an AE. Changes in the severity of an AE will be documented to allow an assessment of the duration of the event at each level of severity to be performed. Aes characterized as intermittent require documentation of onset and duration of each episode. The investigators, the study coordinator, will record all adverse event events with start and stop dates occurring any time after informed consent is obtained to 30 days (for SAEs) after the last dose of IP. Events will be followed for outcome information until resolution or stabilization. Any death occurring within 30 days of the last dose of IP, regardless of attribution to the IP/intervention, requires reporting to sponsor/designee within 24 hours. Deaths due to COVID-19 should be recorded as an outcome on CRT.

8.4.8 Adverse Event Reporting

According to 21 CFR 312.64(b), the investigator must record nonserious Aes and report them to the sponsor. This will be performed per schedule of activity (See Section 1.3).

8.4.9 Serious Adverse Event Reporting

The study investigator will report to the sponsor, any SAE, whether or not considered study intervention related within 24 hours.

Any SAE must be reported by the site to:

- The Sponsor (or designee) within 24 hours of becoming aware of the event
- The investigational site's IRB/Ecs by the investigator in accordance with their regulations

If an investigational site becomes aware of a new SAE or has follow-up information to a previously reported SAE, the site must notify the Sponsor (or designee) within 24 hours of becoming aware. Notification can be accomplished by completing the SAE report form submitting it to the Sponsor via email:

Email: PHV_ DB-EF-PHASEII-001_SO@IQVIA.com

Reporting instructions and the SAE Report Form are provided in the Study Manual.

8.5 Unanticipated Problems

Unanticipated problems (Ups) are problems that involve risks to participants or others to include, in general, any incident, experience, or outcome that meets **all** following criteria:

- Unexpected in terms of nature, severity, or frequency given (1) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and ICF; and (2) the characteristics of the participant population being studied;
- Related or possibly related to participation in the research ("possibly related" means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and

- Suggests that the research places participants or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

The investigator will report Ups to the reviewing IRB. The UP report will include the following information: protocol identifying information: protocol title and number, PI's name, and the IRB project number; detailed description of the event, incident, experience, or outcome; an explanation of the basis for determining that the event, incident, experience, or outcome represents an UP; a description of any changes to the protocol or other corrective actions that have been taken or are proposed in response to the UP.

To satisfy the requirement for prompt reporting, Ups will be reported using the following timeline:
(1) Ups that are SAEs will be reported to the IRB within 24 hours of the investigator becoming aware of the event.
(2) Any other Ups will be reported to the IRB within 7 days of the investigator becoming aware of the problem.

9. STATISTICAL CONSIDERATIONS

9.1 Statistical Hypotheses

Primary Endpoint:

(1) The primary efficacy endpoint, Improvement in partial pressure of arterial oxygen to fraction of inspired oxygen (PaO_2/FiO_2) ratio from pre-infusion baseline (Day 0) to Day 7, will be analyzed using the Analysis of Variance (ANOVA) adjusting for an intubation status. No improvement (+0 mmHg) will be assigned to subjects who die or had negative change in P/F ratio from baseline. The PaO2/FiO2 ratio of patients discharged prior to Day 7 will be imputed as 380. A difference between 2 mean changes from the baseline to Day 7 will be tested at one-sided significance level of 0.075 (overall Type I error rate ≤ 0.1) and estimated with 85% confidence interval.

Ho: $\mu 0 \geq \mu 1$

Ha: $\mu 0 < \mu 1$

$\mu 0$: Mean improvement from baseline to Day 7 in the control arm 1

$\mu 1$: Mean improvement from baseline to Day 7 in the selected experimental arm 2 or mean improvement from baseline to Day 7 in the selected IP arm, or arm 3 if both arms remain open after planned interim safety review with 60 patients. No statistical comparison will be performed for the unselected IP arm.

Secondary Endpoints:

(1) Time to recovery as defined by from the first study treatment until return of oxygenation saturation (SpO2) $\geq$ 93% on room air (or PaO2/FiO2 $\geq$ 300 mmHg) will be estimated using the Kaplan-Meier (KM) method and 95% confidence interval by arm. Median time to recovery and a percentage of recovered patients at Days 14 and 28 will be displayed by arm. A recovery odds ratio between the selected IP and control arm will also be estimated using a cox regression model.

(2) Incidence of Serious Adverse Events (SAEs) will be estimated by arm and displayed by severity and relationship to the study drug. Incidence of SAEs that led to not receiving full doses of the study treatment (IP+saline or saline alone) will also be summarized by am.

(3) All-cause mortality (or overall survival) will be estimated using the KM method as survival rates at Day 14, 28 and 60 by arm. The hazard ratio (HR) between the selected IP and control arms will also be estimated using a cox regression model.

Exploratory Endpoints:

All exploratory endpoints will be analyzed and compared among arms descriptively and estimated at the following time points as listed in Schedule of Activities in Section 1.3.

9.2 Sample Size Determination

For the selected experimental IP arm, approximately 30% higher improvement of P/F ratio from pre-infusion baseline to Day 7 and ≥ a higher recovery rate by 10 percentage point for selected experimental arm is assumed compared to those achieved by the control arm at Day 7. The underlying assumption is that the difference between the two mean changes (experimental and placebo arms) from the baseline to Day 7 is approximately 72 mmHg (see Table 1) with a standard deviation of 150[12]. Total of 80 (40 x 2) subjects for the final 2-arm comparison will generate approximately 80% power with 1-sided alpha of 0.075 using ANOVA adjusting for intubation status.

Table 1. Assumptions used to estimate mean changes from baseline to Day 7

	ExlFlo Arm	Approximate % Patients	Estimated Day 7 Status		Mean Changed from Baseline to Day 7	Overall Mean
ExoFlo	Moderate	50%	Recovered	55%	315	
			Non-recovery	45%	90	
	Severe	50%	Recovered	35%	406	
			Non-recovery	65%	116	216
Control	Moderate	50%	Recovered	45%	235	
			Non-recovery	55%	64	
	Severe	50%	Recovered	25%	320	
			Non-recovery	75%	88	144
					Difference:	72

9.3 Analysis Population

This modified intention-to-treat (mITT) Analysis set will be used for primary and secondary efficacy endpoints and defined as patients who are randomized and received at least a partial dose of the randomly allocated study treatment. Patients will be analyzed according to the randomized treatment arm. Safety Analysis set is defined as patients who received any dose of the study treatment (IP/saline or saline alone) and analyzed by the treatment arm/dose they actually received. Per-protocol Analysis set is defined as patients who are randomized and received full doses of both randomly allocated study treatment infusions (i.e., days 1 and 4). The Per-Protocol Analysis set will be used as a secondary analysis set for the primary and the first secondary efficacy endpoints.

9.4 Statistical Analyses

All estimated results will be presented with 95% confidence intervals except the primary endpoint of the comparison of P/F ratios after the final unblinding occurs.

9.4.1 Analysis of the Safety Endpoint

All safety analyses are based on the Safety Analysis set. Patients will be grouped according to the treatment which they received and summarized for treatment emergent AEs/SAE by severity and relationship to the study drug. All deaths and any AEs that led to not receiving full doses of the study treatment will be listed. All AE incidence rates will be estimated with 95% CI when needed.

