CHEST

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

RESEARCH QUESTION: Do two doses of ExoFlo safely reduce mortality in COVID-19-associated moderate to severe ARDS compared with placebo?

STUDY DESIGN AND METHODS: A prospective phase 2 multicenter double-anonymized randomized placebo-controlled dosing trial was conducted at five sites across the United States with infusions of placebo, 10 mL of ExoFlo, or 15 mL of ExoFlo on days 1 and 4. Patients (N = 102) with COVID-19-associated moderate to severe ARDS were enrolled and randomized to treatment. Adverse events were documented throughout the study. 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 population was reduced with 15 mL ExoFlo mixed with 85 mL normal saline (ExoFlo-15) compared with placebo (not significant, χ^2, $P = .1343$). For the post hoc subgroup analyses, 60-day mortality was decreased with ExoFlo-15 compared with placebo (relative risk, 0.385; 95% CI, 0.159-0.931; $P = .0340$; n = 50). With ExoFlo-15, a relative risk of 0.423 (95% CI, 0.173-1.032; $P = .0588$; n = 24) was determined for participants aged 18 to 65 years with moderate to severe ARDS. Ventilation-free days improved with ExoFlo-15 ($P = .0455$; n = 50) for all participants aged 18 to 65 years.

INTERPRETATION: The 15 mL dose of ExoFlo was found to be safe in patients with severe or critical COVID-19-associated respiratory failure. In participants aged 18 to 65 years, the risk reduction in 60-day mortality was further improved from subjects of all ages in the intention-to-treat population after two doses of 15 mL of ExoFlo compared with placebo.

CLINICAL TRIAL REGISTRATION: ClinicalTrials.gov; No.: NCT04493242; URL: www.clinicaltrials.gov.

CHEST 2023; 164(6):1444-1453

KEY WORDS: COVID-19; bone marrow mesenchymal stem cell; efficacy; extracellular vesicle; safety

FOR EDITORIAL COMMENT, SEE PAGE 1343

Take-home Points

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

Results: No adverse events or serious adverse events related to the investigational product were reported. For participants aged 18 to 65 years with respiratory failure, 60-day mortality was significantly decreased with ExoFlo-15 compared with placebo (relative risk, 0.385; 95% CI, 0.159-0.931; $P = .0340$).

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

Optimal management of ARDS morbidity remains critical. ARDS develops in 33% to 42% of hospitalized patients with COVID-19 and in 61% to 81% of patients admitted to the ICU. Patients with COVID-19-associated ARDS exhibit pathologic changes of diffuse alveolar damage similar to those of classic ARDS.[1,2] Pooled mortality estimates of ARDS cases in patients with COVID-19 showed similar mortality to patients with non-COVID-19 ARDS.[3]

Bone marrow mesenchymal stem cells (BM-MSCs) show promise for the treatment of ARDS. The phase 1 Human Mesenchymal Stem Cells for ARDS Treatment (START) trial monitored outcomes for 60 days following a single IV administration to patients with moderate to severe ARDS; no serious adverse events (SAEs) were observed following infusion of allogeneic BM-MSCs.[4] Transplantation of healthy donor BM-MSCs into patients with COVID-19 pulmonary disease improved functional outcomes without any observed adverse effects, and serum level changes in tumor necrosis factor alpha and IL-10 suggest that BM-MSCs may inhibit cytokine storm.[5] MSCs from other tissue sources also exhibit efficacy.[6] However, 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 (Direct Biologics) is an extracellular vesicle (EV) product manufactured per Current Good Manufacturing Practice regulations from a single-donor BM-MSC culture that conveys the immunomodulatory and regenerative properties of BM-MSCs 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-MSCs used to manufacture ExoFlo are fully characterized to meet the International Society for Cellular Therapy 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 US Food and Drug Administration that includes information about the chemistry, manufacturing, and control requirements for an approved phase 2 Investigational New Drug Application clinical study. The efficacy and safety potential of ExoFlo were evidenced by an investigator-initiated safety study treating patients with COVID-19-associated ARDS.[13] These findings combine with the acellular nature, homogeneity, and scalability of ExoFlo to increase its potential as a practical therapeutic option 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 that ExoFlo would be safe in the treatment of patients with severe and critical COVID-19 and compared the safety and efficacy of two doses of ExoFlo vs placebo.

