STATISTICAL ANALYSIS PLAN

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Study 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 Number: DB-EF-PHASEII-001

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Bone Marrow Mesenchymal Stem Cell Derived Extracellular Vesicles Infusion Treatment for COVID-19 Associated Acute Respiratory Distress Syndrome (ARDS)

Direct Biologics, LLC

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Abbreviation	Definition
ABG	Arterial Blood Gas
ANC	Absolute Neutrophil Count
ARDS	Acute Respiratory Distress Syndrome
ATC	Anatomical Therapeutic Chemical
BMI	Body Mass Index
bmMSC	Bone marrow mesenchymal stem cells
BMP	Basic Metabolic Profile
CBC	Complete Blood Count
CFB	Change from Baseline
CRF	Case Report Form
CRO	Contract Research Organization
CRP	C-Reactive Protein
DMC	Data Management Center
DNA	Deoxyribonucleic Acid
ECG	Electrocardiogram
DSMB	Data Safety Monitoring Board
ECG	Electrocardiogram
EQ-5D-5L	EQ = EuroQoL; 5D = 5 Dimensions; 5L = 5 Levels
ICU	Intensive Care Unit
IL-6	Interleukin-6
IP	Investigational Product
IRT	Interactive Response Technology
IV	Intravenous
LFTs	Liver Function Tests
MedDRA	Medical Dictionary for Regulatory Activities
miRNA	Microcoding Ribonucleic Acid
mRNA	Messenger RNA
MV	Mechanical Ventilation
NIH	National Institutes of Health
PT	Preferred Term
RCT	Randomized Controlled Trial
RNA	Ribonucleic Acid
SAE	Serious Adverse Event
SOC	System Organ Class
SOFA	Sequential Organ Failure Assessment
SpO2	Peripheral Capillary Oxygen Saturation, commonly also referred to as Oxygen Saturation
TEAE	Treatment Emergent Adverse Event
TESAE	Treatment Emergent Serious Adverse Event

1.0 INTRODUCTION

The purpose of this Statistical Analysis Plan (SAP) is to provide a comprehensive and detailed description of methods of the data analyses outlined in the protocol DB-EF-PHASEII-001 version 7.0, dated 8Feb2021. This SAP will include detailed summary specification of efficacy and safety data of ExoFlo infusion treatment for patients with COVID-19 associated ARDS.

Results obtained from the analyses described in this document will provide the basis of the Clinical Study Report (CSR) for this study. Endpoints, sample size, analysis methods specified in this SAP take precedence over those described in the last protocol version dated 8Feb2021. The modifications from the protocol are mainly based on the FDA feedback dated 17Jul2021.

2.0 STUDY OBJECTIVES & ENDPOINTS

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
PRIMARY		
To determine the final optimal safe dose for IP	Overall safety and tolerability data reviewed by the DSMB to determine the final optimal safe dose for IP	*Two IP treatment arms are of comparable safety.
To evaluate the 60-day mortality rate for IP 15ml as treatment for COVID-19 associated moderate to severe ARDS* compared to placebo.	The 30-day mortality rate is defined as the proportion of patients who expires due to any cause within 30 days (up to Day 31) from the date of randomization.	ARDS is the primary cause of death among patients with Covid-19. Reducing the mortality rate for hospitalized patients with Covid-19 associated ARDS is the most relevant measure of the treatment effect.
SECONDARY		
To evaluate the KM estimates of all-cause mortality, the Time to Discharge from hospital, the proportion of discharged patients within 7 days, incidence of SAEs for IP 15ml as treatment for COVID-19 associated moderate to severe ARDS* compared to placebo.	Overall mortality (or overall survival) will be estimated using the KM method as survival rates at Day 16 and 31 by arm. The hazard ratio (HR) between the selected IP and control arms will also be estimated using a cox regression model for a descriptive purpose.	ARDS is the primary cause of death among patients with Covid-19. Reducing the mortality rate for hospitalized patients with Covid-19 associated ARDS is the most relevant measure of the treatment effect.
	The proportion of discharged patients within 7, 30 and 60 days.	Discharge is an unbiased measure of overall clinical improvement which should subsume recovery from a pulmonary standpoint.
	Time to Discharge as defined by the number of days from the date of randomization until documented discharge from the hospital.	
	Incidence of Treatment Emergent Serious Adverse Events (SAE) regardless of relationship to the study drug	Incidence of SAES as reviewed by an independent Data Safety Monitoring Board (DSMB) is a critical part of the safety comparison between IP 15ml and placebo arms.
	Ventilation free days	Number of days for which patients are not on mechanical ventilation within 60 days of the follow-up.

