1 TITLE PAGE

Clinical Study Protocol

Study Title:	A Phase 1/2a Study of Cryopreserved Ex Vivo Expanded Polyclonal $CD4^+CD127^{lo/-}CD25^+$ T **Reg**ulatory Cells (cePolyTregs) for the Treatment of Acute Respiratory Distress Syndrome (**ARDS**) Associated with SARS-CoV-2 Infection (regARDS)
Protocol Number:	UCSF-cePolyTregs-01
Investigational Product(s):	cePolyTregs
Sponsor:	University of California, San Francisco (UCSF) 513 Parnassus Ave., Box 0540 San Francisco, CA 94143-0540 415-716-9117
Development Phase:	Phase 1/2a
Sponsor's Responsible Medical Officer:	Carolyn Hendrickson, MD, MPH carolyn.hendrickson@ucsf.edu
Protocol Version:	2.0
Version Date:	01 April 2022

CONFIDENTIAL

2 SPONSOR SIGNATURE PAGE

A Phase 1/2a Study of Cryopreserved Ex Vivo Expanded Polyclonal $CD4^+CD127^{lo/-}CD25^+$ T **Reg**ulatory Cells (cePolyTregs) for the Treatment of Acute Respiratory Distress Syndrome (**ARDS**) Associated with SARS-CoV-2 Infection (regARDS)

This clinical study protocol was subject to critical review and has been approved by the Sponsor. The following personnel contributed to writing and/or approving this protocol:

Jeffrey Bluestone, PhD A.W. and Mary Margaret Clausen Distinguished Professor, UCSF	Date

3 INVESTIGATOR SIGNATURE PAGE

A Phase 1/2a Study of Cryopreserved Ex Vivo Expanded Polyclonal $CD4^+CD127^{lo/-}CD25^+$ T **Reg**ulatory Cells (cePolyTregs) for the Treatment of Acute Respiratory Distress Syndrome (**ARDS**) Associated with SARS-CoV-2 Infection (regARDS)

I agree to the following:

- To conduct the study in strict accordance with the protocol; contract with the Sponsor; Good Clinical Practice (GCP) guidelines, including the International Council for Harmonisation (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use Guidelines (ICH E6); and regulatory requirements of the US Food and Drug Administration (FDA) and any other applicable regulatory authorities.
- To maintain adequate and accurate records on the study, and to make those records available for inspection by the Sponsor (or its authorized representative), the FDA, or any other applicable regulatory authorities, as authorized by law.
- To report to the Sponsor (or its authorized representative) any adverse events (AEs) or serious AEs that occur during the study.
- To promptly report to the Institutional Review Board (IRB) and the Sponsor all changes in research activity and all unanticipated problems involving risks to subjects or others, and not make any changes in the protocol without approval from the Sponsor and the IRB, except when necessary to eliminate apparent immediate hazards to subjects.
- To personally conduct or supervise the study and ensure that all associates, colleagues, and employees assisting in the conduct of the study are also duly qualified and are informed about their obligations and commitments.
- To ensure that the IRB responsible for initial and continuing review and approval of this study complies with applicable laws.
- To inform potential subjects that the study drug is being used for investigational purposes and to ensure that the requirements for obtaining informed consent and IRB review and approval are met.
- To comply with all other responsibilities of investigators under this protocol and all applicable laws.
- That this protocol and all data and information generated in connection with this study are the exclusive property of the Sponsor.

I have read and understood the Investigator's Brochure, including potential risks and adverse effects of the study drug. I represent that I am a licensed medical practitioner in good standing under applicable law and that I am qualified and duly authorized to conduct the study.
I acknowledge that the Sponsor has the right to terminate the study at any time.

Investigator's Signature	Date
Name (Print) and Institution Name	

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5.1 Synopsis

Title of Study Protocol: A Phase 1/2a Study of Cryopreserved Ex Vivo Expanded Polyclonal $CD4^+CD127^{lo/-}CD25^+$ T **Reg**ulatory Cells (cePolyTregs) for the Treatment of Acute Respiratory Distress Syndrome (**ARDS**) Associated with SARS-CoV-2 Infection (regARDS)

Protocol Number: UCSF-cePolyTregs-01

Name of Sponsor Company:
University of California, San Francisco
513 Parnassus Ave., Box 0540
San Francisco, CA 94143-0540

Name of Finished Product: cePolyTregs

Phase of Development: Phase 1/2a

Investigators/Study Centers: Up to 12 centers

Objectives:

Primary Objectives

Parts 1 and 2: To evaluate the safety and tolerability of cePolyTregs administered as a single intravenous (IV) dose in subjects with ARDS associated with SARS-CoV-2 infection

Part 2 (Phase 2a): To evaluate the efficacy of cePolyTregs administered as a single IV dose in subjects with ARDS associated with SARS-CoV-2 infection

Secondary Objectives

Part 1 (Phase 1): To determine the Recommended Phase 2 Dose (RP2D) of cePolyTregs administered as a single IV dose in subjects with ARDS associated with SARS-CoV-2 infection

Parts 1 and 2: To determine the pharmacodynamics and biomarker changes associated with cePolyTregs administration as a single IV dose in subjects with ARDS associated with SARS-CoV-2 infection

Exploratory Objective

To determine the clinical activity of a single IV dose of cePolyTregs on extrapulmonary manifestations of infection with SARS-CoV-2

Endpoints:

Primary Endpoints

Part 1 (Phase 1):

Safety and tolerability of cePolyTregs as assessed by dose limiting toxicities (DLTs), defined as any related treatment-emergent adverse event (TEAE) with an NCI CTCAE 5.0 grade ≥ 3 which also represents a shift from baseline clinical status of ≥ 1 NCI CTCAE grade.

Part 2 (Phase 2a):

Efficacy of cePolyTregs compared with placebo/SOC, as assessed by time to recovery, defined as the first day during the 28 days after randomization on which a subject meets the criteria for category 0, 1, 2, 3, or 4 on the WHO severity scale for COVID-19 pulmonary syndrome.

Safety of cePolyTregs as assessed by the occurrence of related TEAEs with an NCI CTCAE 5.0 grade ≥ 3.

Secondary Safety Endpoints

Part 1 (Phase 1):

RP2D of cePolyTregs as assessed by cumulative safety, tolerability, pharmacodynamic and biomarker changes associated with administration of cePolyTregs compared with placebo/SOC

Parts 1 and 2:

Safety and tolerability of cePolyTregs as assessed by:

- Type and frequency of TEAEs
- Type and frequency of treatment-emergent serious adverse events (TESAEs)
- Type and frequency of adverse events of special interest (AESIs) including infusion/hypersensitivity reactions, increase in SARS-CoV-2 viremia, and infections including pneumonia and those involving pathogens not anticipated in a COVID-19 setting
- Type and frequency of changes in clinical laboratory values, physical examinations, electrocardiograms (ECGs), and vital signs

Secondary Efficacy Endpoints

Part 2 (Phase 2a):

Efficacy of cePolyTregs compared with placebo/SOC, as assessed by:

- The proportion of subjects with a 2-point or greater improvement in the WHO severity scale for COVID-19 pulmonary syndrome on each of Study Days 7, 14, 21, and 28
- The proportion of subjects with improvement in the WHO severity scale for COVID-19 pulmonary syndrome to Grade 3 or less on each of Study Days 7, 14, 21, and 28
- The proportion of subjects requiring mechanical ventilation on each of Study Days 7, 14, 21 and 28
- Number of days free of mechanical ventilation on Days 29 and 57
- Mean and median Sequential Organ Failure [SOFA] scores on each of Study Days 7, 14, 21 and 28
- Change in chest imaging (radiograph or CT scan) abnormalities
- All-cause mortality on Study Days 29 and 84

Parts 1 and 2:

Pharmacodynamic and biomarker changes associated with administration of cePolyTregs compared with placebo/SOC, including but not limited to:

- Change from baseline in circulating cePolyTregs
- Change from baseline in pulmonary cePolyTregs by endotracheal aspirate and mini bronchoalveolar lavage (BAL),
- Change from baseline in ferritin, C-reactive protein, lactate dehydrogenase, and high-sensitivity troponin I (hs-TnI)
- Change from baseline in d-dimer
- Change from baseline in absolute lymphocyte count
- Change from baseline in C3, C4, and CH50
- Change from baseline in inflammatory cytokines/chemokines, e.g. IL-6, IL-12, IFNγ, TNFα, MCP-1, and MCP-4
- Phenotypic and functional changes from baseline in immune subsets using cytometric, genomic, and/or proteomic analyses of cells harvested from endotracheal tubes, mini BAL, and blood

Study Design:

This is a Phase 1/2a study to evaluate the safety, tolerability, pharmacodynamics (PD), and efficacy of cePolyTregs in subjects with ARDS associated with SARS-CoV-2 infection. The study will be conducted in 2 parts. Part 1 is an open-label Phase 1 study to assess escalating doses of cePolyTregs administered as a single IV dose. Part 2 is a double-blind, randomized, placebo-controlled parallel-group study of the RP2D of a single IV dose of cePolyTregs compared to placebo. Part 1 will include up to 3 cohorts of 3 to 6 subjects/cohort followed for a total of 12 weeks. Part 2 will include 1 cohort of subjects receiving cePolyTregs and 1 cohort of subjects receiving placebo/SOC followed for a total of 12 weeks.

All subjects in both parts of the study will receive standard of care treatment for COVID-19, including dexamethasone per institutional guidelines and other approved therapies for ARDS associated with SARS-CoV-2 infection per institutional guidelines.

Part 1 (Phase 1)

Part 1 will utilize a standard 3 + 3 design. The initial dose, route, and regimen for Part 1 is proposed as a single dose of 1 x 10^8 cells by IV infusion. The product is expected to have a self-limited duration of subject exposure of approximately 2 weeks. Dose escalation to single IV doses of 2 x 10^8 and 4 x 10^8 cells will be undertaken sequentially based on evaluation by a Data Safety Monitoring Board (DSMB). Dose escalation decisions will be based on the required number of subjects in a cohort completing the 7-day DLT (see definition below) assessment period. Moreover, the dose levels are based on prior data with related products in other indications where biological effects have been observed and are intended to provide a rapid onset of Treg activity at the target organs. After the last subject in each cohort has completed the DLT assessment period, the DSMB will conduct a review of all available data (safety and tolerability) from both the current cohort and cumulative data from all cePolyTregs cohorts before determining recommendations for advancement to the next dose level.

Each cohort will initially enroll 3 subjects. If 0 of 3 evaluable subjects experiences a DLT, then advancement to the next cohort may occur. If 1 of 3 subjects experiences a product-related DLT, then additional subjects will be enrolled in the same cohort for a total of up to 6 evaluable subjects. If 1 out of the 6 subjects experiences a product-related DLT, then advancement to the next cohort may occur. If 2 or more subjects in a cohort of up to 6 subjects experience a product-related DLT, no additional subjects will be enrolled in that cohort until a complete evaluation of all available data can be conducted and an opinion can be rendered by the DSMB as to whether the dose has exceeded the maximum tolerated dose (MTD). Based on cumulative safety, tolerability, product manufacturing feasibility, and PD data from all cePolyTregs cohorts, the RP2D will be determined. The cePolyTregs RP2D will include 6 treated subjects.

Part 2 (Phase 2a)

Part 2 is a double-blind, randomized, placebo-controlled parallel group study of a single IV administration of the RP2D of cePolyTregs compared to placebo/SOC. Dose selection for Part 2 will be based on determination of the RP2D from Part 1. Part 2 will consist of 2 cohorts (cePolyTregs and placebo/SOC) randomized 1:1, with randomization stratified by study site and baseline WHO severity scale for COVID-19 pulmonary syndrome. A total of 100 subjects are anticipated for randomization.

For both parts of the study, written informed consent for study participation will be obtained before any study-related procedures or assessments are performed. All potential subjects will be screened for participation, and those meeting all eligibility criteria will be offered participation in the study.

Subject participation in the study will be conducted in the following 3 defined periods:

Screening Period

The Screening Period will begin when the informed consent form is signed. During this period, subjects will undergo baseline assessments to determine eligibility for study participation. The Screening Period duration will be up to 3 days but may be as short as is necessary to complete all screening evaluations; it will end after all evaluations required to meet eligibility have been completed. If a subject meets all eligibility criteria, they will be offered enrollment into the study.

Treatment Period

The Treatment Period will begin on Day 0 with randomization (Part 2 only) and administration of the investigational product (IP; cePolyTregs or placebo) and have a duration of 28 days. During the Treatment Period, subjects will be followed closely for outcomes to define the safety, tolerability, PD/biomarker changes, and efficacy of cePolyTregs. Subjects meeting clinical criteria for intra-hospital or inter-hospital transfer, as well as hospital discharge, will be followed according to the Schedule of Events (SOE) table irrespective of the location of the subject; ambulatory follow-up visits may be conducted at the study center or with in-home visiting study support staff. Following the Treatment Period, subjects will enter the Safety Follow-up Period.

Safety Follow-up Period

The Safety Follow-up Period will have a duration of 56 days, culminating with an end-of-study (EOS) visit on Day 84.

If subjects terminate early from the study for reasons other than death, they will be asked to undergo an Early Termination (ET) Visit within 7 days of their termination; the reasons for early termination will be documented. Since this is a single-dose study, subjects experiencing a product-related DLT will be asked to remain in the study and complete all study visits through the EOS visit. These subjects will not be replaced. If a subject terminates early for a reason other than a toxicity during the Treatment Period, the subject may be replaced at the discretion of the sponsor.

A DSMB will periodically convene and review all available clinical and laboratory data during the study. After its evaluation, the DSMB may recommend study continuation (with or without modification) or termination of a dose; the DSMB may also recommend de-escalation to a lower dose. If escalation is terminated, the next-lower dose may be declared the MTD and/or RP2D. Alternatively, an additional cohort at an intermediate dose may be added to better define the MTD and/or RP2D.

Dose Limiting Toxicity, Maximum Tolerated Dose, Dose Escalation Stopping Rules, and Individual Subject Stopping Rules:

A DLT is defined as any related TEAE of NCI CTCAE 5.0 grade ≥ 3 which also represents a shift from baseline clinical status of ≥ 1 NCI CTCAE grade.

The MTD is defined as the cePolyTregs dose below the dose at which 2 or more of 6 subjects receiving cePolyTregs in a Part 1 cohort experience a DLT, as confirmed by the DSMB.

The RP2D is defined as the lowest dose achieving the target PD effect or the lower of 2 doses demonstrating similar effects on PD biomarkers of activity of cePolyTregs. Determination of the RP2D will be based upon markers that may include, but are not limited to, changes in circulating levels of cytokines and other markers listed in Table 4.

The study may be stopped at the discretion of the sponsor based on recommendations of the DSMB. The study may also be stopped pending DSMB evaluation of all available safety and PD data if any of the following occur:

- Any study drug-related death
- The occurrence of 3 deaths within 28 days post treatment in the first 5 subjects treated or, subsequent to the first 5 subjects, the observed mortality rate across the cumulative population of treated subjects exceeds the anticipated mortality rate of 50%
- Two CTCAE Grade 4 or 5 TEAEs that are deemed related to study drug
- An increase in the incidence of infections of special interest (pneumonia or infections involving pathogens not anticipated in a COVID-19 setting) beyond the expected background incidence

Dosing of cePolyTregs will be permanently discontinued in a subject if any of the following occurs:

- Any DLT (see definition above)
- Subject withdraws consent
- Pregnancy

- Subject is unable to comply with the study requirements
- Sponsor terminates the study
- A regulatory authority mandates a study dosing cessation

In all cases, necessary measures will be taken to ensure appropriate safety follow-up of all subjects in the trial. All deaths occurring during the study will be reviewed promptly by the DSMB as they occur. If at any point following enrollment of the first 5 subjects, the observed mortality rate across the cumulative population of treated subjects exceeds the anticipated mortality rate of 50%, the study will be halted.