ABBREVIATIONS: AE = adverse event; BM-MSC = bone marrow mesenchymal stem cell; EV = extracellular vesicle; ExoFlo-10 = 10 mL ExoFlo mixed with 90 mL normal saline; ExoFlo-15 = 15 mL ExoFlo mixed with 85 mL normal saline; IRB = institutional review board; ITT = intention-to-treat; SAE = serious adverse event; TEAE = treatment-emergent adverse event; VFD = ventilation-free day

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DOI: https://doi.org/10.1016/j.chest.2023.06.024

Study Design and Methods

Study Design and Participants

A prospective multicenter phase 2 randomized double-anonymized placebo-controlled trial was conducted. Enrollment for EXIT COVID-19 began September 24, 2020, and was 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 blood oxygen saturation < 94% on room air at sea level, Pao_2/Fio_2 < 300 mm Hg and a respiratory rate > 30 breaths per minute, or lung infiltrates > 50% were included (e-Table 1).

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 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

Figure 1 presents a CONSORT (Consolidated Standards of Reporting Trials) diagram of patient screening and enrollment. Patients (N = 102) were randomized 1:1:1 by the clinical trial sites to ExoFlo 15 mL, ExoFlo 10 mL, or placebo arms on day 1. ExoFlo is colorless when thawed, and thus 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, messenger RNA, and microRNA characterization. In addition, 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 show that the mechanism of action is functional.

Dosing of ExoFlo was calculated based on the following: (1) the 24-patient preliminary COVID-19 ExoFlo pilot study[13]; (2) the phase 1 START trial using IV administration of BM-MSCs for ARDS, which reported safety at up to 5 million cells/kg and a ceiling dose of 10 million cells/kg[7]; (3) observation of approximately 2,000 EVs