9.4.2 Analysis of the Primary and Secondary Efficacy as well as Exploratory Endpoints

The primary analysis for primary and secondary efficacy endpoints is based on mITT set. All efficacy analyses for an unselected IP arm vs control arm and secondary/exploratory analyses will be descriptive and no formal statistical comparison will be performed. P-values may be displayed for a descriptive purpose only except for the primary endpoint for the final 2-arm comparison. Patients will be grouped according to the treatment to which they were randomized. Appropriate analysis sets for exploratory endpoints will be defined in the statistical analysis plan (SAP).

9.4.3 AE Analysis

Clinical and laboratory AEs will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) as well as Common Terminology Criteria for Adverse Events (CTCAE) v5.0. System organ class, high-level group term, high-level term, preferred term, and lower-level term will be attached to the clinical database. Within each System Organ Class, AE are listed and accompanied by descriptions of severity (Grade 1-5). All AEs will be summarized, including any infusion-related adverse reactions within 4 hours.

9.4.4 Baseline and Disposition Summaries

Demographic and baseline characteristic measurements will be summarized using descriptive methods. Demographic summaries will include sex, race/ethnicity, and age.
Disposition summary will include the portion of patients who did not receive full doses by their primary reason and the portion of patients who did not reach Day 60 by their primary reason of exiting the study early.

9.4.5 Interim Analysis

The IRT will notify the unblinded DSMB such that an interim analysis can be held following day 7 of 60th patient randomized overall (50% of total N of 120), which should also correspond to approximately 20 patients per each treatment arm.

An efficacy analysis will also be performed by the unblinded DSMB. With the primary efficacy variable being the improvement in P/F ratio from pre-infusion baseline (Day 0) to Day 7, an interim analysis will be performed with a 1-sided p-value threshold of 0.025. All randomized patients receiving at least 1 dose of study intervention will be analyzed. If the selected treatment arm containing IP demonstrates superiority over placebo, meeting the allocated Type I error rate as specified, then the DSMB may advise the sponsor to unblind and stop enrollment or unblind and continue enrollment.

9.4.6 Planned Early Unblinding Analysis

Planned final unblinding analysis for the primary endpoint will be performed following day = 7 for last patient randomized. All randomized patients receiving at least 1 dose of study intervention will be analyzed.

9.4.7 Sub-Group Analyses

Sub-group analysis of primary and secondary efficacy will be performed on baseline ventilation status to see if treatment with the IP has more of an effect on outcome when administered to intubated ARDS patients versus non-intubated ARDS patients.

10. REGULATORY ETHICAL AND STUDY OVERSIGHT CONSIDERATIONS

10.1 Informed Consent Process

Consent forms describing in detail the study intervention, study procedures, and risks are given to the participant and written documentation of informed consent is required prior to starting intervention/administering study intervention.

10.2 Consent Procedures & Documentation

Informed consent is a process that is initiated prior to the individual's agreeing to participate in the study and continues throughout the individual's study participation. Consent forms will be IRB-approved, and the participant will be asked to read and review the document. The investigator or trained proxy of the investigator (resident physician, attending physician) will explain the research study to the participant and answer any questions that may arise. A verbal explanation will be provided in terms suited to the participant's comprehension of the purposes, procedures, and potential risks of the study and of their rights as research participants. Participants will have the opportunity to carefully review the written consent form and ask questions prior to signing. The participants should have the opportunity to discuss the study with their family or surrogates or think about it prior to agreeing to participate. The participant will sign the informed consent document prior to any procedures being done specifically for the study. Participants must be informed that participation is voluntary and that they may withdraw from the study at any time, without prejudice. A copy of the ICF will be given to the participants for their records. The informed consent process will be conducted and documented in the source document (including the date), and the form signed, before the participant undergoes any study-specific procedures. The rights and welfare of the participants will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

10.3 Study Discontinuation and Closure

Study drug dosing in an individual subject will be placed on hold and may be discontinued, following a review of all available clinical data by the medical monitor and discussion with the investigator, if any of the following occurs: EITHER Any SAE or ≥ Grade 3 AE suspected to be an adverse reaction to the IP within 4 hours of administration OR patient has already recovered

with $SpO_2 \geq 93\%$ on room air. This study may be suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to study participants, investigators, IRB, FDA and CBER. If the study is prematurely terminated or suspended, the PI will promptly inform study participants, the IRB, and sponsor and will provide the reasons for the termination or suspension. Study participants will be contacted, as applicable, and be informed of changes to study visit schedule. Circumstances that may warrant termination or suspension include but are not limited to: determination of unexpected, significant, or unacceptable risk to participants, demonstration of efficacy that would warrant stopping, insufficient compliance to protocol requirements, data that is not sufficiently complete and/or analyzable, determination that the primary endpoint has been met, and/or determination of futility. Study may resume once concerns about safety, protocol compliance, and data quality are addressed, and satisfy the sponsor, IRB and/or FDA.

10.4 Confidentiality and Privacy

Participant confidentiality and privacy is strictly held in trust by the participating investigators, their staff, and the sponsor(s) and their interventions. This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to participants. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study will be released to any unauthorized third party without prior written approval of the sponsor. All research activities will be conducted in as private a setting as possible. The study monitor, other authorized representatives of the sponsor, representatives of the IRB, regulatory agencies or pharmaceutical company supplying study product may inspect all documents and records required to be maintained by the investigator, including but not limited to, hospital medical records, and pharmacy records for the participants in this study. The clinical study site will permit access to such records. The study participant's contact information will be securely stored at each clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by the reviewing IRB, Institutional policies, or sponsor requirements. Study participant research data, which is for purposes of statistical analysis

and scientific reporting, will be transmitted to and stored at the CRO. This will not include the participant's contact or identifying information. Rather, individual participants and their research data will be identified by a unique study identification number. The study data entry and study management systems used by clinical sites and by CRO will be secured and password protected.

10.5 Data Storage

Data collected for this study will be analyzed and stored at the independent CRO. After the study is completed, the de-identified, archived data will be transmitted to and stored at the Direct Biologics, LLC, for use by other researchers including those outside of the study. During the conduct of the study, an individual participant can choose to withdraw consent to have biological specimens stored for future research.

10.6 DSMB Safety Oversight

The DSMB is an independent safety oversight committee that will operate on "need-to-know" principles and rules. For example, all individuals involved in the conduct of the trial aside from the unblinded pharmacist and the independent CRO will remain unaware of information related to the planned early unblinding analysis, DSMB deliberations, recommendations, and date/time of the meetings. Members of the DSMB will be selected strictly by credentials and expertise. Individuals with a personal or professional interest in the outcome of the trial will be excluded from consideration. The DSMB closed sessions will be attended by members of the DSMB and unblinded supporting statisticians, who will be unblinded to the study intervention for proper safety oversight given the high incidence of adverse events reported in patients with COVID-19 associated ARDS. Only in closed sessions will the presented data be deliberately and privately reviewed. The DSMB will be required to keep detailed meeting minutes, kept in a confidential file inaccessible to sponsors.

10.7 Clinical Monitoring

Clinical site monitoring is conducted to ensure that the rights and well-being of trial participants are protected, that the reported trial data are accurate, complete, and verifiable, and that the conduct of the trial is in compliance with the currently approved protocol/amendment(s), with the International Conference on Harmonisation (ICH) Good Clinical Practice (GCP) guidelines,

and with applicable regulatory requirements. The first clinical monitoring visit will occur within one week of the first dosed subject to verify eligibility and compliance; then routine monitoring visits will occur on a 2-week interval until the database is locked.