Number of Subjects Planned:

Up to 118 subjects with COVID-19 are planned to be enrolled in the study, including up to 18 subjects in Part 1 and 100 subjects in Part 2.

Study Duration:

Up to 87 days for an individual subject

Inclusion Criteria:

Subjects will be required to meet the following inclusion criteria in order to be eligible for study enrollment:

1. Diagnosis of ARDS and respiratory failure requiring mechanical ventilation for less than 72 hours at the time of enrollment
2. $PaO_2/FiO_2 < 300$ and PEEP > 5
3. Male or female, age 18 to 70 years at Screening
4. Weight > 40 kg
5. Documented diagnosis of infection with SARS-CoV-2 virus by PCR
6. Chest imaging (radiograph or CT scan) with abnormalities consistent with COVID-19 pneumonia that could not be explained by effusions, pulmonary collapse, or nodules; and respiratory failure that could not be explained by cardiac failure or fluid overload
7. Females of childbearing potential and males must use effective contraception practices from Screening until 28 days after the EOS visit
8. Females of childbearing potential must have a negative pregnancy test at Screening and within 24 hours prior to dosing of study drug
9. Able to provide Informed Consent, either by self or by medical proxy
10. Willing and able to comply with this protocol for the entire duration of the study

Exclusion Criteria:

Parts 1 and 2:

Subjects will be ineligible for enrollment in the study if they meet any of the following criteria:

1. Any history or sign of significant chronic active or recurrent infection or screening laboratory evidence consistent with a significant chronic active or recurrent infection requiring treatment with antibiotics, antivirals or antifungals (other than SARS-CoV-2); ongoing antimicrobial treatments will not be exclusionary if, in the opinion of the investigator, no active infection is present (other than SARS-CoV-2)
2. Receiving extracorporeal membrane oxygenation therapy
3. Moribund patients not expected to survive 24 hours after enrollment based on clinical assessment
4. History of significant underlying pulmonary disease (requiring home oxygen), renal disease (requiring dialysis for chronic kidney disease), hepatic disease (Child–Pugh score ≥ 7), or known history of cirrhosis.
5. Known or suspected immunodeficiency disease
6. Positive serology for HBV, HCV, or HIV at Screening

7. Abnormal CBC defined by:
    a. Platelet count < 75,000/mm^3
    b. White blood cell count < 2500/mm^3
    c. Absolute neutrophil count < 500/mm^3
8. History of bone marrow or stem cell transplantation
9. Received any type of live attenuated vaccine < 1 month prior to Screening or is planning to receive any such live attenuated vaccine over the course of the study
10. History of lung cancer or any other malignancy requiring active treatment, except adequately treated basal cell carcinoma or in situ carcinoma of the uterine cervix
11. Any female who is pregnant or breastfeeding, or any female who is planning to become pregnant during the study and follow-up period
12. Any condition that, in the investigator's opinion, may compromise study participation, present a safety risk to the subject, or may confound the interpretation of the study results
13. A QT duration corrected for heart rate by Fridericia's formula (QTcF) > 450 millisecond (msec) for males or > 470 msec for females, based on either single or averaged QTcF values of triplicate ECGs obtained over a 3-minute interval
14. Currently enrolled in another investigational device or drug study
15. Greater than 10 days of continuous (>12 hours/day) administration of oxygen via non-invasive positive pressure ventilation or high-flow nasal cannula oxygen for hypoxemic respiratory failure due to COVID-19

Investigational Products, Dosage, and Mode of Administration:

cePolyTregs will be prepared and administered under sterile conditions according to the Cell Therapy Manual.

cePolyTregs are polyclonal Treg cells that have been collected from qualified donors by leukapheresis under GMP conditions, purified to yield a population of $CD4^+CD127^{lo/-}CD25^+$ regulatory T cells, expanded using anti-CD3/anti-CD28-coated magnetic microbeads and IL-2 for 12 to 13 days, debeaded and cryopreserved in Cryostor® CS5 solution at a concentration of 1 x 10^7 cells/mL. cePolyTregs are distributed to clinical study sites and stored in liquid nitrogen until use.

One or more bags are thawed immediately prior to administration, and cePolyTregs are infused by the IV route in unrelated subjects (allogeneic) according to the study protocol.

Reference Therapy, Dose and Mode of Administration:

All subjects in both parts of the study will receive standard of care treatment for COVID-19, including dexamethasone and other approved therapies for ARDS associated with SARS-CoV-2 infection per institutional guidelines.

Subjects randomized to placebo in Part 2 of this study will receive an infusion of normal saline solution at a matched volume to the cePolyTregs infusion.

Statistical Methods:

Details for the conduct of all safety, efficacy, and PD/biomarker analyses will be provided in a Statistical Analysis Plan (SAP) prior to database lock.

Part 1 (Phase 1): Analyses for the primary safety endpoint will be descriptive. AEs will be classified using the Medical Dictionary for Regulatory Activities (MedDRA) classification system version 20.0 or higher. The severity of the toxicities will be graded according to the NCI CTCAE version 5.0. AEs will be summarized by dose cohort using numbers and proportions.

Part 2 (Phase 2a): In the randomized, Phase 2 portion of the study, the primary efficacy endpoint is time to recovery, defined as the first day during the 28 days after randomization on which a subject meets the criteria for category 0, 1, 2, 3, or 4 on the WHO severity scale for COVID-19 pulmonary syndrome. The intent-to-treat (ITT) analysis will be carried out for the primary efficacy endpoint. Subjects will be analyzed according to the assigned

treatment group, regardless of whether the subjects receive any study treatment or receive a different study treatment from that to which they were randomized. The primary analysis will be a stratified log-rank test of time to recovery with cePolyTregs compared with placebo/SOC, with stratification by WHO severity scale for COVID 19 pulmonary syndrome at enrollment. The test will be performed with a two-sided type I error rate of 10%. Data for subjects who fail to recover or die will be censored at Day 29.

Analysis of the primary safety endpoint will be descriptive according to the intervention received and no formal comparisons between the 2 study arms will be performed. The safety analysis set will consist of all subjects who received at least 1 dose of study intervention. TEAEs will be summarized by dose cohort using numbers and proportions.

Sample Size: In Part 2 of the study, 100 subjects will be randomized 1:1 to receive cePolyTregs administered as a single IV dose or placebo/SOC. Randomization will be stratified by study site and WHO severity scale for COVID-19 pulmonary syndrome. Beigel (2020) reported that the rate of achieving a clinical severity score of 4 or better was 26.6% (67 out of 252 patients) within 14 days in the placebo arm among those with a symptom score ≥ 6. Assuming that the distribution of time to recovery follows an exponential distribution, a 26.6% recovery rate within 14 days is equivalent to a median time to recovery of 31.4 days and a 46.1% recovery rate within 28 days. We expect cePolyTregs will reduce the median time to recovery to 16 days, which is equivalent to recovery rates of 45.5% and 70.2% within 14 and 28 days, respectively, under the exponential assumption. With 50 subjects in each study arm in the present study, the power to detect a reduction of 15.4 days in median time to recovery (or, equivalently, an improvement in recovery rate from 46.1% to 70.2% within 28 days) is 80% using the log-rank test at a two-sided type I error rate of 10%, based on Monte-Carlo simulations with 10,000 replicates.

The planned sample size will provide a reasonably accurate estimate of the true serious adverse event (SAE) rate. A previous study evaluating the effect of remdesivir (Beigel 2020) reported that 30% of patients in the placebo group encountered SAEs. If 15 (30%) out of the 50 subjects in a study arm in the present study are observed to have an SAE, it will be reasonable to conclude that the true SAE rate is lower than 45%, at a 5% error rate, because the 95% confidence interval (0.179, 0.446) will exclude 45%.

Efficacy Analyses: Data will be summarized descriptively by treatment arm and overall. The descriptive summary for the categorical variables will include counts and percentages. Chi-squared tests (or Fisher's exact tests, as appropriate) will be used to compare categorical variables between study arms. Descriptive summaries for the continuous variables will include means, medians, standard deviations, and minimum and maximum values. Two-sample t-tests (or Wilcoxon rank-sum tests, as appropriate) will be used to compare continuous variables between the 2 study arms. Time to recovery will be summarized using the Kaplan-Meier method and compared between study arms using the log-rank test.

Safety Analyses: The safety analyses will describe the incidence of adverse events and laboratory abnormalities. All subjects who receive any dose (any amount) of cePolyTregs will be included in the summaries and listings of safety data. Adverse events will be coded according to system organ class and preferred term using MedDRA. AE severity will be graded using the NCI CTCAE. Adverse events will be organized by system organ class and preferred term. Vital signs and physical examination data and change of hematologic and chemistry parameters from baseline will be summarized for each post-baseline visit. Hematologic and chemistry parameters will be categorized as low, normal, or high based on laboratory normal ranges and presented as shifts from baseline.

5.2 Schedule of Events

Study Period	Screening Period	Treatment Period							Safety Follow-up Period		
		Study Day [a]									
Procedure	Up to 72 hours	0 (Pre-dose)	0.5 (Post-dose) [b]	4 ± 1	7 ± 1	14 ±2	21 ±2	28 ±2	42 ±2	56 ±2	84 ±2 or ET
Consent	X										
Eligibility criteria review	X	X									
Demographics [c]	X										
Medical/surgical histories	X										
Vital signs [d]	X	X	X	X	X	X	X	X	X	X	X
Physical examination [e]	X	X	X	X	X	X	X	X	X	X	X
Concomitant medications	X	X	X	X	X	X	X	X	X	X	X
AE Assessment	X	X	X	X	X	X	X	X	X	X	X
12-lead ECG	X		X					X			X
Efficacy assessments [f]	X		X	X	X	X	X	X	X	X	X
Chest imaging [g]	X	X	X			X	X	X	X	X	X
Ventilation Parameters [h]		X	X	X	X	X	X	X			
Randomization (IWRS, Part 2 only)		X									
Investigational Product administration		X									
Local Labs											
Hematology	X	X	X	X	X	X	X	X	X	X	X
Serology [i]	X										
Serum chemistry	X	X	X	X	X	X	X	X	X	X	X
High sensitivity troponin	X	X	X	X	X	X	X	X	X	X	X
d-dimer	X	X	X	X	X	X	X	X	X	X	X
Acute phase reactants (ferritin, CRP)	X	X	X	X	X	X	X	X	X	X	X

Study Period	Screening Period	Treatment Period							Safety Follow-up Period		
		Study Day [a]									
Procedure	**Up to 72 hours**	**0 (Pre-dose)**	**0.5 (Post-dose) [b]**	**4 ± 1**	**7 ± 1**	**14 ±2**	**21 ±2**	**28 ±2**	**42 ±2**	**56 ±2**	**84 ±2 or ET**
Urinalysis	X	X	X	X	X	X	X	X	X	X	X
Pregnancy test (urine) [j]	X								X	X	X
Mechanistic Collections											
Endotracheal Aspirate [k]		X	X	X	X	X	X	X			
Mini BAL [k, l,]		X	X	X	X	X	X	X			
Whole blood		X	X	X	X	X	X	X		X	X
Urine		X	X	X	X	X	X	X			

Abbreviations: AEs = adverse events; CRP = C-reactive protein; D = Day(s); ECG = electrocardiogram; ET = early termination; HBsAg = hepatitis B virus surface antigen; HCV = hepatitis C virus; HIV = human immunodeficiency virus; IP = investigational product; IWRS = Interactive Web Response System; min = minute(s); PD = pharmacodynamic.

[a] Safety (e.g., AE assessments, vital signs, physical examinations) and efficacy assessments will be performed daily during the period in which subjects remain hospitalized.

[b] Assessments should be measured at 12 hours (± 6 hours) post dose.

[c] Includes subject's sex, age, race, and ethnicity.

[d] Vital signs include systolic and diastolic blood pressure, pulse, respiration rate, SpO_2, (oxygen saturation via pulse oximeter) and temperature (includes height and weight at the Screening Visit). Vital signs (except for temperature and weight) will be monitored periodically for a minimum of 30 minutes after administration of IP or placebo. Subject must be seated or in a semi-recumbent position in a rested, calm state for at least 3 minutes before vital signs are collected.

[e] Physical examination will be *complete* at Screening and *targeted* for all other study days. At a minimum, the *complete physical examination* should include assessments of the skin, head and neck, lungs, cardiovascular system, abdomen, and extremities. A *targeted physical examination* will include assessment of any new subject complaints or changes from baseline.

[f] WHO severity scale for COVID-19 pulmonary syndrome, Sequential Organ Failure Score; efficacy will be assessed in both Parts 1 and 2 of the study.

[g] Radiograph or CT imaging should be performed as scheduled to the extent possible and should be included as study data regardless of time of collection; missed scans will not be considered a protocol deviation. Subjects who are ambulatory and discharged from hospital may forego further imaging if impracticable without incurring a protocol deviation.

[h] Mode of ventilation, FiO_2, SpO_2, plateau pressure, peak pressure, PEEP collected daily per local procedure.

[i] Serology includes HBsAg, anti-HCV Ab, anti-HIV Ab (if positive, HCV RNA will be measured).

[j] Pregnancy testing should be conducted at screening and all visits after hospital discharge.

[k] Samples will be collected until the removal of the endotracheal tubes, with the last sample collection at the time of removal.

[l] Mini BAL collections are at PI discretion based on exclusion criteria. Mini BAL **exclusion criteria:** FIO2 > 0.8, PEEP > 14 cm H2O, hemodynamic instability despite fluid and vasopressor support, open external ventricular device or intracranial pressure greater than 15 mmHg, INR greater than 2.0 within 36 hours of mini BAL, platelets <50 × 103/mm3 within 36 h of mini BAL (Hendrickson 2017). Missed collections will not be considered protocol deviations.

6 LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

AE	adverse event
AESI	adverse event of special interest
ARDS	acute respiratory distress syndrome
BAL	bronchoalveolar lavage
BMI	body mass index
CBC	complete blood count
cePolyTregs	Cryopreserved Ex Vivo Expanded Polyclonal $CD4^{+}CD127^{lo/-}CD25^{+}$ T Regulatory Cells
CFR	Code of Federal Regulations
CT	computerized tomography
CTCAE	Common Terminology Criteria for Adverse Events
DLT	dose limiting toxicity
DSMB	Data Safety Monitoring Board
ECG	electrocardiogram
EDC	electronic data capture
eCRF	electronic case report form
EOS	End of Study
ET	Early Termination
GCP	Good Clinical Practice
GVHD	graft-versus-host disease
HBV	hepatitis B Virus
HCV	hepatitis C Virus
HIV	human immunodeficiency virus
ICF	informed consent form
ICH	International Council for Harmonisation
IP	investigational product
IRB	Institutional Review Board
ITT	intent-to-treat
IV	intravenous
IWRS	Interactive Web Response System
LDH	lactate dehydrogenase
LN_2	liquid nitrogen
LPS	lipopolysaccharide
MedDRA	Medical Dictionary for Regulatory Activities
MTD	maximum tolerated dose
NCI	National Cancer Institute
PaO2/FiO2	the ratio of arterial oxygen partial pressure (PaO2 in mmHg) to fractional inspired oxygen (FiO2 expressed as a fraction, not a percentage)
PCR	polymerase chain reaction
PD	pharmacodynamics
PEEP	positive end-expiratory pressure
PK	pharmacokinetic(s)

RP2D	Recommended Phase 2 Dose
SAE	serious adverse event
SAP	statistical analysis plan
SOC	standard of care
SOE	schedule of events
SOFA	Sequential Organ Failure Assessment
TEAE	treatment-emergent adverse event
Treg	regulatory t cells
WHO	World Health Organization

7 INTRODUCTION

7.1 COVID-19 Background

The zoonotic SARS-CoV-2 coronavirus was first identified in Wuhan, China in late 2019 from a set of patients with severe pneumonia (now denoted Corona Virus Disease-2019 or COVID-19). As of late July 2020, the virus has caused a global pandemic with more than 15 million confirmed infections and 635,000 deaths worldwide, >145,000 in the US (Johns Hopkins Coronavirus Resource Center). Approximately 12% of all patients infected with SARS-CoV-2 go on to develop severe disease requiring intensive care (Phua 2020), including a significant proportion who develop acute respiratory distress syndrome (ARDS). This disease is characterized by inflammation and diffuse alveolar damage, and respiratory failure, and can progress to shock and death (Bradley 2020, Xu 2020, Wolfel 2020). This end-stage disease is characterized by elevated inflammatory markers, shock and cardiac failure (Siddiqi 2020).