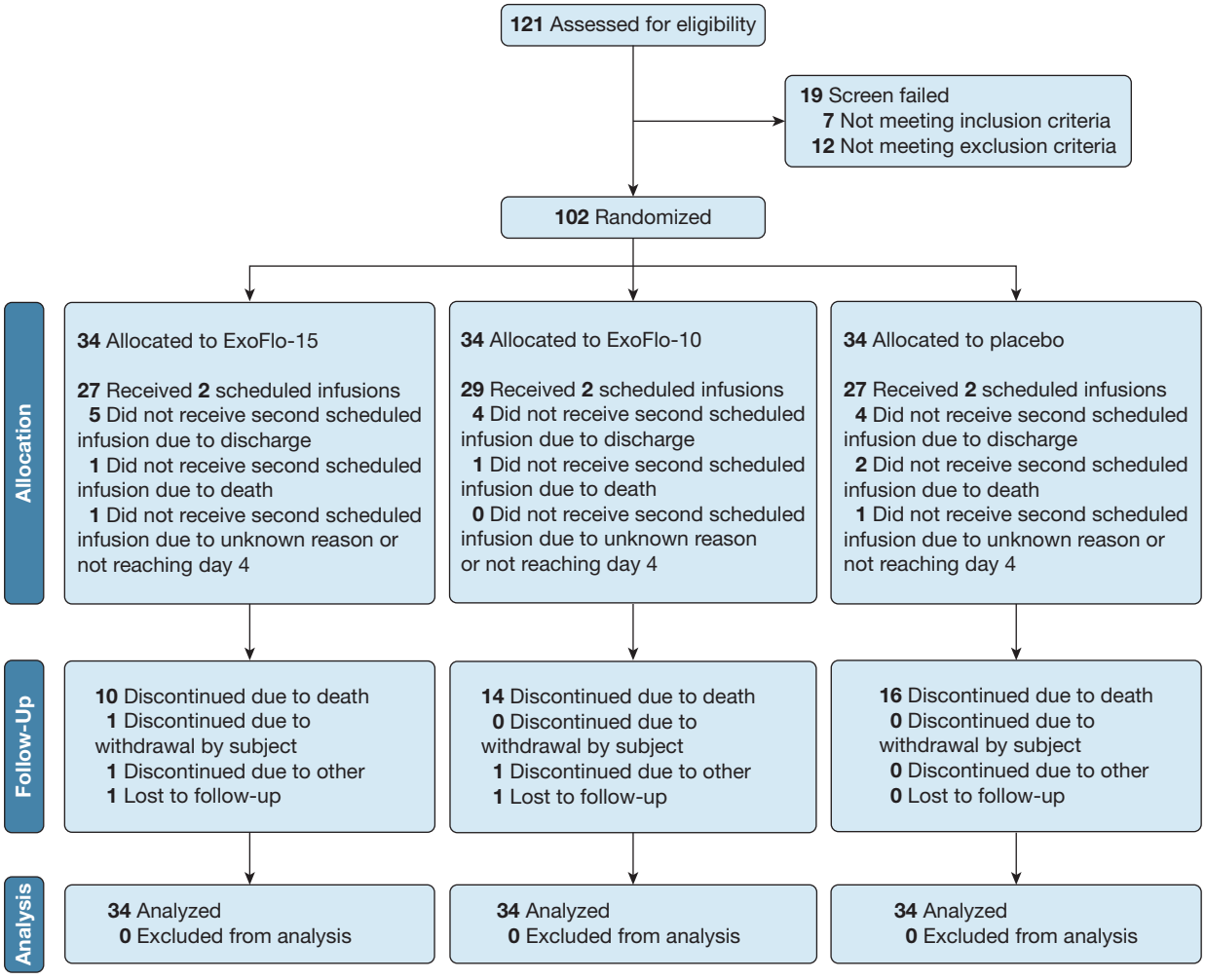

Figure 1 – *CONSORT diagram for study enrollment, allocation of treatment arm and follow-up. ExoFlo-10 = 10 mL ExoFlo mixed with 90 mL normal saline; ExoFlo-15 = 15 mL ExoFlo mixed with 85 mL normal saline.*

secreted per cell; and (4) laboratory analysis indicating 60 to 80 billion EVs/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 IV infusion over 60 minutes on day 1. Treatment arms were: 100 mL normal saline (placebo), 10 mL ExoFlo mixed with 90 mL normal saline (ExoFlo-10), and 15 mL ExoFlo mixed with 85 mL normal saline (ExoFlo-15). A repeat of the same study treatment occurred on day 4 if the patient had not recovered (blood oxygen saturation $\geq$ 93% on room air or $Pa_{O_2}/F_{IO_2} \geq 300$ mm Hg). All patients were followed up 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 the ExoFlo-15 and placebo arms for three types of prior and concomitant medications (remdesivir, plasma, and 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 predefined limits for the test of equivalence. Although all means and percentages between ExoFlo-15 and the placebo arms in e-Table 2 were not significant ($P > .1$) according to a superiority test, we could not make a conclusive statement on equivalence between the ExoFlo-15 and placebo arms. Other experimental treatment or off-label use of marketed medications was prohibited. Patients were assessed daily from day 1 to day 60 during hospitalization. All SAEs and grade 3 or 4 AEs representing increased severity from day 1 as well as any grade 2 or higher suspected drug-related hypersensitivity reactions were recorded.

Outcomes

The primary end point was improvement in the mortality rate within 60 days from randomization. Secondary end points included: time to death; incidence of treatment-emergent SAEs; proportion of discharged patients; time to hospital discharge at 7, 30, and 60 days from randomization; and ventilation free days (VFDs). Exploratory outcome measurements included viremia, serum acute-phase reactants, immune cell subset counts, Sequential Organ Failure Assessment scores, and quality of life (five-level European Quality of Life-5 Dimensions scale) scores.

Statistical Analysis

Calculation of the sample size was based on 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 intention-to-treat (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 toward nominating a safe and effective dose of ExoFlo for the treatment of respiratory failure from COVID-19, as well as 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 using a χ^2 test as a primary method. Predefined subgroup analyses were performed in patients who met criteria for moderate to severe ARDS and/or a post hoc subgroup of patients aged $\geq$ 18 to < 65 years to investigate primary and secondary end points in this disease-specific cohort.

Ethical Principles

The study was conducted in accordance with ethical principles as denoted in the International Council for Harmonisation E6 requirements.

Results

Trial Participants

Thirty-four subjects were randomized to treatment per study arm. There were no significant demographic or clinical differences in the treatment arms based on age, sex, race, BMI, respiratory rate, intubation prior to enrollment, time from first diagnosis of COVID-19 to time of first treatment dose, total Sequential Organ Failure Assessment score, Pa_{O_2}/F_{IO_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 (79.4%) of 34 participants were randomized to receive ExoFlo-15, 29 (85.3%) of 34 participants to ExoFlo-10, and 27 (79.4%) of 34 participants to placebo.