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
EXPLORATORY		
The exploratory objectives are to evaluate whether IV treatment with the IP is associated with significant surrogate markers compared to placebo. To evaluate improvement in P/F ratio from baseline to Day 7 for IP 15ml as treatment for COVID-19 associated moderate to severe ARDS* compared to placebo.	Change from the baseline of CRP, D-dimer, Ferritin; ANC to Day 1, 4, 7, 10, 15, 29 and 61 as descriptively compared between Arms 1 and 3. SOFA Score on days = 1, 15, 29. Improvement in partial pressure of arterial oxygen to fraction of inspired oxygen (PaO2/FiO2) ratio from pre-infusion baseline (Day 0) to Day 7. PaO2 may be calculated from arterial blood gas (ABG) or imputed from the SpO2 daily	SOFA score is a common mortality prediction score used in sepsis research.[84] Individual scores can be useful as a measure of organ dysfunction as pulmonary, cardiovascular, hematologic, hepatic, renal, and neurologic systems are all affected.

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; 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; 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; SpO_2=peripheral capillary oxygen saturation;.

3.0 STUDY DESIGN

3.1 Overview

This is a Phase II multicenter placebo-controlled double-blinded randomized controlled study. All eligible study participants in were randomized by 1:1:1 ratio to one of the following three treatment arms stratified by research site and intubation status:

(1) Placebo, which is IV 100 mL of Normal Saline
(2) IV 10 mL of IP mixed with 90 mL of Normal Saline
(3) IV 15 mL of IP mixed with 85 mL of Normal Saline

As of 18Feb2021, the DSMB reviewed the unblinded safety data of first 60 infused patients and concluded that both IP arms were equally safe to continue. There were no treatment-related AE or SAE reported among 102 treated patients.

3.2 Random Assigning of Subjects to Treatment Arm

Because the intubation status is a known confounding factor and institutions may vary in terms of clinician preferences, hospital resources, patient demographics, and severity of illness, a stratified block randomization by ventilator status (intubated vs. non-intubated) and research site was implemented so that the key patient characteristics are balanced among the treatment arms.

3.3 Blinding

This research study involves blinding of both participant and research investigators. The hospital pharmacists will not be blinded to the study treatment as they must prepare and allocate the study treatment correctly.

Planned Unblinding will occur at the planned interim analyses for the DSMB and after the data are fully collected, the source is verified, and the database is locked.

Unplanned Unblinding: Emergency unblinding of the treatment for an individual patient may be required to protect the participant's safety if knowing the participant's treatment assignment would affect immediate medical management.

The investigator must then inform the sponsor of the emergency unblinding without disclosing the treatment the patient received. The decision to unblind will be the sole discretion of a treating investigator, the fewest number of people will be informed as a need-to-know basis.

3.4 Determination of Sample Size

The number of randomized subjects in this Phase 2 study was 102 patients (34 patients per arm). Calculation of the sample size assumed that the 60-day mortality rates of 32% (Expanded Access 30Jul21, N=50) and 43% (El-Solh 2020, N=643). Sixty-eight patients in the ITT analysis set will generate approximately 38% power based on a one-sided type I error rate of 0.1 to reject the null hypothesis that there is no difference in the two 60-day mortality rates between Arm 1 and Arm 3.

Due to lack of sufficient power to test the primary endpoints, analyses on all endpoints will be descriptive to generate hypotheses for the next Phase 3 study. P-value will be purely for a descriptive purpose if presented.

Other than the safety data review by the DSMB, no interim analysis requiring efficacy data was planned for this study.

4.0 ENDPOINTS

For all binomial rates including mortality, AE incidence, and discharge, will be displayed with 95% exact confidence intervals.

4.1 Primary Endpoints

The DSMB has reviewed the accumulated data for safety, tolerability, dosing, and baseline characteristics twice during this study. Considering the recommendations from the DSMB, the sponsor had decided to proceed with IP 15ml (Arm 3) for a next clinical study.

All-cause mortality within 60 days from the date of randomization will be measured as the proportion of patients who die within 60 day and will be estimated for each treatment arm. The alive status on Day 61 will be rigorously pursued and missing alive status is not expected for in-patients and those who were discharged earlier. The Chi-square (primary) and Cochran-Mantel-Haenszel (sensitivity) test stratified

by the baseline incubation status will be used for a descriptive purpose. The difference between the treatment arms will be estimated with a 95% confidence interval via the Mantel-Haenszel stratum-weighted estimator.

4.2 Secondary Endpoints

Descriptive Comparison

Estimated results will be compared by arm descriptively and no formal statistical comparison will be applied to the following secondary endpoints.

- Overall mortality is defined as time to death estimated by the KM curve and landmark mortality rates at Day 16 and 31 by randomized treatment arm. Patients are alive without a recorded death on study will be censored at the last known alive date. The hazard ratio (HR <1.0 will be favorable outcome for IP) between the selected IP 15ml and control arms will also be estimated with 95% CIs using a cox regression model. A log-rank test will be used to calculate a p-value for a descriptive purpose.