Mortality from COVID-19 is largely due to ARDS. ARDS pathogenesis is characterized by diffuse pulmonary inflammation, altered alveolar permeability, pulmonary edema, and hypoxemic respiratory failure (Thompson, 2017). Although ARDS can be triggered by a variety of predisposing conditions, including pneumonia, aspiration, sepsis, trauma, blood transfusion and pancreatitis, the most common cause of ARDS is infection. ARDS is defined clinically by acute onset hypoxemia (< 1 week and PaO_2/FiO_2 < 300 with a minimum of 5 cm H20 PEEP) and radiographic evidence of pulmonary edema not fully explained by cardiac failure or fluid overload (Force 2012). Hypoxemia can range from mild (PaO_2/FiO_2 < 300) to severe (PaO_2/FiO_2 < 100), and the duration of respiratory failure can be variable. The annual incidence of ARDS is approximately 200 per 100,000 individuals and mortality remains approximately 40% (Eworuke 2018). Despite this burden of disease, little progress has been made in the development of novel therapies for ARDS since it was first described (Ashbaugh 1967).

Some progress has been made in the treatment of ARDS in patients with COVID-19. Studies using dexamethasone have already demonstrated a substantial benefit for hospitalized patients with COVID-19, including a 30% mortality reduction in ventilated patients (The RECOVERY Collaborative Group 2020). However, significant mortality and morbidity continues to be associated with COVID-19 globally, driving the need for novel approaches to inhibit the aberrant immune responses associated with the disease.

7.2 cePolyTregs

7.2.1 Mechanism of Action

Tregs are a subset of $CD4^+$ T cells that function to maintain immune system homeostasis and preserve tolerance to self-antigens. The function of Tregs in maintaining immune tolerance can be harnessed through Treg cell therapy for treating various immunological diseases (Tang 2006). Tregs have a unique and robust therapeutic profile that is distinct from small-molecule and biologic immunosuppressants and anti-inflammatory drugs. Tregs have the ability to migrate to

sites of inflammation and have over 30 different immune regulatory mechanisms to respond to different inflammatory conditions (Tang 2008). While requiring specific T cell receptor-mediated activation to express regulatory activity, Treg effector function appears to work by bystander suppression, regulating local inflammatory responses through a combination of cell-cell contact and suppressive cytokine production. Tregs are long-lived in autologous recipients, and tolerance conferred by autologous Treg therapy can persist even when the therapeutic cells no longer persist (Kendal 2011; Waldmann 2008). In contrast, allogeneic Tregs such as cePolyTregs are eliminated by the recipient host immune system within weeks, with their therapeutic effects having already been manifested as suggested by previous experience in acute and chronic graft-versus-host disease (GVHD; Blazar 2018, Brunstein 2010, Pierini 2015).

Adoptive Tregs therapies have been shown to be effective in dozens of animal models of autoimmunity, organ transplantation, allergy and asthma, and degenerative diseases such as brain dementias, muscular dystrophy and cardiac disease.

7.2.2 Rationale for use of cePolyTregs in ARDS

Early studies in mouse and human ARDS have shown that lymphocyte migration into the lungs is observed during the resolution of inflammation (Wang 2014). Among the lymphocytes, Tregs appear to promote the resolution of ARDS and accelerate tissue repair (D'Alessio 2009, Arpaia 2015). It has been shown that patients with ARDS have increased Tregs in their bronchoalveolar lavage fluid (D'Alessio 2009). In humans, the ratio of Tregs to effector T cells has been identified as an indicator of risk in ARDS, with more Treg infiltration correlating with better outcomes, including less lung injury, increased oxygenation (PaO_2/FiO_2 ratio), and increased survival (Yu 2015, Halter 2020).

Preliminary evidence suggests that the same is true of humans; in a recent pair of case reports, patients with COVID-19-related ARDS and multiorgan failure who had failed treatment with an IL-6 inhibitor (tocilizumab) received adoptive transfer of allogeneic cord blood-derived Tregs. They experienced no SAEs and a decrease in markers of inflammation. Clinical improvement was seen within 48 hours of Treg infusion and correlated with the reduction in cytokines, including IL-6, IFNγ and TNFα (Gladstone 2020).

In patients with ARDS associated with COVID-19 inflammatory syndrome, the administration of Treg cells is a novel therapeutic modality complementary to other pharmacologic interventions that potentially can reduce lung inflammation, mediate lung tissue repair, and significantly improve clinical outcomes.

7.3 Nonclinical Findings

7.3.1 Nonclinical Pharmacology

In vitro pharmacology studies demonstrated that cePolyTregs exhibited the critical attributes of Tregs that predict in vivo functionality, including high levels of FOXP3 expression after expansion; high levels of expression of CD25 (a component of the IL-2 receptor complex),

CTLA-4 (a critical function checkpoint molecule), and LAP (a TGFβ binding protein); enhanced signaling through IL-2R reflected in enhanced phosphorylation of STAT5; and high levels of demethylation of the FOXP3 enhancer, known as the Treg-specific demethylated region (TSDR), that is important for stable FOXP3 expression and a quantitative surrogate marker for stability of FOXP3 expression and immunosuppressive function of Tregs. The in vitro pharmacology studies also confirmed the capacity of Tregs to suppress responder T cell proliferation following polyclonal anti-CD3 and anti-CD28 stimulation.

In vivo pharmacology studies showed that in mouse models of lung disease, depletion of Tregs interfered with the resolution of inflammation. Tregs traveled into the inflamed lung, and adoptive transfer of Tregs significantly promoted survival of the mice. In a humanized mouse model of alloimmune-mediated injury of human skin allografts, co-transfer of PolyTregs, a previous analogous product to cePolyTregs prepared using a similar methodology, led to a significant increase in the ratio of $FOXP3^{+}/CD3^{+}$ T cells in the lesion and blocked skin graft injury. In a mouse model of bone marrow transplantation, Tregs conferred improved overall survival and improved GVHD score.

7.3.2 Nonclinical Pharmacokinetics

Nonclinical pharmacokinetic (PK) studies of cePolyTregs have not been conducted since cePolyTregs are xenogeneic to all nonclinical species and would not be anticipated to demonstrate PK characteristics useful in the nonclinical evaluation of this cellular therapeutic. However, there is clinical experience with the analogous product PolyTregs, including long-term clinical safety data and PK analyses (Section 7.4).

7.3.3 Nonclinical Toxicology

Toxicology studies of cePolyTregs have not been conducted since cePolyTregs are xenogeneic to all nonclinical species and would not be anticipated to demonstrate a toxicology profile useful in the nonclinical safety evaluation of this cellular therapeutic.

The nonclinical safety of species-specific, adoptively transferred Treg cells has been assessed in various disease models following administration of syngeneic Tregs at doses up to 1×10^{6} cells per mouse; specific disease models studied include influenza A virus infection, LPS-induced lung damage, and murine GVHD. While these studies are not strictly toxicology studies, and they were not conducted under GLP standards and protocols, these studies did not show any specific toxicologic findings attributable to the Treg cells. Moreover, these studies did not demonstrate any Treg-specific increase in tumorigenicity risk (Antunes 2010; D'Alessio 2009; Pierini 2015; Nguyen 2007).

Clinical experience with PolyTregs also provides long-term clinical safety data (Section 7.4) to support safe investigational use of the product.

7.4 Clinical Findings

cePolyTregs have not been administered to humans. The analogous PolyTregs autologous product has been administered to > 35 subjects in 7 clinical studies evaluating multiple clinically distinct autoimmune diseases including type 1 diabetes, systemic lupus erythematosus, and pemphigus vulgaris, and the ability of Tregs to prevent renal allograft and islet cell rejection (Ferreira 2019). No significant safety concerns have been observed to date.

In addition, a recent pair of case reports describes the treatment of patients with COVID-19-related ARDS, multiorgan failure, and who had failed treatment with an IL-6 inhibitor (tocilizumab) who received adoptive transfer of allogeneic cord blood-derived Tregs. These patients experienced no SAEs and had a decrease in markers of inflammation (Figure 1). Clinical improvement was seen within 48 hours of Treg infusion and correlated with a reduction in cytokines that included IL-6, IFNγ, and TNFα (Gladstone 2020).

Figure 1. Summary of Disease Characteristics over Time in 2 Patients with SARS-CoV-2-related ARDS Treated with Cord-blood Derived PolyTregs

Patient 1

Characteristic	Day of Hospitalization				
	Day 12	Day 13*	Day 15	Day 17*	Day 22
CB Treg infusion	–	Infusion 1	–	Infusion 2	–
IL-6, *pg/mL*	23 239	–	13 517	–	3981
Lactate, *mmol/L*	–	2.7	1.5	–	
C-reactive protein, *mg/L*	–	124	67	55	14
Ferritin, *µg/L*	–	1963	1119	–	626
Aspartate aminotransferase, *U/L*	180	137	95	–	53
IL-12, *pg/mL*	–	5.7	3.7	–	<0.1
IFNγ, *pg/mL*	–	80.0	13.7	–	3.8
IL-8, *pg/mL*	–	17.5	34.7	–	12.3
MCP-1, *pg/mL*	–	>750	>750	–	329.4
MCP-4, *pg/mL*	–	192.0	377.5	–	88.9
TNFα, *pg/mL*	–	13.0	10.9	–	3.1

Patient 2

Characteristic	Day of Hospitalization					
	Day 1	Day 6	Day 8*	Day 11*	Day 15*	Day 17
CB Treg infusion	–	–	Infusion 1	Infusion 2	Infusion 3	–
IL-6, *pg/mL*	231.0	–	23 544	6047	2498	–
Ferritin, *µg/L*	1743	–	1382	1149	666	–
IL-12, *pg/mL*	–	5.2	–	–	–	1.0
IFNγ, *pg/mL*	–	1258.5	–	–	–	25.8
IL-8, *pg/mL*	–	509.2	–	–	–	64.0
MCP-1, *pg/mL*	–	>750	–	–	–	>750
MCP-4, *pg/mL*	–	495.8	–	–	–	123.3
TNFα, *pg/mL*	–	8.0	–	–	–	5.5

Stable isotope labeling was employed to track PolyTregs in the peripheral vascular space in 6 of the clinical studies noted above. In all trials analyzed to date, a significant percentage of deuterium label in the DNA of circulating Tregs has been observed, supporting engraftment of the cells after adoptive transfer. The maximal percentage of the adoptively transferred PolyTregs was observed by 7 to 14 days, after which point there was a decline in the percentage of labeled Tregs in the circulation. In several studies, the labelled cells were observed in the circulation one

year after transfer. Moreover, there has been no evidence of loss of identity of the PolyTregs over that time as the deuterium label in the DNA was limited to $CD4^{+}CD127^{lo/-}CD25^{+}$ cells with no label observed in other peripheral blood T cell subsets, consistent with long-term PolyTregs stability.

The longevity of cePolyTregs is expected to be much reduced compared to the autologous PolyTregs product described above due to the transfer of the cells into an allogeneic recipient, leading to the recognition and elimination of the adoptively transferred product by the host immune system. This shorter survival has been observed in other studies using an allogeneic Treg product (Blazar 2018, Brunstein 2010, Pierini 2015).

8 INVESTIGATIONAL PLAN

8.1 Overall Study Design and Plan

This is a Phase 1/2a study to evaluate the safety, tolerability, pharmacodynamics (PD), and efficacy of cePolyTregs in subjects with ARDS associated with SARS-CoV-2 infection. The study will be conducted in 2 parts. Part 1 is an open-label Phase 1 study to assess escalating doses of cePolyTregs administered as a single IV dose. Part 2 is a double-blind, randomized, placebo-controlled, parallel-group study of the Recommended Phase 2 Dose (RP2D) of a single IV dose of cePolyTregs compared to placebo. Part 1 will include up to 3 cohorts of 3 to 6 subjects/cohort followed for a total of 12 weeks. Part 2 will include 1 cohort of subjects receiving cePolyTregs and 1 cohort of subjects receiving placebo/standard of care (SOC) followed for a total of 12 weeks. Total study duration is up to 87 days for an individual subject

All subjects in both parts of the study will receive standard of care treatment for COVID-19, including dexamethasone per institutional guidelines and other approved therapies for ARDS associated with SARS-CoV-2 infection per institutional guidelines.

8.1.1 Part 1 (Phase 1)

Part 1 will utilize a standard 3 + 3 design. The initial cePolyTregs dose, route, and regimen for Part 1 is proposed as a single open-label dose of 1 x 10^8 cells by IV infusion; this initial dose is based on prior data with related products in other indications and is intended to provide a rapid onset of Treg activity at the target organs. The product is expected to have a self-limited duration of subject exposure of approximately 2 weeks. Dose escalation to single IV doses of 2 x 10^8 and 4 x 10^8 cells will be undertaken sequentially based on evaluation by a Data Safety Monitoring Board (DSMB). Dose escalation decisions will be based on the required number of subjects in a cohort completing the 7-day dose limiting toxicity (DLT; see Section 8.8.3) assessment period. After the last subject in each cohort has completed the DLT assessment period, the DSMB will conduct a review of all available data (safety and tolerability) from both the current cohort and cumulative data from all cePolyTregs cohorts before determining recommendations for advancement to the next dose level.

Each cohort will initially enroll 3 subjects. If 0 of 3 evaluable subjects experiences a DLT, then advancement to the next cohort may occur. If 1 of 3 subjects experiences a product-related DLT, then additional subjects will be enrolled in the same cohort for a total of up to 6 evaluable subjects. If 1 out of the 6 subjects experiences a product-related DLT, then advancement to the next cohort may occur. If 2 or more subjects in a cohort of up to 6 subjects experience a product-related DLT, no additional subjects will be enrolled in that cohort until a complete evaluation of all available data can be conducted and an opinion can be rendered by the DSMB as to whether the dose has exceeded the maximum tolerated dose (MTD). Based on cumulative safety, tolerability, product manufacturing feasibility, and PD data from all cePolyTregs cohorts, the RP2D will be determined. The cePolyTregs RP2D will include 6 treated subjects.