Safety

The safety analysis set (Table 1) consisted of all 68 enrolled participants 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 in the percentage of subjects with AEs or the distribution of types of AE.

All events occurred > 72 h following treatment and were evaluated by an independent data and safety monitoring board to be reasonably attributable to COVID-19 disease progression or a temporally correlated provoking stimulus. Both treatment-emergent AEs (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 with 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 receiving placebo, 38.2% in the ExoFlo-10

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 TEAE[a]				
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[a]				
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)	0 (0)	1 (2.9)	0 (0)
Study treatment-related serious TEAEs	0 (0)	0 (0)	0 (0)	0 (0)
TEAEs that led to dose interruption	1 (2.9)	0 (0)	0 (0)	1 (1.5)
TEAEs that led to missing dose or discontinued the treatment early	0 (0)	0 (0)	1 (2.9)	0 (0)
TEAEs that led to death	10 (29.4)	13 (38.2)	16 (47.1)	23 (33.8)

Data are presented as No. (%). Related indicates possibly related or probably related. ExoFlo-10 = ExoFlo mixed with 90 mL normal saline; ExoFlo-15 = 15 mL ExoFlo mixed with 85 mL normal saline; TEAE = treatment-emergent adverse events (defined as any adverse event that started between the first dose date and 30 days following the last dose date, inclusively).
[a]Toxicity grades of adverse events are evaluated based on criteria of the National Cancer Institute's Common Terminology Criteria for Adverse Events version 5.0. Each subject is counted once to the worst grade at subject-level.

group, and 29.4% in the ExoFlo-15 group. 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

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 the alpha significance level of 0.2 was suboptimal and may not indicate true statistical significance, the study rejected the null hypothesis for the primary end point (P = .1343) for ExoFlo-15. For all other analyses (including ExoFlo-10 and secondary end points), subgroup analyses were not predefined 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) (Fig 2) was improved at all time points for ExoFlo-15 compared with ExoFlo-10, which was superior at all time points to placebo. The overall mortality comparison between placebo and ExoFlo-15 was measured by the Kaplan-Meier curves and a hazard ratio with 95% CI using a Cox regression model and tested by 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 = .1820) or the hazard ratio (0.59; 95% CI, 0.27-1.30), the Kaplan-Meier curves suggest an increasing reduction in the mortality risk over time with ExoFlo-15 compared with 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 with 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, twenty-nine days for ExoFlo-10, and not reached by placebo when evaluated with Kaplan-Meier curves. The Kaplan-Meier 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 = .5554) or the recovery ratio (hazard ratio, 1.21; 95% CI, 0.63-2.31) as estimated by a Cox regression model comparing time vs hospital discharge between ExoFlo-15 and placebo.

In the ITT population, mean ± SD VFDs were highest for ExoFlo-15 (41.3 ± 25.8) and similar for ExoFlo-10 (32.0 ± 26.2) and placebo (33.9 ± 28.1). The difference

TABLE 2] Summary of Efficacy (ITT Analysis Set)

Study End Point	Statistics	ExoFlo-15 (n = 34)	ExoFlo-10 (n = 34)	Placebo (n = 34)
Subjects discharged within 60 d	No. (%)	20 (58.8)	18 (52.9)	17 (50.0)
Subjects discharged within 30 d	No. (%)	19 (55.9)	17 (50.0)	17 (50.0)
Subjects discharged within 7 d	No. (%)	11 (32.4)	9 (26.5)	11 (32.4)
Median time to discharge (KM)[a]	No.	34	34	34
	Median	22.0 d	29.0 d	NR
	95% CI	(6.0, NE)	(9.0, NE)	(7.0, NE)
Subjects who died within 30 d	No. (%)	9 (26.5)	10 (29.4)	12 (35.3)
Subjects who died within 60 d	No. (%)	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[b]	.1343	…	…
Median time to death (KM)	Median	NR	NR	NR
Mortality rate at 15 d (KM)	%	21.2	22.2	24.2
Mortality rate at 30 d (KM)	%	27.3	32.3	36.3
Mortality rate at 60 d (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, mm Hg[c]	No.	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 d)[d]	No.	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[e]	.3030	…	…

Eighty percent CI and 95% CI of subjects who died within 60 d were calculated by using the exact (Clopper-Pearson) method; 95% CI of P/F ratio increase and ventilation-free days are calculated using the *t* distribution. ExoFlo-10 = ExoFlo mixed with 90 mL normal saline; ExoFlo-15 = 15 mL ExoFlo mixed with 85 mL normal saline; ITT = intention-to-treat; KM = Kaplan-Meier method; Min, max = minimum, maximum; NE = not evaluable; NR = not reached; P/F = Pao_2/Fio_2 (average pressure of arterial oxygen to fraction of inspired oxygen ratio).