- Incidence of Serious Adverse Events (SAEs) among patients in the Safety Analysis set 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, if any, will also be summarized by am.

- A proportion of discharged subjects within 7, 30 and 60 days will be estimated as binomial rates and descriptively compared between IP 15ml and placebo arms using a Chi-square test.

- Time to discharge as defined as the time from the date of randomization until the first documented discharge from the hospital. Time to discharge will be compared between the IP 15ml and placebo and tested using a log-rank test. Any patients who are withdrew from the study early or lost to follow up will be censored at Day 61. All deaths within 60 days from randomization will also be censored at Day 61. The recovery ratio (>1.0 for a favorable outcome) and its 95% confidence intervals (CI) will be estimated using a Cox regression model. Median time to discharge estimated by the Kaplan-Meier (KM) method will also be presented with the corresponding 95% confidence interval.

- Sum of days for which patients are not on mechanical ventilation (i.e., not intubated) within 60 days of the follow-up will be estimated by the treatment arm and descriptive compared between IP 15ml and placebo arms using a normal distribution.

4.3 Exploratory Endpoints

The following parameters will be summarized by treatment group using descriptive statistics for points estimates and their 95% confidence intervals if applicable. Transition of median value per visit over time

will also be graphically presented by treatment arm. Each of the post-baseline visits will use the value entered per scheduled EVENT (Day X) as they are. Only unscheduled visit data will be mapped to Day X and appear as a new visit unless it is close to the missing scheduled visit (within +/- 3 days).

- C-reactive protein (CRP), D-dimer, Ferritin, ANC, and T/NK cells on Day 1, 4, 7, 10, 15, 29 and 61. The result will be graphically presented using mean (SD) and median per visit by treatment arm.
- Sequential Organ Failure Assessment (SOFA) Score on Day 1, 15, and 29 for patients who are still hospitalized. Scores for each SOFA organ system (respiration, coagulation, liver, cardiovascular, CNS, and renal) will be summarized by visit using descriptive statistics on a 0 (normal) to 4 (high degree of dysfunction/failure) scale.
- 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 descriptively compared between Arms 1 and 3. PaO_2 may be calculated from arterial blood gas (ABG) or imputed from the SpO_2 daily.

  No improvement (+0 mmHg) will be assigned to subjects who die or had a negative change in P/F ratio from baseline. The PaO2/FiO2 ratio of patients discharged prior to Day 7 will be imputed as 380 mmHg.

5.0 STATISTICAL CONSIDERATIONS

5.1 General Methodology

The statistical analysis of the data obtained from this study will be performed using SAS® version 9.4 or higher.

Data collected in this study will be documented using summary tables/figures and subject data listings. Continuous variables will be summarized using descriptive statistics, specifically the mean, median, standard deviation, minimum and maximum. Categorical variables will be summarized using frequencies and percentages. For continuous data, the minimum and the maximum will use the same decimal accuracy as the raw data. Mean, median, Q1, and Q3 will use one more decimal place than the raw data and standard deviation will use one more decimal place than mean. For categorical data, percentages will be reported to one decimal place. P-values will be reported to 4 decimal places. P-values less than 0.0001 will be displayed as <0.0001 in the tables.

5.3 Handling of Dropouts and Missing Data

Missing data will not be imputed in general except that 15 will apply to a missing day when year and month are available.

5.4 Safety Data Monitoring

There were two DSMB meeting held during the study. The first meeting included approximate 9 treated patients per arm and the second meeting included 20 treated patients per arm. No efficacy data were shared with the DSMB. The DSMB concluded at the second meeting that there was no difference

between Arm 2 (IP 10ml) and Arm 3 (IP 15ml) with respect to overall safety and recommended continuation of the study without any modification to the protocol.

5.5 Multiple Comparisons / Multiplicity

Multiplicity does not apply to this study.

5.6 Subgroup Analyses

Subgroup analyses of the primary and secondary efficacy endpoints will be performed by the following baseline conditions to see if the treatment effect of the IP 15ml can be enhanced and more desirable for a subset of patients with the Covid-19 associated ARDS:

- Baseline ventilation status
- Severity of ARDS (severe < 100 mmHg, moderate >=100 mmHg by P/F ratio)

5.7 Baseline

Baseline value is defined as the last measurement performed prior to the first infusion of study treatment. For example, if there are two separate results from the screen procedure and on Day 1 right before the first study drug infusion, the result on Day 1 is the baseline value for the patient.

6.0 ANALYSIS POPULATIONS

Intention-to-Treat (ITT) Analysis set will be used for primary and secondary efficacy endpoints and defined as patients who are randomized. Patients will be analyzed according to the randomized treatment arm. This is the primary analysis population for the efficacy endpoints.