8.1.2 Part 2 (Phase 2a)

Part 2 is a double-blind, randomized, placebo-controlled parallel group study of a single IV administration of the RP2D of cePolyTregs compared to placebo/SOC. Dose selection for Part 2 will be based on determination of the RP2D from Part 1. Part 2 will consist of 2 cohorts (cePolyTregs and placebo/SOC) randomized 1:1, with randomization stratified by study site and baseline WHO severity scale for COVID-19 pulmonary syndrome. A total of 100 subjects are anticipated for randomization.

For both parts of the study, written informed consent for study participation will be obtained before any study-related procedures or assessments are performed. All potential subjects will be screened for potential participation, and those meeting all eligibility criteria will be offered participation in the study.

Subject participation in the study will be conducted in the 3 defined periods described in Sections 8.1.3 to 8.1.5.

8.1.3 Screening Period

The Screening Period will begin when the informed consent form (ICF) is signed. During this period, subjects will undergo baseline assessments to determine eligibility for study participation. The Screening Period duration will be up to 3 days but may be as short as is necessary to complete all screening evaluations; it will end after all evaluations required to meet eligibility have been completed. If a subject meets all eligibility criteria, they will be offered enrollment into the study.

8.1.4 Treatment Period

The Treatment Period will begin on Day 0 with randomization (Part 2 only) and administration of the investigational product (IP; cePolyTregs or placebo) and have a duration of 28 days. During the Treatment Period, subjects will be followed closely for outcomes to define the safety, tolerability, PD/biomarker changes, and efficacy of cePolyTregs. Subjects meeting clinical criteria for intra-hospital or inter-hospital transfer, as well as hospital discharge, will be followed according to the Schedule of Events (SOE) table (Section 5.2) irrespective of the location of the subject; ambulatory follow-up visits may be conducted at the study center or with in-home visiting study support staff. Following the Treatment Period, subjects will enter the Safety Follow-up Period.

8.1.5 Safety Follow-up Period

The Safety Follow-up Period will have a duration of 56 days, culminating with an end-of-study (EOS) visit on Day 84.

If subjects terminate early from the study for reasons other than death, they will be asked to undergo an Early Termination (ET) Visit within 7 days of their termination; the reasons for early

termination will be documented. Since this is a single-dose study, subjects experiencing a product-related DLT will be asked to remain in the study and complete all study visits through the EOS visit. These subjects will not be replaced. If a subject terminates early for a reason other than a toxicity during the Treatment Period, the subject may be replaced at the discretion of the sponsor.

A DSMB will periodically convene and review all available clinical and laboratory data during the study. After its evaluation, the DSMB may recommend study continuation (with or without modification) or termination of a dose; the DSMB may also recommend de-escalation to a lower dose. If escalation is terminated, the next-lower dose may be declared the MTD and/or RP2D. Alternatively, an additional cohort at an intermediate dose may be added to better define the MTD and/or RP2D.

8.2 Safety Monitoring and Considerations

Study subjects will be under close medical supervision by the investigator throughout the study. Study product administration will be in the hospital. Scheduled visits for safety assessments will occur weekly during the Treatment Period. During the period of hospitalization, safety and efficacy assessments will be conducted daily.

Ongoing review of safety data for adverse trends will occur throughout the study. An unblinded DSMB with expertise in ARDS and infectious disease will review data prior to dose escalations and will provide recommendations to the Sponsor as described in the DSMB Charter and in Section **Error! Reference source not found.**. In addition, the Sponsor and/or Medical Monitor will perform regular ongoing review of safety data during the study.

To date, there have been no clinical studies using cePolyTregs. However, there has been extensive use of PolyTregs, the analogous autologous product, in a variety of clinical indications. In addition, several studies have evaluated a cord-blood derived allogeneic polyclonal Treg product in patients with GVHD, and a case report of treatment of 2 patients with COVID-19 has also been described. These studies have replicated many of the in vitro characteristics of Tregs found in cePolyTregs. However, the longevity of the Tregs in these clinical settings is much reduced compared to the autologous PolyTregs product, consistent with the suggestion that transfer of the cells into an allogeneic recipient leads to the recognition and elimination of the adoptively transferred product by the host immune system.

Nonclinical studies with autologous, homologous products have not demonstrated specific toxicities related to administration of adoptively transferred Tregs. Potential adverse events based on the biology of cePolyTregs include infusion/hypersensitivity reactions and infection-related events including increase in SARS-CoV-2 viremia, pneumonia, and infections involving pathogens not anticipated in a COVID-19 setting.

8.2.1 Infusion/Hypersensitivity Reactions

All biologic therapeutics, including cellular therapeutics, have the potential for causing infusion/hypersensitivity reactions; therefore, these reactions are regarded as class-specific toxicities for biologic therapeutics. Subjects will be closely observed for such reactions during this study. Appropriate facilities for treating infusion/hypersensitivity reactions and individuals trained in the management of such reactions will be immediately available during administration of cePolyTregs. Sites should be instructed to follow their institutional standard operating procedures for the management of infusion/hypersensitivity reactions, should they occur. These treatments may include, but are not limited to, higher doses of corticosteroids, intravenous antihistamines, etc.

8.2.2 Increase in SARS-CoV-2 Viremia, Pneumonia, and Infections Involving Pathogens Not Anticipated in a COVID-19 Setting

All immunomodulatory therapeutics have the potential for causing increased susceptibility to infections; in the present clinical context, this would most likely be manifest as an increase in SARS-CoV-2 viremia or increased incidence of infections including pneumonia or those involving pathogens not anticipated in a COVID-19 setting. Therefore, these reactions are regarded as class-specific toxicities for immunomodulatory therapeutics. Subjects will be closely observed for infections and increases in SARS-CoV-2 viremia during this study.

Enhanced Treg activity may be associated with an increased incidence and/or severity of infections with a variety of extracellular and intracellular pathogens. At the first indication of nascent or active infection, an aggressive diagnostic workup and subsequent antimicrobial therapy will be initiated according to the clinical protocol and local standard of care. As cePolyTregs are expected to be cleared rapidly following administration, it is anticipated that subjects will regain immune competence and clear their infections with appropriate supportive care.

8.3 Rationale for Study Design

Part 1 of this study will be an open-label, dose escalation trial. On-treatment safety and changes in PD measures will be monitored to determine the safety of cePolyTregs and identify the RP2D for further study in Part 2. Safety and tolerability of cePolyTregs will be evaluated by continuous assessment of AEs, vital signs, ECGs, clinical laboratory parameters, and chest imaging. The PD and biomarker profile associated with cePolyTregs will be evaluated through serial blood sampling. Dose escalation will occur by cohort following review of data by the DSMB to allow emerging safety, PD, and other data to inform dose escalation decisions based on pre-defined DLT criteria and stopping rules.

Part 2 of this study has a randomized, double-blinded, placebo-controlled design to minimize bias in allocation of subjects to treatment assignment and evaluation of safety and clinical activity.

These design features are intended to minimize risk to subjects and to allow exploration of a safe range of doses over relatively short treatment duration as well as to explore doses associated with PD and clinical activity.

8.4 Rationale for Selection of Starting Dose of cePolyTregs

The initial dose, route and regimen of cePolyTregs for Part 1 is proposed as a single dose of 1×10^8 cells by IV infusion over approximately 10 ml/min. This was selected based on a combination of practical considerations of manufacturing capacity, a predicted efficacious dose based on the expected Treg numbers in subjects, and the available safety data of the PolyTregs product currently in clinical trials for various indications.

8.4.1 Dosing Estimate Based on Rodent Models

The effective dose of cePolyTregs for controlling inflammation in ARDS in humans is currently unknown but there are indications from rodent studies. In a mouse model of LPS-induced and viral infection-induced ARDS, a single infusion of 1 million syngeneic Tregs was effective in disease prevention (D'Alessio 2009). Using the traditional exponential allometry for molecular therapy, 1 million cells per dose in a 20 g mouse would translate into ~400 million cells in a 70 kg human with an exponent of 0.75.

There are a number of limitations to this estimate. 1) No dose titration experiments in mice were reported so the minimal effective dose or optimal dose may be different from the estimated 1 million cells. 2) There is no established method for allometric scaling of cellular therapies that have distinct PK and PD from molecular therapies. 3) Mouse models of ARDS used syngeneic (i.e. autologous) Tregs. Allogeneic cePolyTregs proposed for this study contain 10% to 20% of cells that are reactive to mis-matched HLA combined with the existing self-antigen recognition repertoire. These cells are expected to be activated after infusion and proliferate in the recipient, which may potentiate the impact of the infused dose. 4) The Tregs used in studies of mouse models used mouse Tregs whereas the cePolyTregs proposed for use in this study are human Tregs. Due to interspecies incompatibilities of intercellular communications, testing human Treg products limits the utility of mouse models.

Thus, the actual effective dose of cePolyTregs in humans cannot be accurately estimated based on available animal data. The ~400 million cells per dose estimate can be viewed as a reference at best and thus is proposed as the top dose being explored in the current study.

8.4.2 Manufacturing Capacity

Since the inception of UCSF's Treg cell therapy programs, we have produced 43 PolyTregs doses in our GMP facility. For all these products, 1 unit of blood, or ~500×10^6 PBMCs were used as starting materials for Treg cell manufacture. Table 1 summarizes the manufacturing experience which is used to estimate manufacturing capacity.

Table 1. Manufacturing Capacity of Analogous, Autologous Product PolyTregs

	Treg Yield (number of cells)		
Quartile	**Day 0**	**Day 12**	**Day 14**
Median	4.2 million	578 million	1.5 billion
75th percentile	6.0 million	1.0 billion	3.0 billion
25th percentile	2.7 million	299 million	716 million

The manufacturing process for cePolyTregs will be the same as that for PolyTregs with the following 3 major distinctions: 1) cePolyTregs will be sourced from qualified healthy donors; 2) leukapheresis will be used so more Tregs can be collected on Day 0; and 3) products will be harvested on Day 12 instead of Day 14 of manufacture. We expect Day 0 cell yield to be on the higher end of our previous experience since donors can be prescreened for higher numbers of Tregs in blood. Additionally, each leukapheresis will yield 10 billion PBMCs, 20x that in one unit of blood. Thus, we can expect Day 0 cePolyTregs yield to be 20x higher than that in Table 1. Using a conservative estimate of 20 million Tregs on Day 0 and assuming a similar expansion rate as previously observed, we can expect a yield of ~3 billion cells at the Day 12 cePolyTregs harvest. This would translate into 30 doses at 100 million, 15 doses at 200 million, and 7 doses at 400 million cells. More donors can be recruited to repeat the process to scale production.

8.4.3 Safety of T Cell Therapy

Collective experiences with Treg cell therapy during the past 11 years has shown the therapy is generally safe and well tolerated (Esensten 2018).

The highest dose of autologous PolyTregs tested for safety in the Phase 1 Treg study in diabetes was 2.6 x 10^9 (range 2.35 to 2.94 x 10^9). Adverse events in the trial have generally been mild or moderate in severity. The most common adverse events have been related to mild upper respiratory infections. In the 24 hours following infusion, only 4 adverse events in 4 subjects were reported. Two were mild headache, one was mild nausea, and one was mild abdominal pain. Thus, infusion reactions have been limited.

Laboratory abnormalities have reflected protocol-specified phlebotomy. There have been minor elevations in lactate dehydrogenase (LDH) likely due to concomitant infection or minor trauma. One subject had notable transient elevations in transaminases and LDH subsequently determined to be due to intercurrent cytomegalovirus infection.

Finally, in the study described in Section 7.4 in which 2 subjects with COVID-19-related ARDS received infusions of cord-blood-derived Tregs (2 doses of 1 x 10^8 cells/dose in 1 subject and 3 of the same doses in the other) the doses were well-tolerated and clinical improvement was evident within 48 hours of infusion (Gladstone 2020). Although those cells were sourced differently, those results provided a reference point for selecting the starting dose for this trial.

8.5 Study Objectives

8.5.1 Primary Objectives

Parts 1 and 2: To evaluate the safety and tolerability of cePolyTregs administered as a single IV dose in subjects with ARDS associated with SARS-CoV-2 infection

Part 2 (Phase 2a): To evaluate the efficacy of cePolyTregs administered as a single IV dose in subjects with ARDS associated with SARS-CoV-2 infection

8.5.2 Secondary Objectives

Part 1 (Phase 1): To determine the RP2D of cePolyTregs administered as a single IV dose in subjects with ARDS associated with SARS-CoV-2 infection

Parts 1 and 2: To determine the pharmacodynamics and biomarker changes associated with cePolyTregs administration as a single IV dose in subjects with ARDS associated with SARS-CoV-2 infection

8.5.3 Exploratory Objective

To determine the clinical activity of a single IV dose of cePolyTregs on extrapulmonary manifestations of infection with SARS-CoV-2

8.6 Endpoints

8.6.1 Primary Endpoints

Part 1 (Phase 1):

Safety and tolerability of cePolyTregs as assessed by DLTs, defined as any related treatment-emergent adverse event (TEAE) with an NCI CTCAE 5.0 grade ≥ 3 which also represents a shift from baseline clinical status of ≥ 1 NCI CTCAE grade.

Part 2 (Phase 2a):

Efficacy of cePolyTregs compared with placebo/SOC, as assessed by time to recovery, defined as the first day during the 28 days after randomization on which a subject meets the criteria for category 0, 1, 2, 3, or 4 on the WHO severity scale for COVID-19 pulmonary syndrome.

Safety of cePolyTregs as assessed by the occurrence of related TEAEs with an NCI CTCAE 5.0 grade ≥ 3.

8.6.2 Secondary Safety Endpoints

Part 1 (Phase 1):

RP2D of cePolyTregs as assessed by cumulative safety, tolerability, pharmacodynamic and biomarker changes associated with administration of cePolyTregs compared with placebo/SOC

Parts 1 and 2:

Safety and tolerability of cePolyTregs as assessed by:

- Type and frequency of TEAEs
- Type and frequency of treatment-emergent serious adverse events (TESAEs)
- Type and frequency of adverse events of special interest (AESIs) including infusion/hypersensitivity reactions, increase in SARS-CoV-2 viremia, and infections including pneumonia and those involving pathogens not anticipated in a COVID-19 setting
- Type and frequency of changes in clinical laboratory values, physical examinations, electrocardiograms (ECGs), and vital signs

8.6.3 Secondary Efficacy Endpoints

Part 2 (Phase 2a):

Efficacy of cePolyTregs compared with placebo/SOC, as assessed by:

- The proportion of subjects with a 2-point or greater improvement in the WHO severity scale for COVID-19 pulmonary syndrome on each of Study Days 7, 14, 21, and 28
- The proportion of subjects with improvement in the WHO severity scale for COVID-19 pulmonary syndrome to Grade 3 or less on each of Study Days 7, 14, 21, and 28
- The proportion of subjects requiring mechanical ventilation on each of Study Days 7, 14, 21 and 28
- Number of days free of mechanical ventilation on Days 29 and 57
- Mean and median Sequential Organ Failure [SOFA] scores on each of Study Days 7, 14, 21 and 28
- Change in chest imaging (radiograph or CT scan) abnormalities
- All-cause mortality on Study Days 29 and 84

Parts 1 and 2:

Pharmacodynamic and biomarker changes associated with administration of cePolyTregs compared with placebo/SOC, including but not limited to:

- Change from baseline in circulating cePolyTregs
- Change from baseline in pulmonary cePolyTregs by endotracheal aspirate and mini BAL

- Change from baseline in ferritin, C-reactive protein, LDH, and high-sensitivity troponin I (hs-TnI)
- Change from baseline in d-dimer
- Change from baseline in absolute lymphocyte count
- Change from baseline in C3, C4, and CH50
- Change from baseline in inflammatory cytokines/chemokines, e.g. IL-6, IL-12, IFNγ, TNFα, MCP-1 and MCP-4
- Phenotypic and functional changes from baseline in immune subsets using cytometric, genomic and/or proteomic analyses of cells harvested from endotracheal tubes, mini BAL, and blood

8.7 Cohort Dose Escalation

Part 1 of this study will use a cohort dose escalation design. Each cohort will initially enroll 3 subjects. If 0 of 3 evaluable subjects experiences a DLT (Section 8.8.3), then advancement to the next cohort may occur. If 1 of 3 subjects experiences a product-related DLT, then additional subjects will be enrolled in the same cohort for a total of up to 6 evaluable subjects. If 1 out of the 6 subjects experiences a product-related DLT, then advancement to the next cohort may occur. If 2 or more subjects in a cohort of up to 6 subjects experience a product-related DLT, no additional subjects will be enrolled in that cohort until a complete evaluation of all available data can be conducted and an opinion can be rendered by the DSMB as to whether the dose has exceeded the MTD. Dose escalation is allowed to proceed if no predefined stopping criteria are met.