10.8 Quality Assurance and Quality Control

Each clinical site will perform internal quality management of study conduct, data and biological specimen collection, documentation, and completion. An individualized quality management plan will be developed to describe a site's quality management. Quality control (QC) procedures will be implemented beginning with the data entry system and data QC checks that will be run on the database will be generated. Any missing data or data anomalies will be communicated to the sites for clarification/resolution. Following written Standard Operating Procedures (SOPs), the monitors will verify that the clinical trial is conducted and data are generated and biological specimens are collected, documented (recorded), and reported in compliance with the protocol, ICH GCP, and applicable regulatory requirements including current GMP and Good Tissue Practice (GTP).

10.9 Protocol Deviation

A protocol deviation is any noncompliance with the clinical trial protocol, ICH GCP, or Manual of Procedures (MOP) requirements. The noncompliance may be either on the part of the participant, the investigator, or the study site staff. As a result of deviations, corrective actions are to be developed by the site and implemented promptly. These practices are consistent with ICH GCP:

- 4.5 Compliance with Protocol, sections 4.5.1, 4.5.2, and 4.5.3
- 5.1 Quality Assurance and Quality Control, section 5.1.1
- 5.20 Noncompliance, sections 5.20.1, and 5.20.2.

It is the responsibility of the site investigator to use continuous vigilance to identify and report deviations within 5 working days of identification of the protocol deviation, or within 5 working days of the scheduled protocol-required activity. All deviations must be addressed in study source documents, reported to CBER and CRO. Protocol deviations must be sent to the reviewing IRB per their policies. The site investigator is responsible for knowing and adhering to the reviewing

IRB requirements. Further details about the handling of protocol deviations will be included in the MOP.

10.10 Public Access

This study will be conducted in accordance with the following publication and data sharing policies and regulations: NIH Public Access Policy, which ensures that the public has access to the published results of NIH funded research. It requires scientists to submit final peer-reviewed journal manuscripts that arise from NIH funds to the digital archive PubMed Central upon acceptance for publication. This study will comply with the NIH Data Sharing Policy and Policy on the Dissemination of NIH-Funded Clinical Trial Information and the Clinical Trials Registration and Results Information Submission rule. As such, this trial will be registered at ClinicalTrials.gov, and results information from this trial will be submitted to ClinicalTrials.gov. In addition, every attempt will be made to publish results in peer-reviewed journals.

10.11 Conflict of Interest Policy

The independence of this study from any actual or perceived influence, such as by the pharmaceutical industry, is critical. Therefore, any actual conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the design and conduct of this trial.

10.12 Protocol Amendment History

Amendment	Personnel Name	Changes
Amendment 1, 05 August 2020	Sascha Sengupta, MD	1) Sections were reformatted in accordance with GCP standards. Study contact information removed. 2) ExoFlo was removed from the title. The terminology Extracellular Vesicles was used in the title and study description. 3) Protocol authorization page now has signature spaces for both the Chief Medical Officer and the Chief Scientific Officer. 4) Investigator's Agreement was transferred from the end to the section after Protocol authorization. 5) List of Index Tables added after Table of Contents. 6) Both sections 1.2 and 1.3 emphasize that the second infusion is not administered if patient recovers by day = 4.

		7) Section 1.3 was compiled with all evaluations, labs, and procedures required itemized as rows. 8) Section 1.3 footnote specified that patients are to be monitored for 72 hours following the first and the second infusion. If the patient meets the hospital's criteria for discharge and recovers from a respiratory standpoint, there is no need to prolong the inpatient monitoring following day = 7 if the patient received 2 infusions or following day = 4 if the patient received only 1 infusion. 9) Changed terminology of day =0 to day =1 throughout the protocol. Day = 3 changed to day =4 and so forth throughout the protocol. 10) Concomitant medication was removed from the exclusion criteria. Standard of care was updated so that patients with severe COVID-19 will be allowed to remain concurrently on Remdesivir and Dexamethasone. Remdesivir and Dexamethasone were specifically mentioned as part of Standard of Care. 11) Statistical section 9.2 was updated with new assumptions about the distribution curve (given that we're trying to prove the additive effect of ExoFlo on Remdesivir) and percentages of patients recovered by day. The sample size required was 66 for power of 0.8 and 84 for power of 0.9; thus, expanded sample size to N=75 and updated this throughout the protocol. 12) Staggering protocol was updated to simplify it into 3 stages. 13) Statistical basis for stopping rules was changed to Bayesian technique; safety review cycle was specified in a table and changed to reflect a practical but still frequent meeting schedule.
Amendment II, 31 August 2020	Sascha Sengupta, MD Vikram Sengupta, MD	1) Endpoints were revised and restructured in the Section 1.1 protocol synopsis, Section 3 *Objectives & Endpoints*, and Section 9.1 Statistical Hypothesis. Primary endpoint was changed to % recovered by day =5. Safety endpoints were simplified as Incidence of Serious Adverse Events and moved into Secondary Endpoints. VFDs, PaO2/FiO2 which was clarified as improvement in P/F ratio, as well as all-cause mortality was also renamed a secondary endpoint. Median time to recovery was removed. Secondary efficacy endpoints were renamed Exploratory endpoints. 2) NK degranulation assay remains in Section 1.3 Schedule of Activities. Limited this test to day = 1, 4, 7, and 15 given the logistics of the external lab involved. Specified that EKG, microbiology, and rule out labs do not have to be repeated on day=0 if obtained following hospital admission. Specified that flow cytometry labs do not have to be obtained on day = 0. Specified that Il-6 and TNF-alpha do not have to be obtained on day =0. 3) Time windows for vital sign measurements were specified in Section 1.3 as well as Section 6.1.3. 4) EMR removed from the Study Intervention (all of section 6) per CRO request as individual hospital sites may have different protocol. 5) Statistical sample size calculation in Section 9.2 was updated using current primary endpoint. Target N remains 75. 6) Steering committee and specifics of interaction with DSMB are removed from the protocol per CRO request as there are differences with their current Standard Operating Procedures 7) Modified criteria for slowing and criteria for suspending study intervention in section 7.1.1. Original criteria of using 10% of baseline for slowing infusion was very restrictive per CRO.