Safety Analysis set is defined as all patients who received any dose of the study treatment (IP/saline or saline alone) and analyzed by the treatment arm/dose they received. All analyses of safety and dose exposure will be based on this population.

The analysis set for exploratory endpoints will be based on the non-missing measurement or test result at each visit. The change from the baseline value will be based on patients who had valid test results at both visits.

7.0 SUBJECT DISPOSITION

For patients in the Safety Analysis sets, a disposition summary will display the proportion of patients who did not receive full doses by their primary reason by treatment arm. The proportion of patients who did not reach Day 60 will also be summarized by treatment arm and by their primary reason of withdrawing from the study early.

8.0 PROTOCOL DEVIATIONS

The number and percentage of subjects within each category of important (major) protocol deviation will be listed for the ITT Analysis set by the clinical study team.

9.0 DEMOGRAPHICS AND BASELINE CHARACTERISTICS

Demographics and baseline characteristics will be summarized. Descriptive statistics using mean, standard deviation, minimum, maximum will be presented for the continuous variable and frequencies / percentages will be presented for the categorical variables with 95% confidence intervals when appropriate. Age at the date of randomization will be used in general unless age at the time of informed consent is relevant to a specific summary.

Summaries will include following factors in general while the TFL shell document may display fewer factors depending on the nature of analyses (e.g., DSUR, IB, DSMB, etc).

Demographics

- Age (continuous)
- Age (>=65, <65)
- Sex
- Ethnicity
- Race

Baseline Characteristics

- Weight
- BMI
- SpO2* by position measuring SpO2
- Time (days) from the first diagnosis of Covid-19 to the first study treatment infusion
- P/F ratio as continuous variable*
- ARDS Severity (P/F Ratio < 100, >=100 mmHg)*

*SpO2 and P/F Ratio must be based on the newly added data field (March 2021) in the Clinical Assessment Form (Daily) based on the highest SpO2 between 0:00 and 7:00 am, which are considered as the most stable measure of the day.

All of these results will be summarized by treatment group for the ITT and Safety Analysis sets if these analysis sets are different.

10.0 TREATMENT ADMINISTRATION

Study Drug:

Exposure to the study drug, IP 10ml, IP 15ml, or placebo will be summarized by arm for the safety analysis set for the following parameters.

- Total number of infusions patients received
- Total cumulative dose administered (if not fully dosed, read first 4 letters from “specify” (e.g., 50 cc) and converted to ml.

- Total number of patients who received at least 1 partial dose due to any reason

The number of subjects with each type of dose modification (reduction, interruption, early discontinuation) will be summarized. Subjects who did not receive two full doses will be summarized by the corresponding reason.

Concomitant Medications:

In general, concomitant medications including the standard of care for Covid-19 will be classified and summarized by prior, concomitant, and post. A patient may be counted for multiple categories.

Prior: A drug started prior to the first infusion of study treatment regardless of its end date.
Concomitant: A drug started between the first infusion of study treatment and Day 31 regardless of its end date.
Post: A drug started on or after Day 32 (i.e., outside the treatment-emergent period)

Concomitant medications will be summarized by ATC class 4/preferred term using frequencies and percentages for prior and concomitant medications.

11 Safety Analyses

Treatment-emergent adverse events (TEAEs) are defined as AEs that started after the first study drug infusion up to Day 31.

The number and percentage of patients with any TEAEs, TESAEs, TEAEs that are related to the study drug, TEAEs leading to a modification (reduction, interruption, withdrawal) of the study drug, and TEAE of special interest, if applicable, will be presented by severity. AEs that are possibly or probably related to the study treatment as well as events with a missing assessment of relationship to study treatment will be considered related to the study treatment. All deaths that occurred on study will be listed separately by treatment arm.

All AEs will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) and will be summarized at the subject level using frequencies and percentages by MedDRA system organ class (SOC) and preferred term (PT) by severity.

For exploratory endpoint laboratory parameters, the actual values and the change from baseline to each post-baseline visit will be graphically presented by treatment group for each laboratory test.

12 TFL (table, figure, listing) Shell Document

A TFL shell document that contains a list of table/figure/listings (TFLs) and their mock shells were prepared for the clinical study report (CSR) and can serve for other purposes when needed (DSMB, publication, DSUR, IB, IND update, etc). The TFL shell document may contain further programming instructions and specifications that are not covered in this SAP.

13 REFERENCES

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2. CDER Combined Cross-Discipline Team Leader, Division Director, and ODE Director Summary Review, Remdesivir, August 2020.

3. El-Solh, et al. Clinical Course and Outcome of Covid-19 Acute Respiratory Distress Syndrome Data from A National Repository, October 2020.

4. US FDA, CBER, CDER, Covid-19: Developing Drugs and Biological Products for Treatment or Prevention Guidance for Industry, February 2021