Based on cumulative safety, tolerability, product manufacturing feasibility, and PD data from all Part 1 cePolyTregs cohorts (including biomarker data listed in Table 4), the RP2D will be determined for use in Part 2 of the study. The cePolyTregs RP2D will be received by 6 treated subjects in Part 1.

Part 1 cohort enrollment will proceed in a sequential manner to evaluate escalating dose levels of cePolyTregs. The first dose level to be studied will be 1 x 10^8 cells by IV infusion over approximately 1 hour and the maximum dose level to be studied will be 4 x 10^8 cells. An intermediate dose level of 2 x 10^8 cells is planned. Alternatively, an additional cohort at an intermediate dose may be added to better define the MTD and/or RP2D.

8.8 Data Safety Monitoring Board, Maximum Tolerated Dose Determination, Dose-Limiting Toxicity, and Stopping Criteria

8.8.1 Data Safety Monitoring Board

The DSMB membership will consist of independent medical experts with expertise in infectious disease, pulmonary medicine, and/or clinical research. The full details of the DSMB structure and responsibilities are provided in the DSMB Charter.

The RP2D determination will be made based on Part 1 (Phase 1) cohorts in which each new higher dose will be studied until the RP2D is identified; that RP2D will then be studied in Part 2 (Phase 2).

Dose escalation is allowed to proceed if no predefined stopping criteria are met. Dose escalation decisions will be based on the required number of subjects in a cohort completing the 7-day DLT assessment period. After the last subject in each cohort has completed the DLT assessment period, the DSMB will conduct a review of all available data (safety and tolerability) from both the current cohort and cumulative data from all cePolyTregs cohorts before determining recommendations for advancement to the next dose level. Details of cohort dose escalation are provided in Section 8.7.

Additional subjects may be enrolled in a cohort if considered necessary by the DSMB to further characterize product safety, tolerability, or efficacy. Also, the DSMB may recommend modification of the doses to be evaluated including de-escalation to a lower dose, to not initiate specific dose cohorts, to initiate additional dose cohorts, or to terminate the study if it is deemed necessary. If escalation is terminated, the next-lower dose may be declared the MTD and/or RP2D. Appropriate regulatory approvals will be obtained prior to initiation of any DSMB-recommended protocol changes, as required.

8.8.2 Maximum Tolerated Dose

The MTD is defined as the cePolyTregs dose below the dose at which 2 or more of 6 subjects receiving cePolyTregs in a Part 1 cohort experience a DLT, as confirmed by the DSMB.

8.8.3 Dose-Limiting Toxicities

A DLT is defined as any related TEAE ≥ NCI CTCAE 5.0 Grade 3 in a subject who has received treatment with cePolyTregs which also represents a shift from baseline clinical status of ≥ 1 NCI CTCAE grade.

Adverse events related to the standard of care (e.g., dexamethasone) are not considered DLTs. TEAEs related to ARDS will not be considered DLTs, unless they are judged to be related (or also related) to cePolyTregs.

8.8.4 Individual Subject Study Drug Dosing Discontinuation

Dosing of cePolyTregs will be permanently discontinued in a study subject if any of the following occur:

- Any DLT
- Subject withdraws consent
- Pregnancy
- Subject is unable to comply with the protocol requirements
- Sponsor terminates the study

- A regulatory authority mandates dosing cessation

In all cases, necessary measures will be taken to ensure appropriate safety follow-up of all subjects in the trial.

8.8.5 Subject Replacement

Subjects experiencing a product-related DLT will discontinue dosing and be asked to remain in the study and complete all study visits through the EOS visit; these subjects will not be replaced. If a subject terminates early for a reason other than a toxicity during the Treatment Period, the subject may be replaced at the discretion of the Sponsor.

8.8.6 Study Discontinuation

The study may be stopped at the discretion of the Sponsor based on recommendations of the DSMB. The study may also be stopped pending DSMB evaluation of all available safety and PD data if any of the following occur:

- Any study drug-related death
- The occurrence of 3 deaths within 28 days post treatment in the first 5 subjects treated or, subsequent to the first 5 subjects, the observed mortality rate across the cumulative population of treated subjects exceeds the anticipated mortality rate of 50%
- Two CTCAE Grade 4 or 5 TEAEs that are deemed related to study drug
- An increase in the incidence of infections of special interest (pneumonia or infections involving pathogens not anticipated in a COVID-19 setting) beyond the expected background incidence

If the DSMB recommendation is to interrupt study dosing for all subjects or to pause enrollment, study dosing can be resumed, based on the recommendation of the DSMB, only after further review of all available safety data and applicable notifications of/submission to appropriate regulatory agencies and institutional review committees, in line with country-specific regulations.

All deaths occurring during the study will be reviewed promptly by the DSMB as they occur. If at any point following enrollment of the first 5 subjects, the observed mortality rate across the cumulative population of treated subjects exceeds the anticipated mortality rate of 50%, the study will be halted.

9 STUDY POPULATION

Each cohort will enroll unique subjects who may not be treated in more than 1 cohort.

9.1 Inclusion Criteria

1. Diagnosis of ARDS and respiratory failure requiring mechanical ventilation for less than 72 hours at the time of enrollment
2. $PaO_2/FiO_2 < 300$ and PEEP > 5
3. Male or female, age 18 to 70 years at Screening
4. Weight > 40 kg
5. Documented diagnosis of infection with SARS-CoV-2 virus by PCR
6. Chest imaging (radiograph or CT scan) with abnormalities consistent with COVID-19 pneumonia that could not be explained by effusions, pulmonary collapse, or nodules; and respiratory failure that could not be explained by cardiac failure or fluid overload
7. Females of childbearing potential and males must use effective contraception practices from Screening until 28 days after the EOS visit
8. Females of childbearing potential must have a negative pregnancy test at Screening and within 24 hours prior to dosing of study drug
9. Able to provide Informed Consent, either by self or by medical proxy
10. Willing and able to comply with this protocol for the entire duration of the study

9.2 Exclusion Criteria (Parts 1 and 2)

Subjects will be ineligible for enrollment in the study if they meet any of the following criteria:

1. Any history or sign of significant chronic active or recurrent infection or screening laboratory evidence consistent with a significant chronic active or recurrent infection requiring treatment with antibiotics, antivirals or antifungals (other than SARS-CoV-2); ongoing antimicrobial treatments will not be exclusionary if, in the opinion of the investigator, no active infection is present (other than SARS-CoV-2)
2. Receiving extracorporeal membrane oxygenation therapy
3. Moribund patients not expected to survive 24 hours after enrollment based on clinical assessment
4. History of significant underlying pulmonary disease (requiring home oxygen), renal disease (requiring dialysis for chronic kidney disease), hepatic disease (Child-Pugh score ≥ 7), or known history of cirrhosis.
5. Known or suspected immunodeficiency disease
6. Positive serology for HBV, HCV, or HIV at Screening

7. Abnormal CBC defined by:
   a. Platelet count < 75,000/mm^3
   b. White blood cell count < 2500/mm^3
   c. Absolute neutrophil count < 500/mm^3
8. History of bone marrow or stem cell transplantation
9. Received any type of live attenuated vaccine < 1 month prior to Screening or is planning to receive any such live attenuated vaccine over the course of the study
10. History of lung cancer or any other malignancy requiring active treatment, except adequately treated basal cell carcinoma or in situ carcinoma of the uterine cervix
11. Any female who is pregnant or breastfeeding, or any female who is planning to become pregnant during the study and follow-up period
12. Any condition that, in the investigator's opinion, may compromise study participation, present a safety risk to the subject, or may confound the interpretation of the study results
13. A QT duration corrected for heart rate by Fridericia's formula (QTcF) > 450 millisecond (msec) for males or > 470 msec for females, based on either single or averaged QTcF values of triplicate ECGs obtained over a 3-minute interval
14. Currently enrolled in another investigational device or drug study
15. Greater than 10 days of continuous (>12 hours/day) administration of oxygen via non-invasive positive pressure ventilation or high-flow nasal cannula oxygen for hypoxemic respiratory failure due to COVID-19

10 STUDY TREATMENTS

10.1 Description of Treatments

Detailed information regarding the storage, preparation, and administration of cePolyTregs and placebo for this study is contained in the cePolyTregs Cell Therapy Manual.

All subjects in both parts of the study will receive standard of care treatment for COVID-19, including dexamethasone per institutional guidelines and other approved therapies for ARDS associated with SARS-CoV-2 infection per institutional guidelines.

In addition, all subjects in the Part 1 cohorts will receive open-label cePolyTregs. All subjects in the Part 2 cohorts will be randomized in a blinded fashion to receive either cePolyTregs or matching placebo using a 1:1 (active:placebo) allocation ratio.

The study drugs are:

- cePolyTregs containing polyclonal Treg cells that have been collected from qualified donors by leukopheresis under GMP conditions, purified to yield a population of $CD4^+CD127^{lo/-}CD25^+$ regulatory T cells, expanded using anti-CD3/anti-CD28-coated magnetic microbeads and IL-2 for 12 to 13 days, debeaded and cryopreserved in Cryostor® CS5 solution at a concentration of $1x10^7$ cells/mL.
- Placebo, containing an inactive substance designed to mimic the excipient of the investigational product, at a matched volume to the cePolyTregs infusion.

Planned single doses of cePolyTregs in Part 1 of the study are as follows:

- 1×10^8 cells by manual IV infusion;
- 2×10^8 cells by manual IV infusion;
- 4×10^8 cells by manual IV infusion

Single doses of cePolyTregs in the placebo-controlled Part 2 of the study will be determined based on the results observed in Part 1.

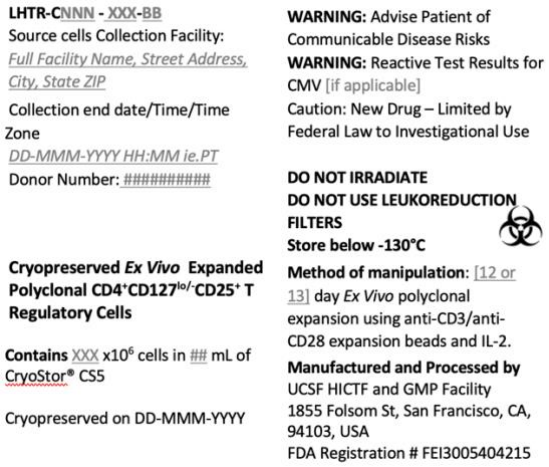
10.2 Packaging and Labeling

cePolyTregs are cryopreserved in either CryoMACS® Freezing Bag 50 or CryoMACS® Freezing Bag 250 depending on the dose. The CryoMACS® Freezing Bag consists of a freezing bag and corresponding overwrap bag. Both the freezing bag and overwrap bags are composed of ethylene vinyl acetate tubular film and are individually packed and sterilized.

cePolyTregs are distributed to clinical study sites and stored in liquid nitrogen until use.

cePolyTregs will be labeled by the manufacturing site with product information including: product name and identifiers, method of manipulation, formulation, source cell material details,

date of cryopreservation, storage instructions, and all applicable statements and warnings. Product will be issued to each subject by the clinical site prior to infusion.



As is generally the case with blood products and cellular therapies, the labels on the cePolyTregs bags and the placebo bags will be different and will need to be checked at bedside as part of the infusion process. Consequently, study product identity will be blinded for the subjects and investigators but will not be blinded for other site staff.

10.3 Study Drug Dosage Preparation

cePolyTregs will be kept in a monitored freezer in vapor phase liquid nitrogen (LN_2) until the time of infusion. Stability after thawing is 4 hours. The long-term stability of cePolyTregs in LN_2 freezers is provided in the current Investigator's Brochure.

cePolyTregs will be prepared and administered under sterile conditions according to the Cell Therapy Manual. The cells will be thawed at the bedside using a water bath or comparable device maintained at 34°C to 38°C by trained personnel. There should be no frozen clumps left in the container at the time it is infused. If the cePolyTregs cell product appears to be damaged or the bag appears to be compromised, it should not be infused, and the site should notify HICTF and the GMP facility immediately.

10.4 Study Drug Administration

The cePolyTregs will be infused intravenously into an 18-gauge (or larger) intravenous catheter, either through a peripheral vein (preferred) or central vein. The cells are drawn into a 60 mL syringe via a plasma transfer set and manually infused. A leukoreduction filter must not be used for the infusion.

Emergency medical equipment (i.e., emergency crash cart) must be available during the infusion in case the subject has an allergic response, severe hypotensive crisis, or any other reaction to the infusion. Vital signs (temperature, respiration rate, pulse, SpO2, and blood pressure) will be

taken before and for a minimum of 30 minutes after infusion and until these signs are satisfactory and stable.

10.5 Management of Infusion/Hypersensitivity Reactions

As noted above, emergency medical equipment (i.e., emergency crash cart) must be available during the infusion in case the subject has an allergic response, severe hypotensive crisis, or any other reaction to the infusion. Vital signs (temperature, respiration rate, pulse, SpO2, and blood pressure) will be taken before and for a minimum of 30 minutes after infusion and until these signs are satisfactory and stable. Sites should be instructed to follow their institutional standard operating procedures for the management of infusion/hypersensitivity reactions, should they occur.

10.6 Prior and Concomitant Medications

Restrictions that apply to the period before the first admission are described in Section 9.2. Details of prior and concomitant medications and treatments will be collected at the time of enrollment and throughout the study.

All subjects in both parts of the study will receive standard of care treatment for COVID-19, including dexamethasone and other approved therapies for ARDS associated with SARS-CoV-2 infection per institutional guidelines. Concomitant use of any investigational treatments other than cePolyTregs for COVID-19 will not be permitted.

10.7 Enrollment or Randomization and Blinding

It is the investigator's responsibility to ensure that subjects are eligible to participate in the study prior to enrollment and continue to remain eligible throughout the study.

In Part 1 of the study, subjects will be enrolled and treated with open-label cePolyTregs sequentially in the order in which they are screened.

In Part 2 of the study, subjects will be assigned a subject identification number after the informed consent is signed using a centralized interactive web response system (IWRS) or other secure methodology. At randomization (Day 0), a centralized schema will be applied to randomize subjects in a 1:1 ratio to cePolyTregs or placebo. The system will stratify the randomized subjects by study site. All subjects and investigators will be blinded to treatment group throughout Part 2 of the study. Other study center staff will not be blinded because of differences in the product labels for active vs placebo product (see Section 10.2). The investigator may request emergency unblinding for safety reasons; subjects unblinded for safety reasons should continue in the study for assessment of outcomes. Subjects who are randomized but do not receive study drug may be replaced.