[a]Subjects who died or were discontinued from the study due to a reason other than discharge prior to reaching 60 d (day 61) are censored at day 61.

[b]χ^2 test for 60-day mortality rates. *P* value is displayed for a descriptive purpose.

[c]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 mm Hg was assigned for discharged patients, and no change (0) was assigned to patients with negative change from the baseline or died prior to day 7.

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

[e]Wilcoxon rank sum test. *P* value is displayed for a descriptive purpose.

in VFDs between ExoFlo-15 and placebo failed to reach statistical significance (Wilcoxon rank sum test, $P = .303$); however, encouraging trends in several end points emerged in analyses of subpopulations of the ITT population that were not predefined (e-Tables 3-5).

Subpopulations: Important post hoc subanalyses were conducted in patients aged 18 to 65 years with respiratory failure or moderate to severe ARDS. Those with respiratory failure had a 60-day mortality of 50% in the placebo arm and 19.2% in the ExoFlo-15 arm, representing absolute risk reduction of 30.8% and a relative risk of 0.385 (95% CI, 0.159-0.931; $P = .0340$) (Table 3). For this age group who met modified Berlin criteria for moderate to severe ARDS, 60-day mortality was 72.7% in the placebo arm and 30.8% in the ExoFlo-15 arm, yielding an absolute risk reduction of 41.9% and a relative risk of 0.423 (95% CI, 0.173-1.032; $P = .0588$). These findings indicated a trend toward improvement.

For the group aged 18 to 65 years, the number of VFDs with ExoFlo-15 (47.6 days) was improved (Wilcoxon rank sum test, $P = .0455$,) compared with placebo

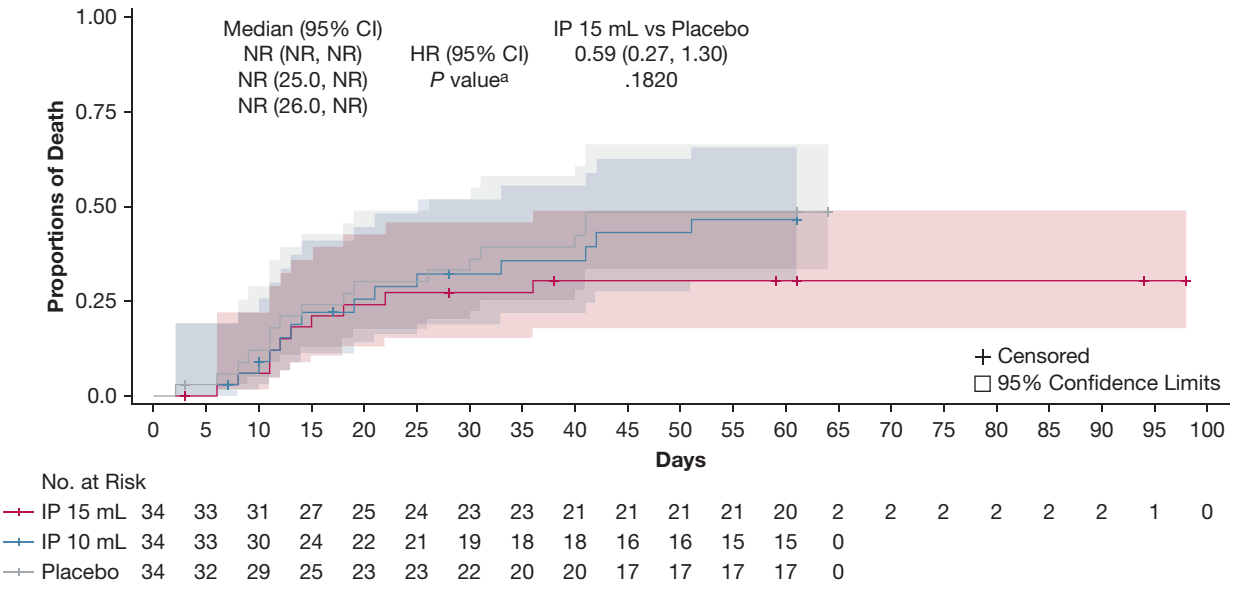

Figure 2 – *Mortality (intention-to-treat population). Time to death was compared between investigational product 15 mL and placebo by using a log-rank test. Median time to death was estimated by using the Kaplan-Meier method. Time to death is the interval in days from randomization to the subject's death. The interval is censored to study discontinuation or completion if the subject is alive. The HR of investigational product 15 mL to placebo was estimated by using a Cox regression model with a 95% CI. [a]P value is from the log-rank test. HR = hazard ratio; IP = investigational product; NR = not reached.*