		8) Study Procedure by Visit section 8.1 was added per request by CRO for clarification. 9) Definition Adverse Events of Special Interests (AESI) was added as section 8.4.3. 10) Reporting events to participants in section 8.4.9 was updated to reflect that the CRO typically only informs patients when they have an unexpected or severe AE. 11) Data Safety Review Cycles are updated in the Section 7.1.4 Stopping Rules. There will be 3 safety review cycles for which the DSMB will convene, also specified in section 10.6: The first is following day =5 for the 9th enrolled participant; the second is following day =5 for the 37th enrolled patient. The third is following day = 15 for the 75th enrolled participant (stopping rules does not apply here as 100% of target N is enrolled). Additional times for meeting(s) if any will be based on the DSMB charter. 12) Interim analysis following day =5 enrollment of the 37th patient was added as section 9.4.6. Planned early unblinding was updated in section 9.4.7 to specify that unblinding may occur if there's superiority with either a primary or secondary endpoint.
Amendment III, 07 October 2020	Sascha Sengupta, MD	1) Range of research sites increased to 2-5 in section 1.1. 2) N corrected to 75 in section 1.2 3) Quantitative SARS-CoV-2 RT PCR unavailable at sites; changed to qualitative SARS-CoV-2 RT PCR for screening, day=15, 29, & 61. Updated in Sections 1.1, 1.3, 3, 8.1, and 8.2. 4) NK degranulation assay removed throughout the protocol due to lack of site feasibility. Updated in Sections 1.1, 1.3, 3, 8.1, 8.2 and 9.1. 5) Section 7.1.3 on stopping rules was revised to a non-Bayesian version of the previous stopping rules due to request of the biostatisticians. 6) Due to site request to clarify section 7.1.2 as all patients will be selected for the study due to overall clinical deterioration including respiratory deterioration, the clinical criteria for not receive the repeat study administration has been specified as the scenario in which a serious and suspected adverse reaction occurred in response to the Investigational Product within 4 hours of administration.
Amendment IV 2 November 2020	Sascha Sengupta, MD	1) Range of research sites increased to 2-15 in section 1.1 and 4.1. 2) Days of exploratory endpoints updated in section 1.1 to match 1.3. 3) Primary endpoint was updated from proportion of patients recovered by day =5 back to median time to recovery in days given uncertainty regarding anticipated recovery distributions. This was updated in Sections 1.1, 1.3, 4, 5, 9.1. Sample size calculation was performed for the primary endpoint without change in N and updated in Section 9.2. 4) Ventilator-free days removed as a secondary endpoint given unpredictable patient characteristics at active RCT site. This was updated in Sections 1.1, 1.3, 4, 9.1. 5) Schedule of Activities 1.3 updated to reflect EDC design, which is PaO2/FiO2 will remain a daily record in the EDC. While the hospital site will routinely requiring O2 support recording in the EMR, for ease of use of the EDC, O2 support will only be recorded for days of interest in the EDC. 6) Overall study design in Section 4.1 was updated to remove intubation status from the stratification blocks. Due to need for increased enrollment and

		unpredictability of the COVID-19 ARDS patient demographic, patients will be randomized to treatment arm and stratified by research site only. 7) Inclusion criteria in Section 5.1 was revised to remove redundant language. For example, it is unnecessary to state again that patients must be rapidly clinically deteriorating when screening for EXIT COVID-19. Inclusion criteria already includes the modified definition of ARDS as well as hypoxia requiring noninvasive or invasive oxygen support despite Standard of Care, which can include Dexamethasone and Remdesivir. 8) All concomitant medications should be logged into the EDC due to CRO preference. This was updated in Section 6.6. 9) Section 10.6 was update such that board members of the DSMB were changed from blinded to unblinded in order to provide proper safety oversight. 10) In Section 7.1.3, stopping rules were updated with change in day from 5 to 15 for the trial milestones used for data safety review cycle. 11) In Section 7.1.3, AEs of special interests are revised to count towards the stopping rules only if occurring within 72 hours of IP administration. To employ this disproportionate incidence of grade 3, 4, and 5 SAE between treatment arms without specifying the acute time frame will be to exercise unnecessary bias against most novel products in general—suppose if a new biologic is protective and extends survival (thus possibly hospitalization) for some patients, the biologic treatment arms will accumulate disproportionate incidence of grade 3, 4, and 5 SAE compared to placebo simply due to the survival of the treated patients and the nature of the severe COVID-19 disease state itself, which has been shown to be prothrombotic and hyperinflammatory in nature. 12) AEs of Special Interests were revised in Section 7.1.3. Given that multiorgan failure and cytokine storm are well known and frequently reported sequalae of refractory COVID-19, these two particular AE of special interests were removed to reduce false signals in the COVID-19 patient population. DSMB will still be able to make assessments on data provided and take action based on clinical risk. 13) Language in Section 8.3 was corrected to reflect that it is the hospital site research staff that collects data at the site, not the CRO.
Amendment V 31 December 2020	Sascha Sengupta, MD	1) Primary endpoint was changed from median time to recovery to improvement in P/F ratio from pre-infusion baseline (Day 0) to Day 7 in Sections 1.1, 3, and 9.1. Time to recovery was revised to a secondary endpoint. 2) EQ-5D-5L will be collected only on Days 29 and 61. This was updated in Sections 1.1, 1.3, 3, and 9.1. 3) *Schedule of Activities* in Section 1.3 was updated to reflect the addition to days of COVID-19 symptoms prior to the current admission in addition to flexibility with SARS-CoV-2 qualitative PCR given the shortage of testing at certain hospital sites. In the event of test shortage, documentation of positive SARS-CoV-2 qualitative or quantitative within 14 days prior to admission will suffice for Day 0 or 1. The frequency of temperature monitoring was reduced to 5 minutes prior to the infusion and every 15 minutes during the infusion to reduce staff exposure. 4) *Schedule of Activities* in Section 1.3 was updated to reflect the increase in sample size from N=75 to 120. Please note that while the initial IRT plan will be to randomize to the three treatment arms 1:1:1—following review of safety after the 60th patient has been randomized overall and assuming no significant safety issues (i.e. SUSAR), the unblinded DSMB may request that the IRT be

		reprogrammed to randomize to Treatment Arm 1 and selected experimental Arm only, such that the final sample size can be reduced from 120 to 100 with, for example: Arm 1 (N=40), Arm 2 (N=20), and Arm 3 (N=40). This strategy is justified in the calculation of sample size Section 9.2 and permits N to remain appropriately low in number for a Phase II Clinical Trial. 5) *Schedule of Activities* in Section 1.3 was also updated to clarify a common site question. Screening and dosing can be on the same day. Therefore, screening can occur on day =0 or day =1. 6) Stratification in *Overall Study Design* in Sections 4.1 and 6.3 was changed back to stratifying by both research site and intubation status. 7) Exclusion Criteria in section 5.3 revised to reduce the duration of intubation from 72 hours to 24 hours. 8) To allow for flexible adoption of medications approved by the FDA for Severe COVID-19, Standard of Care was updated with link to NIH treatment guidelines for COVID-19 in Sections 5.1 and 6.6. Concomitant Medications Section 6.6 was specifically updated with the NIH treatment guideline for COVID-19 link. 9) Method of notification was updated to the appropriate telephone and/or email for both sections 6.3 and 8.4.9. 10) Criteria for slowing or suspending the study administration in Section 7.1.1 was broadened more as clinical guidance rather than strict clinical algorithms. 11) The Staggering Protocol in Section 6.4 was updated such that a 24-hour pause is implemented following day 7 following the 60th patient randomized overall. This corresponds to the updated Interim Analysis (Section 9.4.5). The Stopping Rules (Section 7.1.3) was also updated to reflect this important trial milestone. 12) Methods for Statistical Analysis in Section 9.1, 9.2, and 9.3 were revised given the change in primary endpoint and further refined overall. 13) Interim Analysis (Section 9.4.5) was revised to reflect the efficacy analysis will be performed at Day 7 following the 60th patient randomized overall into the trial. 14) Planned Early Unblinding (Section 9.4.6) was revised to reflect that efficacy analysis will be performed at Day 7 following the final patient randomized overall into the trial.
Amendment VI 8 February 2021	Sascha Sengupta, MD	1) IRT plan for randomizing only to treatment arm 1 and treatment arm 3 was removed from Sections 1.1, 1.3, and 9.2 such that target N is up to 120 for the overall study. 2) Acceptable IP for clinical trials were updated with a second lot # P-441-2004-C5, which was included in previous CMC administrative amendment submission to the FDA, demonstrating comparable data and laboratory analysis. Lot numbers will continue to be logged per IP manual. This was updated in RCT Protocol sections 1.1, 2.24, and 4.3.1. 3) Language in Section 8.1 clarified and specified such that the hospital sites did not misinterpret the assessment as conflicting with Section 7.1.2. To be clear, on Day 4, patients' eligibility criteria are reassessed such that only a change of code status and placement of ECMO (aside from criteria clarified in 7.1.2) would then disqualify patients from receiving the patient from receiving the repeat study administration. 4) Stratification in *Overall Study Design* in Sections 4.1 and 6.3 was changed back to stratifying by research site only.