10.8 Storage and Accountability

cePolyTregs will be kept in a monitored freezer in vapor phase LN2 until time of infusion. Stability after thawing is 4 hours. The long-term stability of cePolyTregs in LN2 freezers is provided in the current Investigator's Brochure.

The Sponsor or designee will supply the study drug. The site will maintain the following records: receipt of shipments, administration to subjects, and return of partially used, or unused study drug. Upon completion or early termination of the study, all unused and partially used investigational product must be returned to the Sponsor (or designated agent), unless the Sponsor authorizes the study site to destroy the investigational product.

11 STUDY PROCEDURES

The following assessments will be conducted during screening and at the time points specified in the Schedule of Events (SOE) (Section 5.2). All missed visits must be documented in the subject's medical record and the appropriate eCRF.

11.1 Screening and Informed Consent

Each subject must sign and date the ICF before participating in any study-specific activities. The ICF may be signed prior to the Screening Visit.

After the ICF is signed, subjects in Part 2 will be assigned a unique subject identification number by IWRS or other secure methodology.

The subject number, assigned at the time of screening, will be used to identify the subject during the study and must be used on all study documentation related to that subject. The subject identification number will remain constant throughout the entire study and must not be changed after initial assignment. Each study site will maintain a list identifying all subjects by subject identification number and subject initials.

After completing the Screening Period, the subject will be evaluated by the investigator to confirm eligibility. A subject is considered to have started screening on the date that the Screening Visit is recorded. Screen failure subjects will be entered into the study records. Investigators will maintain a screening log of all potential study subjects that includes limited information about each candidate, including dates of screening and procedures, and the outcome of the screening process (e.g., enrolled into the study, reason for ineligibility, or withdrawal of consent).

At Baseline and before any study drug is administered, potential subjects will be reviewed by the site to reconfirm their eligibility.

11.2 Demographics and Medical/Surgical History

Demographic data will be collected, including each subject's sex, age, race, and ethnicity. Where local regulations do not permit certain demographic data to be collected, collection of those data will not be required.

The investigator or designee will collect the subject's medical and surgical histories. All findings will be recorded on the medical history and surgical history eCRFs.

11.3 Physical Examination

The investigator or designee will conduct a complete physical examination as outlined in the SOE. Physical examination clinically significant findings prior to the first dose of study drug administration will be recorded on the medical history eCRF; clinically significant, treatment-emergent findings after the first study drug dose will be recorded as AEs.

At a minimum, the *complete physical examination* (Baseline) should include assessments of the skin, head and neck, lungs, cardiovascular system, abdomen, and extremities. A *targeted physical examination* (all other visits) will include assessment of any new subject complaints or changes from baseline.

11.4 Vital Signs

The following vital sign measurements will be performed: systolic and diastolic blood pressure, pulse, respiration rate, SpO2, and temperature (height and weight at the Screening Visit only). Vital signs (except for temperature and weight) will be monitored periodically for a minimum of 30 minutes after administration of IP. Subject must be seated or in a semi-recumbent position in a rested, calm state for at least 3 minutes before vital signs are collected.

11.5 12-Lead Electrocardiography

12-Lead ECGs will be obtained locally on standard ECG equipment available at the study site. The subject must be in a semi-recumbent or supine position in a rested, calm state for at least 10 minutes before ECG assessment is performed. Each 12-lead ECG should be performed prior to blood draws, dosing, or other invasive procedures whenever possible.

The investigator or designated study site physician will review, sign, and date all ECGs.

Any clinically significant, treatment-emergent abnormal ECG findings will be recorded as an AE.

11.6 WHO Severity Scale for COVID-19 Pulmonary Syndrome

The WHO ordinal severity scale (Figure 2) was derived by a special WHO committee to measure illness severity over time (World Health Organization 2020). Subject status will be assessed using the severity scale at the intervals described in the SOE.

Figure 2. WHO Ordinal Scale for COVID-19 Clinical Improvement

Patient State	Descriptor	Score
Uninfected	No clinical or virological evidence of infection	0
Ambulatory	No limitation of activities	1
	Limitation of activities	2
Hospitalized Mild disease	Hospitalized, no oxygen therapy	3
	Oxygen by mask or nasal prongs	4
Hospitalized Severe Disease	Non-invasive ventilation or high-flow oxygen	5
	Intubation and mechanical ventilation	6
	Ventilation + additional organ support – pressors, RRT, ECMO	7
Dead	Death	8

11.7 Sequential Organ Failure Score (SOFA)

The SOFA score is an objective score that allows for calculation of both the number and severity of organ dysfunction in 6 organ systems (respiratory, coagulation, liver, cardiovascular, renal, and neurologic; Jones 2009) (Table 2). The score can measure individual or aggregate organ dysfunction. The SOFA will be assessed at the intervals specified in the SOE.

Table 2. The Sequential Organ Failure Assessment (SOFA) Score

SOFA score	1	2	3	4
Respiration [a]				
PaO_2/FIO_2 (mm Hg)	<400	<300	<220	<100
SaO_2/FIO_2	221-301	142-220	67-141	<67
Coagulation				
Platelets $\times 10^3/mm^3$	<150	<100	<50	<20
Liver				
Bilirubin (mg/dL)	1.2-1.9	2.0-5.9	6.0-11.9	>12.0
Cardiovascular [b]				
Hypotension	MAP <70	Dopamine ≤5 or dobutamine (any)	Dopamine >5 or norepinephrine ≤0.1	Dopamine >15 or norepinephrine >0.1
CNS				
Glasgow Coma Score	13-14	10-12	6-9	<6
Renal				
Creatinine (mg/dL) or urine output (mL/d)	1.2-1.9	2.0-3.4	3.5-4.9 or <500	>5.0 or <200

CNS = central nervous system; MAP = mean arterial pressure; SaO_2 = peripheral arterial oxygen saturation.

[a] PaO_2/FIO_2 ratio was used preferentially. If not available, the SaO_2/FIO_2 ratio was used.

[b] Vasoactive medications administered for at least 1 hr (dopamine and norepinephrine µg/kg/min).

11.8 Mechanical Ventilation Status

If the patient is receiving invasive mechanical ventilation, ventilatory parameters will be collected daily between 06:00 and 10:00, including the following, per local procedure: Mode of ventilation, FiO2(Fraction of inspired oxygen), SpO2 , plateau pressure, peak pressure, PEEP (Positive End-Expiratory Pressure). If an ABG (arterial blood gas) is obtained during that time, then the PaO2 (Partial pressure of arterial oxygen) will also be collected.

If the patient is receiving non-invasive ventilation, the level of inspiratory pressure, FIO2, and PEEP will be registered. If the patient is receiving high-flow oxygen, the flow rate and the FIO2 will be recorded.

The proportion of subjects requiring mechanical ventilation will be assessed on Study Days 7, 14, 21, and 28, and the number of days free of mechanical ventilation will be assessed on Days 28 and 56.

11.9 Chest Imaging

Radiograph or CT imaging will be performed as scheduled in the SOE to the extent feasible. Subjects who are ambulatory and discharged from hospital may forego further imaging if impracticable without incurring a protocol deviation.

11.10 Clinical Laboratory Tests

Blood and urine samples will be collected according to the SOE and below. All laboratory tests will be performed at the local laboratory. Samples will be collected pre-dose on Day 0 as per SOE. Repeat laboratory testing will not be required if a test was already performed within the specified time window for collection. Samples may be analyzed for the tests outlined in this protocol and for any additional tests (with the Sponsor's approval) necessary to further evaluate subject safety. These may include, but are not limited to, investigation of unexpected results. Subjects will be in a seated, semi-recumbent, or supine position during blood collection. Collection procedures are described in the study Laboratory Manual.

Table 3 presents the clinical laboratory assessments to be performed.

Table 3. Clinical Laboratory Parameters

Chemistry	Hematology	Urinalysis	Viral Studies
• Sodium • Potassium • Chloride • Bicarbonate • Calcium • Magnesium • Phosphorus • Glucose • BUN • Creatinine • Total protein • Albumin • Uric acid • LDH • Total bilirubin • Direct bilirubin • Indirect bilirubin • Alkaline phosphatase • ALT • AST • GGT	• RBC count • Hgb • Hct • Platelet count • WBC count with differential: neutrophils, lymphocytes including absolute CD4 and CD8 count, monocytes, eosinophils, and basophils (absolute counts and percentages)	• Appearance • Color • Specific gravity • pH • Blood • Protein • Glucose • Bilirubin • Urobilinogen • Leukocyte esterase • Nitrite Microscopic analysis: • WBC • RBC • Epithelial cells • Bacteria • Casts and/or crystals[a]	• Anti-HIV Ab • HBsAg • Anti-HCV Ab (if positive, HCV RNA will be measured) **Other** • Urine pregnancy test[b] • Ferritin • C-reactive protein • d-dimer • high-sensitivity troponin

Abbreviations: ALT = alanine aminotransferase; AST (SGOT) = aspartate aminotransferase; BUN = blood urea nitrogen; GGT = gamma-glutamyl transferase; HBsAg = hepatitis B surface antigen; Hct = hematocrit; HCV = hepatitis C virus; Hgb = hemoglobin; HIV = human immunodeficiency virus; LDH = lactate dehydrogenase; RBC = red blood cell; RNA= ribonucleic acid; WBC = white blood cell

[a] Microscopic analysis of urine should only be performed if clinically indicated.

[b] Pregnancy testing only for women of childbearing potential.

11.11 Exploratory Pharmacokinetic and Pharmacodynamic Biomarkers

Table 4 summarizes laboratory mechanistic analyses for assessing PK and PD of cePolyTregs using samples collected as described in the SOE.

Table 4. Mechanistic Assessments of cePolyTregs PK and PD

PK Markers
Change from baseline in circulating cePolyTregs
Change from baseline in pulmonary cePolyTregs by endotracheal aspirate and mini BAL
cePolyTregs expansion and activation using scRNA/TCR-sequencing
PD Markers
Change from baseline in acute phase reactants, e.g. ferritin, C-reactive protein, LDH, and albumin
Change from baseline in endothelial cell injury markers, e.g. d-dimer, soluble thrombomodulin, VWF, and angiopoietin-2
Change from baseline in lung epithelial cell and other tissue injury markers, e.g. RAGE, KL-6, SF-D, and troponin I
Change from baseline in absolute lymphocyte count
Change from baseline in inflammatory markers, e.g. IL-6, IL-12, IFNγ, GM-CSF, TNFα, IP10, MCP-1, MCP3, C3, C4, CH50, and MCP-4
Phenotypic and functional changes from baseline in immune subsets using cytometric, genomic and/or proteomic analyses of cells harvested from endotracheal tubes, mini BAL and blood
Anti-SARS-CoV-2 T cell immunity
Anti-SARS-CoV-2 humoral immunity

11.12 Removal of Subjects from the Study

At any time, the investigator can remove a subject from the study or discontinue study drug dosing, if deemed necessary for the subject's safety. The Sponsor also reserves the right to terminate the study at any time. The reason for early treatment discontinuation and early withdrawal from the study will be collected in the eCRFs. Subjects who discontinue treatment early should continue to attend all Follow-up study visits unless they have withdrawn consent for any further follow-up.

If a subject requests or decides to withdraw consent from the study, all efforts will be made to complete and report the observations as thoroughly as possible up to the subject's date of study withdrawal.

11.13 Ventilator Procedures

Ventilator management, including weaning, shall follow the modified ARDS Network lower tidal volume (6 ml/kg PBW) protocol (Appendix A **Ventilator Protocol**). If not already being used, this low tidal volume protocol for mechanical ventilation shall be initiated within one hour of randomization. Since the time a subject achieves unassisted ventilation affects the co-primary endpoint, days free of ventilation, and because recent evidence-based consensus

recommendations have identified a best practice for weaning, weaning strategy will also be controlled by protocol rules in accordance with these evidence-based recommendations (Appendix B **Ventilator Weaning Procedure**). This will assure similar weaning methods and provide potential benefit to both study groups. This newer weaning strategy is a simplified version of the protocolized weaning strategy used in prior ARDS Network studies.

11.14 Assessment of Concomitant Medications

Reportable concomitant medications will be captured in the electronic database system as defined below based on timing of study drug administration. Medication doses can be reported as ranges and changes in dose can be reported if deemed clinically significant by the investigator.

Prior to study drug administration:

- Vaccine status
- COVID-specific therapies
- Immunosuppressive therapies (steroids, DMARDS, biologics)
- Antibiotics
- Vasopressors

Following study drug administration:

- COVID-specific therapies
- Immunosuppressive therapies
- Antibiotics
- Vasopressors
- Neuromuscular blockade
- Any treatment for an AE
- Changes in BP meds or addition of new meds (hypotension + hypoxemia)
- Change in oxygen/ventilator

12 ADVERSE EVENTS

An AE is defined as any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease or any worsening of a pre-existing condition temporally associated with the use of a study drug, whether or not related to study drug. From signing the ICF to study drug administration, all SAEs and nonserious AEs related to protocol-mandated procedures will be recorded on the SAE/AE eCRF. All other untoward medical occurrences observed during screening, including exacerbation or changes in the medical and surgical history will be captured on the medical and surgical history eCRF.

An AE that occurs before the first administration of the study drug will be considered a pre-treatment AE. A TEAE is defined as any adverse event that was not present before the first administration of the study drug, or any event already present, that worsens in either severity or frequency, following exposure to the study drug. AEs that occur after treatment with IP or during the Safety Follow-up Period will be documented on the AE eCRF. The investigator will assess AE severity, the causality relationship of the AE to study drug, and will treat the subject as medically required to ensure the subject's safety.

AEs will be captured through the EOS Visit at Day 56 or the ET Visit. Reportable adverse events are defined based on timing of study drug administration, as follows:

- Treatment – Day 7: AEs grade 3+ that are not expected to occur in critically ill patients, per the discretion of the investigator.
- Treatment Day 8 – Day84/EOT: AEs grade 4+ that are not expected to occur in critically ill patients, per the discretion of the investigator.

Events expected in the critically ill and are being captured in the eCRF should not be reported as adverse events unless considered to be related to the study drug or unexpectedly severe or frequent per the discretion of the investigator. These include but are not limited to; need for vasoactive drugs or fluids for hypotension, decreased PaO2/FiO2, transient hypoxemia, need for renal replacement therapy, or worsening acute respiratory distress syndrome.

Investigators should use their clinical judgment to determine whether a subject is to be withdrawn due to an AE. In the event the subject requests to withdraw from study-related treatment or the study due to an AE, the subject should be asked to return for all remaining study visits. In the event a subject is withdrawn from the study, an ET Visit should be completed. All subjects experiencing AEs, including clinically significant abnormal laboratory values, whether associated with the study drug, must be monitored until the condition (1) returns to normal, (2) returns to the subject's baseline, (3) the investigator determines the AE has reached a stable outcome and is no longer clinically significant, or (4) the subject is considered lost to follow-up.

12.1 Serious Adverse Events

A *serious adverse event* (SAE) is defined as any AE occurring at any dose that results in any of the following outcomes:

- Death
- Life-threatening AE (Note: A life-threatening AE is one that, in the view of the investigator places the subject at immediate risk of death from the reaction as it occurred)
- Hospitalization or prolongation of existing hospitalization
- A persistent or significant disability/incapacity
- A congenital anomaly/birth defect.