(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), and 13.3 (placebo); severe ARDS, 34.6 (ExoFlo-15), 26.5 (ExoFlo-10), and 19.6 (placebo).

Discussion

This prospective double-anonymized randomized placebo-controlled phase 2 trial is the first to show that 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 AEs or SAEs related to ExoFlo at either the 10 mL or 15 mL treatment dose. Given the severity of illness in this patient population, the overwhelming safety profile is highly encouraging for the regulatory path in patients severely impaired with COVID-19. There were no differences in the safety profile at either dose despite the difference in efficacy trends observed between ExoFlo-10 compared with ExoFlo-15, and no AEs were related to the investigational product. The rate of TEAEs and SAEs of any severity grade did not increase

TABLE 3] Sixty-Day Mortality Rate for Patients Aged 18 to 65 Years With Respiratory Failure or Moderate to Severe ARDS

Indication	Placebo, n/N (%)	ExoFlo 15 mL, n/N (%)	Absolute Risk Reduction[a]	Relative Risk (95% CI)[b]	N	*P* Value for Relative Risk
Respiratory failure due to severe or critical COVID-19, aged 18-65 y	12/24 (50.0)	5/26 (19.2)	30.8%	0.385 (0.159-0.931)	50	.0340
Moderate to severe ARDS subgroup (CPAP, BiPAP, MV), all ages[c]	11/17 (64.7)	6/16 (37.5)	27.2%	0.580 (0.281-1.195)	33	.1394
Moderate to severe ARDS subgroup (CPAP, BiPAP, MV), aged 18-65 y[c]	8/11 (72.7)	4/13 (30.8)	41.9%	0.423 (0.173-1.032)	24	.0588

MV = mechanical ventilation.
[a]Absolute Risk Reduction = (60-day mortality placebo) – (60-d mortality ExoFlo).
[b]Relative Risk = 60-d mortality rate with ExoFlo/60-d mortality rate with placebo.
[c]Moderate to severe ARDS defined per the modified Berlin definition in which moderate ARDS is 100 mm Hg < P/F ratio ≤ 200 mm Hg, and severe ARDS is P/F (Pao_2/Fio_2) ratio ≤ 100 mm Hg.

beyond placebo with either dose of ExoFlo. The number of patients in treatment arms who died was lower with treatment relative to placebo. In fact, overall mortality trended lowest in the ExoFlo-15 group 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,[19] wherein two treatments of ExoFlo-15 resulted in a mortality reduction among patients hospitalized with severe or critical COVID-19. Additional secondary end points here supporting the benefit of ExoFlo-15 included confirmation of overall mortality according to Kaplan-Meier curves, shorter time to hospital discharge, increased VFDs, and biomarker trends. Dose-response trends were observed in the ITT population for 60-day mortality rate, overall mortality (Kaplan-Meier), median time to discharge (Kaplan-Meier), and VFDs. In the subgroup of patients aged 18 to 65 years with moderate or severe ARDS, 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. This study was not adequately powered for a mortality benefit between treatment arms; however, a larger mortality risk reduction was identified in subjects aged 18 to 65 years 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 to 65 years who met the modified Berlin criteria for moderate to severe ARDS, mortality was 30.8% for ExoFlo-15 (n = 11) compared with 72.7% (n = 13) in the placebo group; there was 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 comorbidities in the aging population (age is a known independent prognostic factor that affects subjects aged > 65 years overwhelmingly to decrease treatment effects on mortality) or to the 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, showing the value of ExoFlo in a critically ill patient population. Importantly, this scenario 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.