11. APPENDICES

11.1 Imputing PaO_2 from SpO_2 and Estimating FiO_2

The relationship between PaO_2 and SpO_2 is sigmoidal. While many studies employed linear or log-linear regression modeling, the following equation, which is technically the Ellis inversion of the Severinghaus equation, provides a non-linear method for imputing PaO_2 from SpO_2 and was proven to be superior in accuracy in analysis of data from three ARDS Network Studies with total N = 1,184 as well as in prospective, observational study with N = 703.[89, 90]

$$PO_2 = \{\frac{11{,}700}{(^1/_S - 1)} + [50^3 + (\frac{11{,}700}{^1/_S - 1})^2]^{1/2}\}^{1/3} + \{\frac{11{,}700}{(^1/_S - 1)} - [50^3 + (\frac{11{,}700}{^1/_S - 1})^2]^{1/2}\}^{1/3}$$

Non-linear*

$PO_2 = PaO_2$; $S = SaO_2$ or SpO_2; $F = FiO_2$

The following table is the lookup table for PaO_2 for given SpO_2 and is derived from the supplemental data of the 2017 prospective, observational study, where * is generally considered unreliable on the basis of the sigmoidal shape of the hemoglobin-oxygen dissociation curve and § is based on SpO_2 99.5%.[89]

Measured SpO_2 (%)	Imputed PaO_2 (mmHg)
100*	167*§
99*	132*
98*	104*
97*	91*
96	82
95	76
94	71
93	67
92	64
91	61
90	59
89	57
88	55
87	53

Measured SpO_2 (%)	Imputed PaO_2 (mmHg)
86	51
85	50
84	49
83	47
82	46
81	45
80	44
79	43
78	42
77	42
76	41
75	40
74	39
73	39
72	38
71	37
70	37

The following table for imputing PaO_2 from SpO_2 and FiO_2 is from the retrospective analysis of ARDSnet data.[90] Overall, these tables allow for (1) calculation of estimated PaO_2/FiO_2 when an ABG or arterial line (A-line) is not available, (2) practical use of PaO_2/FiO_2 as a standardized measure of oxygenation when there is institutional and physician variability in mode of oxygen support, aggressiveness of early intubation, and speed of ventilator weaning. Although an imputed PaO_2/FiO_2 may not be as precise as ABG derived PaO_2/FiO_2, it is still more precise than other metrics, such as ordinal scales of oxygen support.

eTable 2: Imputed PF ratio (cells) for combinations of SpO_2 (rows) and FiO_2 (columns)

	0.30	0.35	0.40	0.45	0.50	0.55	0.60	0.65	0.70	0.75	0.80	0.85	0.90	0.95	1.00
80%	148	127	111	98	89	81	74	68	63	59	55	52	49	47	44
81%	151	129	113	101	91	82	76	70	65	60	57	53	50	48	45
82%	155	132	116	103	93	84	77	71	66	62	58	55	52	49	46
83%	158	136	119	106	95	86	79	73	68	63	59	56	53	50	47
84%	162	139	122	108	97	89	81	75	70	65	61	57	54	51	49
85%	167	143	125	111	100	91	83	77	71	67	63	59	56	53	50
86%	171	147	129	114	103	94	86	79	73	69	64	61	57	54	51
87%	177	151	132	118	106	96	88	81	76	71	66	62	59	56	53
88%	182	156	137	121	109	99	91	84	78	73	68	64	61	58	55
89%	189	162	141	126	113	103	94	87	81	75	71	67	63	60	57
90%	196	168	147	130	117	107	98	90	84	78	73	69	65	62	59
91%	203	174	153	136	122	111	102	94	87	81	76	72	68	64	61
92%	213	182	159	142	128	116	106	98	91	85	80	75	71	67	64
93%	223	191	168	149	134	122	112	103	96	89	84	79	74	71	67
94%	236	202	177	157	142	129	118	109	101	94	89	83	79	75	71
95%	252	216	189	168	151	138	126	116	108	101	95	89	84	80	76
96%	273	234	205	182	164	149	136	126	117	109	102	96	91	86	82

The following table for estimating FiO_2 from mode of oxygen support is from International Symposium on Intensive Care and Emergency Medicine (*https://www.intensive.org/epic2/Documents/ Estimation%20of%20PO2%20and%20FiO2.pdf*).

Method	**O_2 (L/min)**	**Estimated FiO_2 (%)**
Nasal Cannula	1	24
	2	28
	3	32
	4	36
	5	40
	6	44
Face Mask	5	40
	6-7	50
	7-8	60
Nonrebreather	10	95

11.2 Sample EQ-5D-5L and Scoring

The 5-level EQ-5D version (EQ-5D-5L) consists of a descriptive system, including the 5 dimensions of mobility, self-care, usual activities, pain/discomfort, and anxiety/depression, as well as EQ visual analogue scale (EQ-VAS). Because the EQ-5D will be administered over the phone, only the descriptive system will be administered and not the EQ-VAS. Each dimension has 5 levels: no problems, slight problems, moderate problems, severe problems and extreme problems. The patient will be asked to indicate his/her health state verbally. This decision results in a 1-digit number that expresses the level selected for that dimension. The digits for the 5 dimensions can be combined into a 5-digit number that describes the patient's health state. Valuation study for EQ-5D-5L has already been performed in the US in 2019.

Under each heading, please tick the ONE box that best describes your health TODAY.

MOBILITY

I have no problems in walking about ❑
I have slight problems in walking about ❑
I have moderate problems in walking about ❑
I have severe problems in walking about ❑
I am unable to walk about ❑

SELF-CARE

I have no problems washing or dressing myself ❑
I have slight problems washing or dressing myself ❑
I have moderate problems washing or dressing myself ❑
I have severe problems washing or dressing myself ❑
I am unable to wash or dress myself ❑

USUAL ACTIVITIES (*e.g. work, study, housework, family or leisure activities*)

I have no problems doing my usual activities ❑
I have slight problems doing my usual activities ❑
I have moderate problems doing my usual activities ❑
I have severe problems doing my usual activities ❑
I am unable to do my usual activities ❑

PAIN / DISCOMFORT

I have no pain or discomfort ❑
I have slight pain or discomfort ❑
I have moderate pain or discomfort ❑
I have severe pain or discomfort ❑
I have extreme pain or discomfort ❑

ANXIETY / DEPRESSION

I am not anxious or depressed ❑
I am slightly anxious or depressed ❑
I am moderately anxious or depressed ❑
I am severely anxious or depressed ❑
I am extremely anxious or depressed ❑

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2. Scoring the EQ-5D-5L descriptive system

This example shows how a health state is described using the EQ-5D-5L descriptive system:

Under each heading, please tick the ONE box that best describes your health TODAY.