Important medical events that may not result in death, be life threatening, or require hospitalization may be considered an SAE when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include blood dyscrasias or convulsions that do not result in subject hospitalization, or the development of drug dependency or drug abuse.

12.2 Adverse Events of Special Interest

To date, there have been no clinical studies using cePolyTregs, and nonclinical studies with autologous, homologous products have not demonstrated specific toxicities related to administration of adoptively transferred Tregs. Potential adverse events of interest in the COVID-19 setting based on the biology of cePolyTregs include infusion/hypersensitivity reactions and infections including pneumonia and those involving pathogens not anticipated in a COVID-19 setting; these clinical parameters will be closely monitored in the current study.

The following events are defined as AESIs that should be reported by completing the Adverse Event eCRF and AESI eCRFs within 24 hours of the site's awareness of them, even if they do not meet criteria for an SAE:

- Evidence of hypersensitivity or transfusion incompatibility (e.g. new rash, urticaria, new onset bronchospasm).
- Diagnostically or clinically confirmed respiratory infections that were not present before study drug infusion AND that require a treatment course with antimicrobial agents, operative management, or procedural drainage.
- Within 6 hours of IP exposure:
    - New ventricular tachycardia, ventricular fibrillation, or asystole or any cardiac arrhythmia requiring cardioversion.
    - Hypoxemia requiring an increase in FiO2 of 0.2 or more and/or an increase in PEEP of 5 cm H2O or more to maintain SpO2 in the target range of 88–95%, for a sustained duration (>2 hours) and not associated with a care procedure.

- Pulmonary Embolism

12.3 Clinical Laboratory Adverse Events

The investigator is responsible for reviewing the results of all laboratory tests as they become available and determining whether an abnormal value in an individual subject is a clinically significant change from the subject's baseline value(s). The investigator may repeat the laboratory test or request additional tests to verify the results of the original laboratory tests. In general, abnormal laboratory findings without clinical significance (based on the investigator's judgment) are not to be recorded as AEs. Laboratory values that are outside the laboratory reference range should be reported as AEs only if considered by the investigator to be clinically significant. Laboratory value changes that require treatment or adjustment in current therapy are to be considered AEs. When applicable, clinical sequelae (not the laboratory abnormality) are to be recorded as the AE.

12.4 Severity

All AEs, both serious and nonserious, will be assessed for severity using the NCI CTCAE v 5.0. The CTCAE scale includes unique clinical descriptions of AEs categorized by anatomy and/or pathophysiology. Reference the following website:

https://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/CTCAE_v5_Quick_Reference_8.5x11.pdf

The CTCAE scale displays Grades 1 through 5 with unique clinical descriptions of severity for each AE (including abnormal laboratory values), based on this general guideline provided in the scale. For AEs not covered by CTCAE, the conventional definition of severity will be used, as follows:

- Grade 1 (mild) AE: Minor; no specific medical intervention; marginal clinical relevance.
- Grade 2 (moderate) AE: Minimal intervention; local intervention; noninvasive intervention.
- Grade 3 (severe) AE: Significant symptoms requiring hospitalization or invasive intervention.
- Grade 4 (life-threatening or disabling) AE: Complicated by acute, life-threatening complications; need for intensive care or emergent invasive procedure.
- Grade 5: Fatal AE

12.5 Causality Assessment

The investigator or qualified sub-investigator is responsible for assessing and assigning the causality relationship of the event to study drug or study-related procedures (e.g., invasive

procedures, such as venipuncture) using clinical judgment and the following categories of relatedness:

- Related: There is at least some possibility that the AE could be related to study medication administration.
- Not related: There is a high degree of certainty that the AE is NOT related to study medication administration.

In making a causality assessment of an AE, it should be considered as to whether or not the AE is expected to occur due to the underlying disease, based on the investigator's clinical experience.

12.6 Reporting Adverse Events

12.6.1 Reporting Procedures for Nonserious Adverse Events

The investigator is responsible for ensuring that all reportable AEs observed by the investigator or designee or reported by the subject are reported using the AE eCRF.

The investigator will assign the following AE attributes:

- AE diagnosis or syndrome(s), if known (if not known, signs or symptoms)
- Dates of onset and resolution (if resolved)
- Severity
- Causality relationship to study drug or study-related procedures
- Action taken
- Outcome

Follow-up of nonserious AEs will continue through the last day on the study and/or until a definitive outcome (e.g., resolved, resolved with sequelae, lost to follow-up) is achieved.

When a subject is withdrawn from the study because of a nonserious AE, the Sponsor and/or designee must be notified by email or phone within 48 hours.

12.6.2 Reporting Procedures for Serious Adverse Events

All SAEs, regardless of cause(s) or causal relationship to study drug, must be reported within 24 hours to the study Sponsor and/or designee. The investigator is responsible for ensuring that all SAEs observed by the investigator or reported by the subject are promptly assessed and reported to the Sponsor and/or designee. The investigator must assess the causality relationship of the SAE to study drug or any study-related procedure.

The procedures for reporting SAEs are as follows:

- Within 24 hours of the investigator's knowledge of the event, enter data into the AE/SAE eCRF. If the electronic data capture system (EDC) is not available, the paper SAE Report Form may be used, but all data must be entered into the EDC once the EDC is available.
- Additional contact numbers and AE/SAE reporting instructions will be provided in the eCRF Completion Guidelines.
- For fatal or life-threatening events and all SAEs, also email and/or fax redacted copies of hospital records, autopsy reports, and other documents, when requested and applicable. Transmission of such documents should occur with personal subject details de-identified (redacted), without losing the traceability of a document to the subject identifiers. Entry of the initial report of the event into EDC should not be delayed in order to include these additional documents.
- The Sponsor and/or designee may request additional information from the investigator to ensure the timely completion of accurate safety reports.

The investigator must take all necessary therapeutic measures for resolution of the SAE. Any medications or therapies necessary for treatment of the SAE must be recorded in the event description section of the SAE form and the concomitant medication eCRF.

Follow-up of SAEs will continue through the last day on the study and/or until a definitive outcome (e.g., resolved, resolved with sequelae, lost to follow-up, fatal) is achieved.

While pregnancy is not considered an AE, all cases of fetal drug exposure via a maternal parent as study subject or pregnancy of a partner of a male study subject will be reported immediately to the Sponsor or its designee. Information related to the pregnancy must be documented on a Pregnancy Confirmation and Outcome Form, provided by the Sponsor or its designee, and the pregnancy should be followed until a definitive outcome has been determined.

The Sponsor or its designee will report SAEs as required to regulatory authorities and investigators in compliance with all reporting requirements, according to local regulations and ICH Good Clinical Practice (GCP).

The investigator will notify the appropriate Institutional Review Board (IRB) of SAEs occurring at the study site and other AE reports received from the Sponsor, in accordance with local requirements. The investigator or designee at each study site is responsible for submitting safety reports (initial and follow-up) and other safety information (e.g., in the revised cePolyTregs Investigator's Brochure) to the IRB and/or other applicable regulatory authorities and for retaining a copy in the study files.

13 PLANNED STATISTICAL METHODS

A detailed description of data analysis and statistical methods to be used will be outlined separately in the statistical analysis plan.

13.1 General Considerations

Part 1 (Phase 1)

Analyses for the primary safety endpoint will be descriptive. AEs will be classified using MedDRA version 20.0 or higher. The severity of the toxicities will be graded according to the NCI CTCAE version 5.0.

Part 2 (Phase 2a)

In the randomized, Phase 2 portion of the study, the primary efficacy endpoint is time to recovery, defined as the first day during the 28 days after randomization on which a subject meets the criteria for category 0, 1, 2, 3, or 4 on the WHO severity scale for COVID-19 pulmonary syndrome. The intent-to-treat (ITT) analysis will be carried out for the primary efficacy endpoint. Subjects will be analyzed according to the assigned treatment group, regardless of whether the subjects receive any study treatment or receive a different study treatment from that to which they were randomized. The primary analysis will be a stratified log-rank test of time to recovery with cePolyTregs compared with placebo/SOC, with stratification by WHO severity scale for COVID-19 pulmonary syndrome at enrollment. The test will be performed with a two-sided type I error rate of 10%. Data for subjects who fail to recover or die will be censored at Day 29.

Analysis of the primary safety endpoint will be descriptive according to the intervention received and no formal comparisons between the 2 study arms will be performed. The safety analysis set will consist of all subjects who received at least 1 dose of study drug. AEs will be summarized by dose cohort using numbers and proportions.

The assessment of PD and biomarker data are described in Appendix C **Mechanistic Assessments**.

13.2 Determination of Sample Size

In Part 2 of the study, a total of 100 subjects will be randomized 1:1 to receive cePolyTregs or placebo/SOC administered as a single IV dose. Randomization will be stratified by study site and baseline WHO severity scale for COVID-19 pulmonary syndrome. Beigel (2020) reported that the rate of achieving a clinical severity score of 4 or better was 26.6% (67 out of 252 patients) within 14 days in the placebo arm among those with a symptom score ≥ 6. Assuming that the distribution of time to recovery follows an exponential distribution, a 26.6% recovery rate within 14 days is equivalent to a median time to recovery of 31.4 days and a 46.1% recovery rate within 28 days. We expect cePolyTregs will halve the median time to recovery to 16 days, which is

equivalent to recovery rates of 45.5% and 70.2% within 14 and 28 days, respectively, under the exponential assumption. A sample size of 100 subjects (50 per group) will have 80% power to detect a reduction of 15.4 days in median time to recovery (or, equivalently, an improvement in recovery rate from 46.1% to 70.2% within 28 days) using the log-rank test at a two-sided type I error rate of 10%, based on Monte-Carlo simulations with 10,000 replicates.

The planned sample size will also provide a reasonably accurate estimate of the true SAE rate. The remdesivir study (Beigel 2020) reported that 30% of patients in the placebo group encountered SAEs. If 15 (30%) out of the 50 subjects in a study arm of the present study are observed to have an SAE, it will be possible to conclude that the true SAE rate is lower than 45%, at a 5% error rate, because the 95% confidence interval (0.179, 0.446) will exclude 45%.

13.3 Analysis Populations

13.3.1 Safety Population

The *safety population* consists of all subjects who receive any amount of study medication. All data summaries except PK-data related summaries will use this population.

13.3.2 Pharmacodynamic Population

The *pharmacodynamic population* includes those subjects in the safety population who have at least one measurable post-IP exposure sample for a PD parameter.

13.3.3 Efficacy Population

Subjects in the safety population who have at least 1 posttreatment assessment for a marker of clinical activity will be included in the efficacy population.

13.4 Subject Disposition

Subject disposition data will be summarized and will include the number and percent of enrolled subjects; number and percent of subjects initiating and completing treatment; and number and percent of subjects discontinuing treatment and discontinuing the study, further broken down by the reasons for discontinuation. Subject enrollment will also be summarized by study site. A summary of major protocol deviations will be tabulated for each cohort and cohorts combined by treatment group.

13.5 Demographics and Baseline Characteristics

Demographics and other baseline characteristics will be summarized for the subjects in the safety population. Demographic data will include age, gender, race, and ethnicity.

13.6 Safety Analyses

All safety data summaries will use the safety population.

The number and percent of subjects experiencing any DLTs will be presented by cohort. A listing of DLTs will also be provided.

Adverse events (including DLTs) will be coded using MedDRA (v 20.1, or the current version) and will be graded by the investigator using the NCI CTCAE v 5.0 or the current version. Subject incidence of TEAEs, TESAEs, TEAEs leading to treatment discontinuation, AESIs, and TEAEs with an outcome of death will be summarized by SOC and preferred term. Adverse events will also be further summarized by worst CTCAE severity grade and relationship to study drug. In addition, AESIs as described in Section **Error! Reference source not found.** will also be summarized by SOC, preferred term, and worst severity grade.

Clinical laboratory data will be summarized descriptively, and the summaries will include observed values at collection time points and their changes from baseline. All laboratory parameters that can be graded using the CTCAE v 5.0 will be graded. For selected parameters, the following summaries may be produced:

- Worst postbaseline severity grade
- Shift summary of baseline grade to worst postbaseline severity grade.

Safety evaluations may also include changes in the subject's vital signs and ECG findings.

13.7 Pharmacodynamic Analyses

All PD data summaries will use the PD population.

Detailed PD analyses are outlined in Appendix C **Mechanistic Assessments**.

13.8 Efficacy Analyses

Data will be summarized descriptively by treatment arm and overall. The descriptive summary for the categorical variables will include counts and percentages. Chi-squared tests (or Fisher's exact tests, as appropriate) will be used to compare categorical variables between study arms. Descriptive summaries for the continuous variables will include means, medians, standard deviations and minimum and maximum values. Two-sample t-tests (or Wilcoxon rank-sum tests, as appropriate) will be used to compare continuous variables between the two study arms. Time to recovery will be summarized using the Kaplan-Meier method and compared between study arms using the log-rank test.

14 ADMINISTRATIVE CONSIDERATIONS

14.1 Investigator Responsibilities

The investigator is responsible for complying with all regulatory requirements relating to performing clinical research with an investigational product. The investigator is responsible for ensuring that the investigation is conducted according to the signed Investigator Agreement (FDA Form 1572, or equivalent), the approved protocol, and applicable regulations for protecting the rights, safety, and welfare of study subject under the investigator's care. The investigator is additionally responsible for the control of investigational product and for providing accurate and verifiable data to the Sponsor.

The investigator must obtain the written informed consent of each subject before participation in the study. The investigator must assure initial and continuing review of the study by an IRB that complies with applicable national and local regulations.

The investigator will ensure adequate documentation of the training of research study personnel for conduct of the study, including qualifications, experience, and study role.

The investigator will be given a copy of the most current version of the cePolyTregs Investigator's Brochure and appropriate study process manuals and plans. The investigator is obligated to become familiar with these documents prior to initiation of the study.

Other investigator responsibilities relative to the IRB include, but are not limited to, the following:

- Submit to the IRB for review any advertisements that will be used to recruit subjects, as applicable
- Submit all protocol amendments, revisions of the Investigator's Brochure, or revisions of the Informed Consent to the IRB for review
- If the Sponsor notifies the investigator about SAEs reported in other studies associated with this or closely related investigational products, report that information to the IRB if required per local regulations
- Provide the IRB with any other information it requests before or during the conduct of the study
- Report to the IRB all adverse drug reactions that are serious, unexpected, and related to investigational product as per local regulations
- Maintain a file of study-related information
- Update the IRB on a minimum of a yearly basis as per local regulations

14.2 Independent Ethics Committee Approval

Before initiation of the study at a study site, the protocol, including the final version of the ICF, the subject information sheet (if applicable), and any other applicable/relevant study documentation, will be submitted to the appropriate IRB. In addition, the IRB must approve all advertising used to recruit subjects for the study prior to use. Written approval of the study documentation must be obtained and sent to the Sponsor or its designee before the study drug can be released to the investigator.

The investigator is responsible for informing the IRB of any amendments to the protocol, ICF, written information provided to subjects, and/or other procedures in accordance with local requirements. The protocol must be re-approved by the IRB upon receipt of amendments, in accordance with applicable law. The investigator must send a copy of the approval letter from the IRB to the Sponsor or its designee.

The investigator will report promptly to the IRB and the Sponsor any new information that may adversely affect the health or safety of past or current subjects or the conduct of the study, including deviations from the protocol or reports of any reportable SAEs, during and for one (1) year after study completion.