Although this is the first completed prospective randomized placebo-controlled trial of an EV product for the treatment of respiratory failure from COVID-19, several other randomized clinical trials have been conducted with antiviral and immunomodulatory therapeutics for the treatment of COVID-19.[15,20-30] Those with documented effects on mortality include remdesivir (antiviral) and dexamethasone and IL-6 antagonists (immunomodulatory). The current trial is the first, to the best of our knowledge, to show an EV product with potential mortality benefit that, in phase 3, may be superior to the aforementioned clinical trial results.

Weaknesses of the current study include that insufficient power was proposed for the primary end point and indications of efficacy may arise from the small sample size and post hoc subgroup analyses. However, preliminary efficacy inferences may be drawn from trends in the end point data to guide generation of hypotheses for the future phase III 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 vs placebo. In addition, these trends seemed to be improved in the younger patient cohort with ARDS.

Interpretation

Based on preliminary results shown with ExoFlo, the US Food and Drug Administration issued a regenerative medicine advanced therapeutic 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 this phase 3 trial will be critical to the ongoing treatment of patients with ARDS. 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.

Funding/Support

Funding for this study was provided by Direct Biologics, LLC.

Financial/Nonfinancial Disclosures

The authors have reported to *CHEST* the following: A. L. L. is CMO at Direct Biologics. J. T. R. is a PhD at Direct Biologics. S. Q. and V. S. are prior associate CMOs at Direct Biologics. None declared (S. S., D. J. P., T. I. M., B. P. W., J. J. W., M. A.).

Acknowledgments

Author contributions: A. L. L. is responsible for the content of the article. Specific author contributions were as follows: J. T. R., protocol design, interpretation of data, and editing the manuscript; S. S., protocol design, interpretation of data, and editing the manuscript; S. S. and V. S., protocol design, interpretation of data, editing manuscript; and D. J. P., T. I. M., B. P. W., J. J. W., and M. A., enrollment of patients, protocol design, and editing the manuscript. All authors contributed to protocol design, interpretation of data, and drafting and finalizing manuscript.

Role of sponsors: 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.

Additional information: The e-Tables are available online under "Supplementary Data."