MOBILITY
- ☑ I have no problems in walking about
- ☐ I have slight problems in walking about
- ☐ I have moderate problems in walking about
- ☐ I have severe problems in walking about
- ☐ I am unable to walk about

SELF-CARE
- ☐ I have no problems washing or dressing myself
- ☑ I have slight problems washing or dressing myself
- ☐ I have moderate problems washing or dressing myself
- ☐ I have severe problems washing or dressing myself
- ☐ I am unable to wash or dress myself

USUAL ACTIVITIES (*e.g. work, study, housework, family or leisure activities*)
- ☐ I have no problems doing my usual activities
- ☐ I have slight problems doing my usual activities
- ☑ I have moderate problems doing my usual activities
- ☐ I have severe problems doing my usual activities
- ☐ I am unable to do my usual activities

PAIN / DISCOMFORT
- ☐ I have no pain or discomfort
- ☐ I have slight pain or discomfort
- ☐ I have moderate pain or discomfort
- ☑ I have severe pain or discomfort
- ☐ I have extreme pain or discomfort

ANXIETY / DEPRESSION
- ☐ I am not anxious or depressed
- ☐ I am slightly anxious or depressed
- ☐ I am moderately anxious or depressed
- ☐ I am severely anxious or depressed
- ☑ I am extremely anxious or depressed

Levels of perceived problems are coded as follows:

- ☑ Level 1 is coded as a '1'
- ☑ Level 2 is coded as a '2'
- ☑ Level 3 is coded as a '3'
- ☑ Level 4 is coded as a '4'
- ☑ Level 5 is coded as a '5'

This example identifies the health state '12345'.

Notes:
- **There should be only ONE response for each dimension**
- **Missing values are preferably coded as '9'.**
- **Ambiguous values (e.g. two boxes are ticked for a single dimension) should be treated as missing values.**
- **This example is for the EQ-5D-5L Paper Self-Complete. Instructions for the interview and proxy versions are provided with those instruments.**

10

12. REFERENCES

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e-Table 1. Inclusion and Exclusion Criteria for EXIT COVID-19

Inclusion Criteria – must have all of the following:

- Male or female aged 18-85.
- COVID-19 positive as defined by positive Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) SARS-CoV-2.
- Hypoxia requiring noninvasive oxygen support such as Nasal Cannula (NC), Nonrebreather (NRB), Bilevel Positive Airway Pressure (BIPAP), Continuous Positive Airway Pressure (CPAP), high flow nasal cannula oxygen (HFNC O2) or mechanical ventilation (MV) despite initiating standard of care.

Inclusion Criteria for Subgroup Analysis – must also meet the following specification:

- Moderate to severe ARDS as defined by modified Berlin definition (non-ventilated patients did not have to have PEEP specifications), which includes timing within 1 week of known clinical insult or new or worsening respiratory symptoms; bilateral opacities not fully explained by effusions, or lung collapse; respiratory failure not fully explained by cardiac failure or fluid overload; $PaO_2/FiO_2 \leq 200$ mm Hg.

Exclusion Criteria - one or more of the following

- Vulnerable populations such as pregnant patients, children, individuals with severe physical or mental disabilities who cannot provide meaningful consent.
- Active malignancy requiring treatment within the last five years.
- Major physical trauma in the last 5 days, including motor vehicle accidents, assaults, mechanical falls with sequalae of significant bleeding or craniofacial bruising, and surgeries.
- Active tuberculosis or cystic fibrosis.
- Severe chronic respiratory disease including chronic obstructive pulmonary disease or pulmonary fibrosis requiring home oxygen > 5L/min.
- Use of extracorporeal membrane oxygenation (ECMO) during the current hospitalization.
- Pre-existing pulmonary hypertension.
- Severe pre-existing hepatic impairment (presence of cirrhosis, liver function tests (LFTs) ≥ 6x baseline, INR ≥ 2.0).
- Pre-existing Chronic Kidney Disease (CKD) stage IIIb or End Stage Renal Disease (ESRD) prior to onset of COVID-19 (stage I, II, and IIIa are acceptable)
- Irreversible coagulopathy (e.g., frequently occluded vascular access despite anticoagulation, precipitous platelet drops concurrent with end-organ damage suggesting consumptive process) or irreversible bleeding disorder (e.g., frequent bleeding from vascular access, endotracheal tubes, and foley).
- Pneumonia clearly attributable to a non-COVID-19 related process, including aspiration pneumonia or pneumonia that is exclusively bacterial, or originating from a diagnosed alternative virus (e.g., influenza).
- Patients who are not full code.
- Endotracheal intubation duration ≤ 24 hours.
- Moribund—expected survival < 24 hours.
- Severe metabolic disturbances on presentation (e.g., ketoacidosis, pH < 7.3)

e-Table 2. Demographic and Clinical Characteristics of the Patients at Baseline (ITT Analysis Set)

	Statistics	ExoFlo 15 mL (N=34)	ExoFlo 10 mL (N=34)	Placebo (N=34)
Age	n	34	34	34
	Mean (SD)	56.8 (14.97)	62.1 (13.47)	58.5 (11.76)
	Min, Max	24, 81	29, 79	32, 78
Age ≥ 65	n (%)	8 (23.5)	14 (41.2)	10 (29.4)
Age < 65	n (%)	26 (76.5)	20 (58.8)	24 (70.6)
Gender				
Male	n (%)	22 (64.7)	21 (61.8)	24 (70.6)
Female	n (%)	12 (35.3)	13 (38.2)	10 (29.4)
Race				
American Indian or Alaska Native	n (%)	0	0	0
Asian	n (%)	4 (11.8)	1 (2.9)	1 (2.9)
Black or African American	n (%)	5 (14.7)	1 (2.9)	4 (11.8)
Native Hawaiian or Other Pacific Islander	n (%)	0	0	0
White	n (%)	21 (61.8)	31 (91.2)	26 (76.5)
Unknown or Other	n (%)	4 (11.8)	1 (2.9)	3 (8.8)
BMI (kg/m^2) [1]	n	33	34	34
	Mean (SD)	34.98 (9.459)	35.63 (10.939)	34.24 (8.526)
	Min, Max	19, 60.6	18.9, 75.5	21.5, 63.5
Respiratory Rate (breaths/min) [1]	n	34	34	34
	Mean (SD)	23.8 (5.38)	24.4 (5.41)	25.2 (7.90)
	Min, Max	17, 37	14, 39	16, 45
Intubated Prior to Enrolling the Study	n (%)	2 (5.9)	1 (2.9)	4 (11.8)
Time from the First COVID-19 Diagnosis to First ExoFlo Dose Date (days)	n	34	34	34
	Mean (SD)	10.0 (6.55)	9.1 (4.36)	9.5 (4.12)
	Min, Max	2, 38	1, 23	2, 18
Total SOFA Score [1]	n	34	34	33
	Mean (SD)	3.2 (1.78)	2.9 (1.20)	3.2 (1.88)
	Min, Max	2, 9	0, 6	2, 9
PaO_2/FiO_2 Ratio (mmHg) [1]	n	17	19	18
	Mean (SD)	115.202 (61.5299)	113.824 (48.5386)	102.952 (43.0002)
	Min, Max	50, 211.1	60, 208.8	61, 205
PaO_2/FiO_2 Ratio < 100 mmHg	n (%)	10 (29.4)	9 (26.5)	10 (29.4)
PaO_2/FiO_2 Ratio ≥ 100 mmHg	n (%)	7 (20.6)	10 (29.4)	8 (23.5)
Prior Therapy [2]	n	30	27	30
Remdesivir	n (%)	17 (50.0)	21 (61.8)	23 (67.6)
Plasma	n (%)	7 (20.6)	9 (26.5)	9 (26.5)
Dexamethasone	n (%)	26 (76.5)	25 (73.5)	27 (79.4)