The investigator should submit written reports of clinical study status to their IRB annually or more frequently if required. A final study notification will also be forwarded to the IRB after the study is completed or in the event of premature termination of the study in accordance with the applicable regulations. After completion of the study, the investigator will provide the IRB with a report of the outcome of the study. Copies of all contacts with the IRB should be maintained in the study file. Copies of clinical study status reports (including termination) should be provided to the Sponsor.

14.3 Ethical Conduct of Study

The investigator(s) and all parties involved in this study should conduct the study in adherence to the ethical principles based on the Declaration of Helsinki, GCP, ICH guidelines, and the applicable national and local laws and regulatory requirements (referred to herein as “applicable law”).

Investigators and all sub-investigators will comply with 21 Code of Federal Regulations (CFR), Part 54, 1998 and similar conflicts of interest laws requiring documentation of financial interests or arrangements with the Sponsor, or proprietary interests in the drug under study and any other local regulatory requirements as applicable. Any required documentation must be provided prior to the investigator’s (and any sub-investigator’s) participation in the study. The investigator and sub-investigator(s) will notify the Sponsor or its designee of any change in reportable interests during the study and for 1 year following completion of the study. Study completion is defined as the date when the last subject completes all protocol-defined activities.

14.4 Subject Information and Consent

Prior to conducting any study-related procedures, the investigator must obtain informed consent for each subject, in accordance with applicable law. Consent will be documented on a written ICF. The ICF must be approved both by the Sponsor and by the reviewing IRB prior to presenting it to a subject. Each ICF must comply with the ICH GCP Guidelines and applicable regulatory requirements.

Investigators may discuss study availability and the possibility for entry with a potential subject without first obtaining consent. However, informed consent must be obtained and documented prior to initiation of any procedures that are performed solely for the purpose of determining eligibility for research, including withdrawal from current medication(s). The informed consent process should take place under conditions where the subject has adequate time to consider the risks and benefits associated with his/her participation in the study. The investigator or qualified designee must explain to each subject the aims, methods, reasonably anticipated benefits, and potential hazards of the study.

Once appropriate essential information has been provided and fully explained in layman's language to the subject by the investigator (or a qualified designee), the approved ICF will be signed and dated by both the subject and the person obtaining consent (investigator or designee), as well as by any other parties required by the IRB. The subject will receive a copy of the signed ICF; the original will be retained in the study files. The investigator must document the consent interview and place the record in the study files. The investigator shall also maintain a log of all subjects who sign the ICF and indicate if the subject was enrolled into the study or reason for non-enrollment.

14.5 Confidentiality

The investigator must assure that subjects' anonymity will be strictly maintained and that their identities are protected from unauthorized parties. Only subject initials, age, another unique identifier (as allowed by local law) and an identification code will be recorded on any form or biological sample submitted to the Sponsor, IRB, or laboratory. Laboratory specimens must be labeled in such a way as to protect subject identity while allowing the results to be recorded to the proper subject. Refer to specific laboratory instructions (or in accordance with local regulations). NOTE: The investigator must keep a screening log showing codes, names, and addresses for all subjects screened and for all subjects enrolled in the study. Subject data will be processed in accordance with all applicable regulations; some studies may require double-coding of samples.

The investigator agrees that all information received from the Sponsor, including but not limited to the cePolyTregs Investigator's Brochure, this protocol, CRF/eCRF, the study drug, and any other study information, remain the sole and exclusive property of the Sponsor during the conduct of the study and thereafter. This information is not to be disclosed to any third party (except employees or agents directly involved in the conduct of the study or as required by law)

without prior written consent from the Sponsor. The investigator further agrees to take all reasonable precautions to prevent the disclosure by any employee or agent of the study site to any third party or otherwise into the public domain. In compliance with applicable law and/or ICH GCP Guidelines, the investigator will permit the Sponsor's representatives and, when necessary, representatives of the regulatory authorities, direct access to any medical records relevant to the study for verification of study-related procedures and data. Direct access includes examining, analyzing, verifying, and reproducing any records and reports that are important to the evaluation of the study.

Investigators will obtain authorization from the subject to permit access to study-related records, including personal information.

Authorization is required from each subject (e.g., specific permission granted by such individual to a covered entity for the use or disclosure of an individual's protected health information). The investigator and institution must obtain such waiver/authorization in writing from the subject. Valid authorization must meet the implementation specifications under the applicable privacy laws. Authorization may be combined in the ICF (approved by the IRB), or it may be a separate document (approved by the IRB) or provided by the investigator or Sponsor (without IRB approval).

14.6 Study Initiation

Before the study drug can be shipped to the study site and before enrollment can begin, all applicable documentation and approvals as per local regulations must be in place.

The Sponsor or designee will notify the site when they are activated.

14.7 Case Report Forms and Other Study Records

The investigator will comply with the requirements for all assessments and data collection for each subject, as specified in the protocol.

During each subject's visit to the study site, the investigator or qualified designee will record progress notes in the subject's medical record to document all significant observations. At a minimum, these notes will contain the following:

- Documentation of the informed consent process, including any revised consents.
- The date of the visit and the corresponding visit or day in the study schedule.
- General subject status remarks, including any significant medical findings. The severity, frequency, and duration of any AEs and the investigator's assessment of relationship to study drug must also be recorded.
- Any changes in concomitant medications.
- A general reference to the procedures completed.

In addition, any contact with the subject via telephone or other means that provides significant clinical information will also be documented in the progress notes, as described above.

Data for this study will be captured in electronic eCRFs. Study auditing, data entry, verification and validation, and subsequent analysis will be performed by the Sponsor, or its designees, in accordance with GCPs and established Standard Operating Procedures.

Clinical data (including AEs, concomitant medications, and applicable clinical laboratory data) will be entered into an electronic database. The creation and validation of the database, data entry, validation, and verification will be performed according to 21 CFR part 11 and other applicable local regulations. The data system includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate.

14.8 Study Monitoring

The Sponsor or designee will assign monitors who will perform monitoring as frequently as necessary and in accordance with ICH GCP.

Source documents and eCRFs will be reviewed at monitoring visits and any findings will be discussed with the investigational staff. The Sponsor expects that at monitoring visits study documents and staff will be available and a suitable space will be provided for review of the study documents. The monitor will meet with the investigator on a regular basis to provide feedback on the conduct of the study.

14.9 Access to Source Documentation

The study may be subject to audit by the Sponsor, its designee, or by regulatory authorities. If such an audit occurs, the investigator must agree to allow access to required subject records. The investigator should notify the Sponsor promptly of regulatory authority audits that are scheduled and must forward copies of any findings or audit reports to the Sponsor promptly.

By signing this protocol, the investigator grants permission to personnel from the Sponsor, its representatives, and appropriate regulatory authorities for on-site monitoring and review of all appropriate study documentation, as well as on-site review of the procedures employed in data collection, where clinically appropriate.

14.10 Study or Study Site Termination

The Sponsor may suspend or stop the study at all centers or at specific study centers due to (but not limited to) the discovery of an unexpected, serious, or unacceptable risk to the subjects enrolled in the study, a decision on the part of the Sponsor to suspend or discontinue development of the product, failure of the investigator to enroll subjects into the study at an acceptable rate, failure of the investigator to comply with regulatory authority or ICH Guidelines, or submission of knowingly false information.

14.11 Protocol Amendments

The investigator will not make any changes to this protocol without the Sponsor's prior written consent and subsequent approval by the IRB. Any permanent change to the protocol, whether an overall change or a change for specific study site(s), must be handled as a protocol amendment. The Sponsor will write any amendment(s). The investigator will submit each amendment to the IRB. However, a protocol change intended to eliminate an apparent immediate hazard to subjects should be implemented immediately, followed by IRB notification within 5 working days.

If the IRB, investigators, and/or Sponsor conclude that the protocol amendment substantially alters the study design and/or increases the potential risk to the subject, the currently approved written ICF will require modification. In such cases, after the approval of the new ICF by the IRB, the investigator will repeat the informed consent process to obtain the subject's signature on the new ICF before permitting continued participation in the study.

If the Sponsor amends the protocol, agreement from the investigator must be obtained. The IRB must be informed of all amendments and give approval. The investigator must promptly send a copy of the approval letter from the IRB to the Sponsor or its designee.

14.12 Protocol Violations/Deviations

This study should be conducted as specified in this protocol. In the event of a significant deviation from the protocol, the investigator or designee will contact the Sponsor or its designee at the earliest possible time to determine the disposition of the subject involved. If necessary, the Sponsor, Medical Monitor, and investigator will decide whether the subject may continue in the study. Such a decision will be documented by the investigator and Sponsor, as appropriate.

14.13 Quality Assurance

Authorized representatives of the Sponsor, a regulatory authority, or IRB may visit the study site to perform audits or inspections, including source data verification. The purpose of any such audit or inspection is to systematically and independently examine all study-related activities and documents to determine whether these activities were conducted, and data were recorded, analyzed, and accurately reported, according to the protocol, ICH GCP guidelines, and any other applicable regulatory requirements.

The investigator should contact the Sponsor immediately if contacted by a regulatory agency about an inspection.

It is important that the investigator and relevant personnel are available during the possible audits or inspections and that sufficient time is devoted to the process.

14.14 Retention of Data

When the study is completed, the investigator must retain the essential documents for as long as needed to comply with regulatory guidelines and Sponsor requirements. The investigator will

notify the Sponsor prior to moving or destroying any of the study documents. It is the responsibility of the Sponsor to inform the investigator/institution as to when these documents no longer need to be retained. The investigator should take measures to prevent any accidental or premature destruction of these documents.

14.15 Study Report and Publications

A clinical study report will be prepared and submitted to the appropriate regulatory agency or agencies. The Sponsor will ensure that the report meets the standards set out in the ICH Guideline for Structure and Content of Clinical Study Reports (ICH E3). Note that an abbreviated report may be prepared in certain cases.

Investigators in this study may communicate, orally present, or publish in scientific journals or other scholarly media only after written consent has been obtained from the Sponsor.

The investigator will submit to the Sponsor any proposed publication or presentation along with the respective scientific journal or presentation forum at least 60 days before submission of the publication or presentation.

No such communication, presentation, or publication will include the Sponsor's confidential information (see Section 14.5).

The investigator will comply with the Sponsor's request to delete references to its confidential information (other than the study results) in any paper or presentation and agrees to withhold publication or presentation for an additional 60 days in order to obtain patent protection if deemed necessary.

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APPENDIX A Ventilator Protocol (modified from ARDSnet)

Controlled modes of ventilation will be required.

Adjust ventilator to achieve a Vt of 6 ml/kg of predicted body weight. At the physician's discretion, tidal volume can be adjusted +/- 2ml/kg.

Predicted body weight is calculated from age, gender, and height (heel to crown) according to the following equations:

Males: PBW (kg) = 50 + 2.3 [height (inches) – 60]

Females: PBW (kg) = 45.5 + 2.3 [eight (inches) – 60]

APPENDIX B Ventilator Weaning Procedure

Commencement of Weaning (applicable to subjects ventilated invasively or non-invasively)

Subjects will be assessed for the following weaning readiness criteria each day between 0600 and 1000 hours. If a subject's procedure, test, or other extenuating circumstance prevents assessment for these criteria between 0600 and 1000, then the assessment and initiation of subsequent weaning procedures may be delayed for up to 6 hours.

1. At least 12 hours since enrollment in the trial
2. $FiO_2 \leq 0.40$ and PEEP $\leq$ 8 cm H_2O or $FiO_2 \leq 0.50$ and PEEP = 5 cm H_2O
3. Values of both PEEP and $FiO_2 \leq$ values from previous day
4. Not receiving neuromuscular blocking agents and without neuromuscular blockade
5. Subject exhibiting inspiratory efforts. If no efforts are evident at baseline, ventilator set rate will be decreased to 50% of baseline level for up to 5 minutes to detect inspiratory efforts.
6. Systolic arterial pressure $\geq$ 90 mm Hg without vasopressor support ($\leq$ 5 µg/kg/min dopamine or dobutamine will not be considered a vasopressor)

Spontaneous Breathing Trial Procedure and Assessment for Unassisted Breathing

If criteria 1 to 6 above are met, then initiate a trial of up to 120 minutes of spontaneous breathing with $FiO_2 < 0.5$ using any of the following approaches:

1. Pressure support (PS) < 5 cm H_2O, PEEP < 5 cm H_2O
2. CPAP < 5 cm H_2O
3. T-piece
4. Tracheostomy mask

The clinical team may decide to change mode during spontaneous breathing (PS = 5, CPAP, tracheostomy mask, or T-piece) at any time during the spontaneous breathing trial.

Monitor for tolerance using the following:

1. $SpO_2 \geq 90\%$ and / or $PaO_2 \geq 60$ mm Hg
2. Mean spontaneous tidal volume $\geq$ 4 ml/kg PBW (if measured)
3. Respiratory Rate $\leq$ 35 / min
4. pH $\geq$ 7.30 (if measured)
5. No respiratory distress (defined as 2 or more of the following):
   a. Heart rate $\geq$ 120% of the 0600 rate ($\leq$ 5 min at > 120% may be tolerated)
   b. Marked use of accessory muscles

c. Abdominal paradox
d. Diaphoresis
e. Marked subjective dyspnea

If any of the goals a. to e. are not met, revert to previous ventilator settings or to PS ≥ 10 cm H_2O with Positive End-expiratory Pressure and FiO_2 = previous settings and reassess for weaning the next morning. The subject will be reassessed for weaning the following day.

Decision to remove ventilatory support:

If tolerance criteria for spontaneous breathing trial (a. to e. above) are met for at least 30 minutes, the clinical team may decide to discontinue mechanical ventilation. However, the spontaneous breathing trial can continue for up to120 minutes if tolerance remains in question.

APPENDIX C Mechanistic Assessments

Assay	Specific Readout	Comparisons
Assays based on flow cytometry and qPCR to detect cePolyTregs in blood of treated subjects	Percent of endogenous CD4 T cells and Treg	% change from baseline among treatment groups and temporal PK information
Assays based on flow cytometry and qPCR to detect cePolyTregs in the Pulmonary tissue (endotracheal aspirate and mini BAL)	Percent of endogenous CD4 T cells and Treg	% change from baseline among treatment groups and temporal PK information
Flow cytometric, genomic and/or proteomic analyses of cells harvested from endotracheal tubes and mini BAL and blood to determine phenotypic changes in immune subsets	Percent of individual immune subset, expression of activation and memory markers	% change from baseline among treatment groups
Flow cytometric, cell culture and expression analyses of cells harvested from endotracheal tubes and mini BAL and blood to determine functional changes in immune subsets	Functional activities of individual immune subset including cytokine production, cytotoxicity and other immune activities	% change from baseline among treatment groups
C-reactive protein and LDH per clinical laboratory standard methods	Concentrations	% change from baseline among treatment groups and temporal biomarker information
d-dimer per clinical laboratory standard methods	Concentrations	% change from baseline among treatment groups and temporal biomarker information
Albumin per clinical laboratory standard methods	Concentrations	% change from baseline among treatment groups
Troponin I per clinical laboratory standard methods	Concentrations	% change from baseline among treatment groups
Assays for inflammatory cytokines/chemokines, e.g. IL-6, IL-12, IFNγ, GM-CSF, TNFα, IP10, MCP-1, MCP3, and MCP-4	Concentrations	% change from baseline among treatment groups
Frequencies and neutralization activities of SARS-CoV-2-specific antibodies	Titer	% change from baseline among treatment groups
Frequencies and effector function of SARS-CoV-2-specific T cell responses	Percentages of CD4 and CD8 T cells that respond to SARS-Cov-2 antigens, effector molecule expression	% change from baseline among treatment groups