References

1. Tian S, Xiong Y, Liu H, et al. Pathological study of the 2019 novel coronavirus disease (COVID-19) through postmortem core biopsies. *Modern Pathol*. 2020;33(6):1007-1014.
2. Xu Z, Shi L, Wang Y, et al. Pathological findings of COVID-19 associated with acute respiratory distress syndrome. *Lancet Respir Med*. 2020;8(4):420-422.
3. Hasan SS, Capstick T, Ahmed R, et al. Mortality in COVID-19 patients with acute respiratory distress syndrome and corticosteroids use: a systematic review and meta-analysis. *Expert Rev Respir Med*. 2020;14(11):1149-1163.
4. Wilson JG, Liu KD, Zhuo H, et al. Mesenchymal stem (stromal) cells for treatment of ARDS: a phase 1 clinical trial. *Lancet Respir Med*. 2015;3(1):24-32.
5. Leng Z, Zhu R, Hou W, et al. Transplantation of ACE2(-) mesenchymal stem cells improves the outcome of patients with COVID-19 pneumonia. *Aging Dis*. 2020;11(2):216-228.
6. Kirkham AM, Bailey AJM, Shorr R, Lalu MM, Fergusson DA, Allan DS. Systematic review and meta-analysis of randomized controlled trials of mesenchymal stromal cells to treat coronavirus disease 2019: is it too late? *Cytotherapy*. 2023;25(3):341-352.
7. Matthay MA, Calfee CS, Zhuo H, et al. Treatment with allogeneic mesenchymal stromal cells for moderate to severe acute respiratory distress syndrome (START study): a randomised phase 2a safety trial. *Lancet Respir Med*. 2019;7(2):154-162.
8. Zhou T, Yuan Z, Weng J, et al. Challenges and advances in clinical applications of mesenchymal stromal cells. *J Hematol Oncol*. 2021;14(1):24.
9. De Jong OG, Van Balkom BW, Schiffelers RM, Bouten CV, Verhaar MC. Extracellular vesicles: potential roles in regenerative medicine. *Front Immunol*. 2014;5:608.
10. Kaspi H, Semo J, Abramov N, et al. MSC-NTF (NurOwn®) exosomes: a novel therapeutic modality in the mouse LPS-induced ARDS model. *Stem Cell Res Ther*. 2021;12(1):72.
11. Krishnan A, Muthusamy S, Fernandez FB, Kasoju N. Mesenchymal stem cell-derived extracellular vesicles in the management of COVID19-associated lung injury: a review on publications, clinical trials and patent landscape. *Tissue Eng Regen Med*. 2022;19(4):659-673.
12. Tang Y, Zhou Y, Li HJ. Advances in mesenchymal stem cell exosomes: a review. *Stem Cell Res Ther*. 2021;12(1):71.
13. Sengupta V, Sengupta S, Lazo A, Woods P, Nolan A, Bremer N. Exosomes derived from bone marrow mesenchymal stem cells as treatment for severe COVID-19. *Stem Cells Develop*. 2020;29(12):747-754.
14. Sengupta V, Sengupta S, Lazo A Jr, Hicok KC, Moseley T. Response to Lim et al. re: "Exosomes Derived from Bone Marrow Mesenchymal Stem Cells as Treatment for Severe COVID-19.". *Stem Cells Develop*. 2020;29(14):879-881.
15. Horby P, Lim WS, Emberson JR, et al. Dexamethasone in hospitalized patients with Covid-19. *N Engl J Med*. 2021;384(8):693-704.
16. El-Solh AA, Meduri UG, Lawson Y, Carter M, Mergenhagen KA. Clinical course and outcome of COVID-19 acute respiratory distress syndrome: data from a national repository. *J Intensive Care Med*. 2021;36(6):664-672.
17. De Clercq E. Remdesivir: quo vadis? *Biochemical Pharmacology*. 2021;193:114800.
18. Malgie J, Schoones JW, Zeegers MP, Pijls BG. Decreased mortality and increased side effects in COVID-19 patients treated with IL-6 receptor antagonists: systematic review and meta-analysis. *Sci Rep*. 2021;11(1):21522.
19. National Institutes of Health Clinical Center. Expanded access for use of bmMSC-derived extracellular vesicles in patients with COVID-19 associated ARDS. NCT04657458. ClinicalTrials.gov. National Institutes of Health; 2023. Updated April 18, 2023. https://www.clinicaltrials.gov/search?intr=NCT04657458
20. Beigel JH, Tomashek KM, Dodd LE, et al. Remdesivir for the treatment of Covid-19—final report. *N Engl J Med*. 2020;383(19):1813-1826.
21. Bramante CT, Huling JD, Tignanelli CJ, et al. Randomized trial of metformin, ivermectin, and fluvoxamine for Covid-19. *N Engl J Med*. 2022;387(7):599-610.
22. Gordon AC, Mouncey PR, Al-Beidh F, et al. Interleukin-6 receptor antagonists in critically ill patients with Covid-19. *N Engl J Med*. 2021;384(16):1491-1502.
23. Group RC. Tocilizumab in patients admitted to hospital with COVID-19 (RECOVERY): a randomised, controlled, open-label, platform trial. *Lancet*. 2021;397(10285):1637-1645.
24. Higgins AM, Berry LR, Lorenzi E, et al. Long-term (180-day) outcomes in critically ill patients with COVID-19 in the REMAP-CAP randomized clinical trial. *JAMA*. 2023;329(1):39-51.
25. Li G, Hilgenfeld R, Whitley R, De Clercq E. Therapeutic strategies for COVID-19: progress and lessons learned. *Nat Rev Drug Discov*. 2023;22(6):449-475.
26. Reis G, Silva E, Silva DCM, et al. Effect of early treatment with ivermectin among patients with Covid-19. *N Engl J Med*. 2022;386(18):1721-1731.
27. Shankar-Hari M, Vale CL, Godolphin PJ, et al. Association between administration of IL-6 antagonists and mortality among

patients hospitalized for COVID-19: a meta-analysis. *JAMA*. 2021;326(6):499-518.

28. Spinner CD, Gottlieb RL, Criner GJ, et al. Effect of remdesivir vs standard care on clinical status at 11 days in patients with moderate COVID-19: a randomized clinical trial. *JAMA*. 2020;324(11):1048-1057.

29. Wang Y, Zhang D, Du G, et al. Remdesivir in adults with severe COVID-19: a randomised, double-blind, placebo-controlled, multicentre trial. *Lancet*. 2020;395(10236):1569-1578.

30. Yu SY, Koh DH, Choi M, et al. Clinical efficacy and safety of interleukin-6 receptor antagonists (tocilizumab and sarilumab) in patients with COVID-19: a systematic review and meta-analysis. *Emerg Microbes Infect*. 2022;11(1):1154-1165.