[1] Baseline is the last measure prior to the first dose of ExoFlo (Day 0 or Day 1, 5 min before dosing)

[2] Started prior to the first dose of ExoFlo regardless of its end date.

e-Table 3. Summary of Efficacy by ARDS Status (Moderate to Severe ARDS in ITT Analysis Set)

		Moderate*			Severe*		
Study Endpoints	Statistics	IP 15 mL (N=6)	IP 10 mL (N=9)	Placebo (N=7)	IP 15 mL (N=10)	IP 10 mL (N=9)	Placebo (N=10)
Subjects Discharged	n (%)	3 (50.0)	3 (33.3)	3 (42.9)	4 (40.0)	4 (44.4)	3 (30.0)
Median Time to Discharge (KM) [1]	n	6	9	7	10	9	10
	Median (1st, 3rd Quartiles)	NR (8.0, NR)	NR (27.0, NR)	NR (24.0, NR)	NR (24.0, NR)	NR (22.0, NR)	NR (21.0, NR)
Mean Time to Discharge (Restricted to Discharged Subjects)	n	3	3	3	4	4	3
	Mean (SD)	7.3 days (1.15)	16.3 days (10.50)	21.3 days (6.43)	24.0 days (25.46)	18.0 days (10.55)	11.7 days (8.14)
	Min, Max	6, 8	6, 27	14, 26	6, 60	8, 31	6, 21
Subjects Who Died Within 30 Days	n (%)	1 (16.7)	2 (22.2)	4 (57.1)	4 (40.0)	3 (33.3)	3 (30.0)
Subjects Who Died Within 60 Days	n (%)	1 (16.7)	4 (44.4)	4 (57.1)	5 (50.0)	5 (55.6)	7 (70.0)
Median Time to Death (KM)	Median	NR	41.0 days	19.0 days	NR	42.0 days	40.5 days
Mortality Rate at 15 Days (KM)	%	16.7	28.6	42.9	30.0	25.0	10.0
Mortality Rate at 30 Days (KM)	%	16.7	28.6	57.1	40.0	37.5	30.0
Mortality Rate at 60 Days (KM)	%	16.7	76.2	57.1	50.0	62.5	70.0
Mean Time to Death (Restricted to Subjects Who Died)	n	1	4	4	5	5	7
	Mean (SD)	6.0 days (NE)	26.8 days (17.06)	10.5 days (5.80)	19.2 days (10.33)	25.0 days (17.10)	30.1 days (11.25)
	Min, Max	6, 6	11, 42	6, 19	11, 36	10, 51	14, 41
P/F Ratio Increase from Baseline to Day 7 (mmHg) [2]	n	6	9	7	10	8	10
	Mean (SD)	66.333 (124.8113)	41.874 (65.7479)	5.457 (9.3842)	54.573 (64.7043)	49.443 (40.8447)	84.214 (91.7971)

Study Endpoints	Statistics	Moderate* IP 15 mL (N=6)	Moderate* IP 10 mL (N=9)	Moderate* Placebo (N=7)	Severe* IP 15 mL (N=10)	Severe* IP 10 mL (N=9)	Severe* Placebo (N=10)
	Min, Max	0, 311	0, 176	0, 21	0, 171.9	0, 121.2	0, 303.16
#Ventilation-Free Days (within 60 Days)	n	6	9	7	10	9	10
	Mean (SD)	41.2 (24.42)	13.2 (19.72)	25.0 (31.02)	32.6 (28.19)	20.7 (21.45)	25.1 (27.44)
	Min, Max	0, 61	0, 61	0, 61	3, 61	2, 61	1, 61
Median Time to Recovery (KM) [3]	n	6	9	7	10	9	10
	Median (1st, 3rd Quartiles)	NR (7.0, NR)	NR (NR, NR)	NR (15.0, NR)	NR (NR, NR)	NR (18.0, NR)	NR (25.0, NR)
Mean Time to Recovery (Restricted to Recovered Subjects)	n	2	2	2	1	3	4
	Mean (SD)	6.0 days (1.41)	13.5 days (13.44)	13.5 days (2.12)	24.0 days (NE)	15.0 days (3.61)	18.8 days (9.95)
	Min, Max	5, 7	4, 23	12, 15	24, 24	11, 18	5, 27

*According to modified Berlin definition, moderate ARDS is defined as 100 mmHg < P/F ratio <= 200 mmHg, while severe ARDS is defined as P/F ratio <= 100 mmHg.

KM = Kaplan Meier method, NE = Not Evaluable, NR = Not Reached

[1] Subjects who died or discontinued from the study due to a reason other than discharge before reaching 60 days (Day 61) are censored at Day 61.

[2] P/F ratio: All treated subjects with baseline and at least one P/F ratio measured at Day 4 or 7. For missing Day 7 data, 380 mmHg was assigned for discharged patients, and no change (0) was assigned to patients with negative change from the baseline or died before Day 7.

#Ventilation-free days: days when patients are not on mechanical ventilation within 60 days of follow-up.

[3] Subjects who died or discontinued from the study due to a reason other than recover before reaching 60 days (Day 61) are censored at Day 61.

e-Table 4. Summary of Efficacy by Age Group (ITT Analysis Set)

		Age ≥ 65			Age < 65		
Study Endpoints	Statistics	IP 15 mL (N=8)	IP 10 mL (N=14)	Placebo (N=10)	IP 15 mL (N=26)	IP 10 mL (N=20)	Placebo (N=24)
Subjects Discharged	n (%)	2 (25.0)	8 (57.1)	6 (60.0)	18 (69.2)	10 (50.0)	11 (45.8)
Median Time to Discharge (KM) [1]	n	8	14	10	26	20	24
	Median	NR	12.0 days	23.5 days	13.0 days	NR	NR
	(1st, 3rd Quartiles)	(NR, NR)	(6.0, NR)	(6.0, NR)	(6.0, NR)	(9.0, NR)	(6.5, NR)
Subjects Who Died Within 30 Days	n (%)	5 (62.5)	4 (28.6)	3 (30.0)	4 (15.4)	6 (30.0)	9 (37.5)
Subjects Who Died Within 60 Days	n (%)	5 (62.5)	6 (42.9)	4 (40.0)	5 (19.2)	8 (40.0)	12 (50.0)
IP 15 mL vs Placebo	P-value [2]	0.3428			0.0218		
Median Time to Death (KM)	Median	13.0 days	NR	NR	NR	NR	41.0 days
Mortality Rate at 15 Days (KM)	%	71.4	22.6	20.0	7.7	21.8	25.9
Mortality Rate at 30 Days (KM)	%	71.4	30.4	30.0	15.4	33.8	39.0
Mortality Rate at 60 Days (KM)	%	71.4	45.8	40.0	19.4	47.0	52.1
P/F Ratio Increase from Baseline to Day 7 (mmHg) [3]	n	3	9	6	14	9	12
	Mean (SD)	32.690 (47.3584)	28.219 (41.8297)	21.947 (39.7214)	60.404 (93.2238)	57.604 (61.8153)	62.388 (90.4587)
	Min, Max	0, 87	0, 133	0, 102	0, 311	0, 176	0, 303.16
[#]Ventilation-Free Days (within 60 Days)	n	8	14	10	26	20	24
	Mean (SD)	20.8 (25.27)	34.7 (27.77)	42.8 (25.50)	47.6 (22.85)	30.1 (25.64)	30.3 (28.75)
	Min, Max	0, 61	0, 61	0, 61	3, 61	0, 61	0, 61

Study Endpoints	Statistics	Age ≥ 65 IP 15 mL (N=8)	Age ≥ 65 IP 10 mL (N=14)	Age ≥ 65 Placebo (N=10)	Age < 65 IP 15 mL (N=26)	Age < 65 IP 10 mL (N=20)	Age < 65 Placebo (N=24)
IP 15 mL vs Placebo	P-value [4]	0.0792			0.0455		

KM = Kaplan Meier method, NR = Not Reached

[1] Subjects who died or discontinued from the study due to a reason other than discharge before reaching 60 days (Day 61) are censored at Day 61.

[2] Chi-square test for 60-day mortality rates. P-value is displayed for a descriptive purpose.

[3] P/F ratio: All treated subjects with baseline and at least one P/F ratio measured at Day 4 or 7. For missing Day 7 data, 380 mmHg was assigned for discharged patients, and no change (0) was assigned to patients with negative change from the baseline or died before Day 7.

[#]Ventilation-free days: days when patients are not on mechanical ventilation within 60 days of follow-up.

[4] Wilcoxon rank-sum test. P-value is displayed for a descriptive purpose.

e-Table 5. Summary of Efficacy by ARDS Status (Moderate to Severe ARDS aged 18 to 65 in ITT Analysis Set)

		Moderate*			Severe*		
Study Endpoints	Statistics	IP 15 mL (N=4)	IP 10 mL (N=4)	Placebo (N=4)	IP 15 mL (N=9)	IP 10 mL (N=6)	Placebo (N=7)
Subjects Discharged	n (%)	2 (50.0)	2 (50.0)	1 (25.0)	4 (44.4)	3 (50.0)	2 (28.6)
Median Time to Discharge (KM) [1]	n	4	4	4	9	6	7
	Median (1st, 3rd Quartiles)	NR (7.0, NR)	NR (21.5, NR)	NR (NR, NR)	NR (24.0, NR)	NR (22.0, NR)	NR (8.0, NR)
Mean Time to Discharge (Restricted to Discharged Subjects)	n	2	2	1	4	3	2
	Mean (SD)	7.0 days (1.41)	21.5 days (7.78)	24.0 days (NE)	24.0 days (25.46)	20.3 days (11.59)	7.0 days (1.41)
	Min, Max	6, 8	16, 27	24, 24	6, 60	8, 31	6, 8
Subjects Who Died Within 30 Days	n (%)	0	1 (25.0)	3 (75.0)	3 (33.3)	0	2 (28.6)
Subjects Who Died Within 60 Days	n (%)	0	1 (25.0)	3 (75.0)	4 (44.4)	2 (33.3)	5 (71.4)
Median Time to Death (KM)	Median	NR	NR	14.0 days	NR	NR	40.0 days
Mortality Rate at 15 Days (KM)	%	0.0	33.3	50.0	22.2	0.0	14.3
Mortality Rate at 30 Days (KM)	%	0.0	NR	75.0	33.3	0.0	28.6
Mortality Rate at 60 Days (KM)	%	0.0	NR	75.0	44.4	40.0	71.4
Mean Time to Death (Restricted to Subjects Who Died)	n	0	1	3	4	2	5
	Mean (SD)		13.0 days (NE)	12.0 days (6.08)	20.3 days (11.62)	42.0 days (12.73)	28.8 days (12.40)
	Min, Max		13, 13	8, 19	11, 36	33, 51	14, 41
P/F Ratio Increase from Baseline to Day 7 (mmHg) [2]	n	4	4	4	9	5	7
	Mean (SD)	77.750 (155.5000)	44.000 (88.0000)	5.250 (10.5000)	59.407 (66.6868)	68.488 (38.7443)	103.951 (100.5286)

Study Endpoints	Statistics	Moderate* IP 15 mL (N=4)	Moderate* IP 10 mL (N=4)	Moderate* Placebo (N=4)	Severe* IP 15 mL (N=9)	Severe* IP 10 mL (N=6)	Severe* Placebo (N=7)
	Min, Max	0, 311	0, 176	0, 21	0, 171.9	33.6, 121.2	0, 303.16
#Ventilation-Free Days (within 60 Days)	n	4	4	4	9	6	7
	Mean (SD)	47.0 (16.67)	25.3 (27.74)	13.3 (25.84)	34.6 (29.17)	26.5 (24.66)	19.6 (28.44)
	Min, Max	28, 61	0, 61	0, 52	3, 61	2, 61	1, 61
Median Time to Recovery (KM) [3]	n	4	4	4	9	6	7
	Median (1st, 3rd Quartiles)	NR (NR, NR)	NR (13.5, NR)	NR (NR, NR)	NR (NR, NR)	NR (16.0, NR)	NR (18.0, NR)
Mean Time to Recovery (Restricted to Recovered Subjects)	n	1	2	1	1	3	3
	Mean (SD)	5.0 days (NE)	13.5 days (13.44)	15.0 days (NE)	24.0 days (NE)	15.0 days (3.61)	16.0 days (10.15)
	Min, Max	5, 5	4, 23	15, 15	24, 24	11, 18	5, 25

*According to modified Berlin definition, moderate ARDS is defined as 100 mmHg < P/F ratio <= 200 mmHg, while severe ARDS is defined as P/F ratio <= 100 mmHg.

KM = Kaplan Meier method, NE = Not Evaluable, NR = Not Reached

[1] Subjects who died or discontinued from the study due to a reason other than discharge before reaching 60 days (Day 61) are censored at Day 61.

[2] P/F ratio: All treated subjects with baseline and at least one P/F ratio measured at Day 4 or 7. For missing Day 7 data, 380 mmHg was assigned for discharged patients, and no change (0) was assigned to patients with negative change from the baseline or died before Day 7.

#Ventilation-free days: days when patients are not on mechanical ventilation within 60 days of follow-up.

[3] Subjects who died or discontinued from the study due to a reason other than recover before reaching 60 days (Day 61) are censored at Day 61.