MULTI-CENTER, RANDOMIZED, PLACEBO CONTROLLED, INTERVENTIONAL PHASE 2A CLINICAL TRIAL EVALUATING THE SAFETY AND POTENTIAL EFFICACY OF MULTIPLE DOSING OF MESENCHYMAL STROMAL CELLS IN PATIENTS WITH SEVERE ACUTE RESPIRATORY SYNDROME CORONAVIRUS 2 (SARS-Cov-2)

Version 10-21-2020

IND# 21779

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Revision History

Revision #	Version Date	Summary of Changes	Consent Revision
	04/27/2020	Original to CPRC	
V2	5/20/2020	Per CPRC stipulation: Clarified primary and secondary endpoints and statistical consideration. Administrative change – each institution will use their own IRBs. • Section 2.3 added background and additional references • Section 4: Updated inclusion and exclusion criteria • Section 5.5 Updated definition of high risk stratification • Section 9: Safety updates: added monitoring during infusion; added to definition of reportable events; added unblinding procedures; added medical monitor; updated monitoring guidelines • section 8.2 added arterial blood gasses • Appendix I and Appendix II added Apache II and Sofa Scoring, and Dyspnea Index Questionnaire • Updated study procedures to remove single IRB. Minor edits: Added IND number and additional investigators to cover page Synopsis, section 4.6 clarified HIV exclusion criteria Section 8.1 and section 8.2, clarified timing of follow up procedures, added clinical pulmonary follow up procedures	N/A
V3	6/16/2020	Revised after FDA Review: • Updated study title • Synopsis, section 1.3, section 12.1.1 – clarified primary endpoint • Synopsis, Section 4.1 – updated inclusion criteria to clarify definitions of ARDS and SARS-CoV-2, increased upper age limit to 80 • Synopsis, Section 4.2 – updated exclusions to allow for use of other agents • Section 1.1, Section 3 – Clarified hypothesis and design to include efficacy • Section 5 – enrollment will be staggered so that patients cannot be enrolled until 48 hours after the last infusion in the most recently treated patient • Section 6.2 – Updated management of SAEs • Section 8.1 – clarified testing time points • Section 9.2.2 – Updated documentation of events to include supportive interventions, vitals, oxygenation, number of ventilator and ICU days • Section 9.6 – added more detail to stopping rule event monitoring • Section 12.4 – minor clarification to stopping rule language Minor administrative edits to contact information, section 5.4, section 8.1, and section 10.8	N/A

V4	6/26/2020	Section 6.2 added that if patient condition is deteriorating they will be permitted to enter another study 7 days after their last dose of MSCs	NA
V5	10/21/2020	Synopsis – Clarified procedure for post-treatment consent; removed "in the supine position" from exclusion criteria Section 4.1 - Clarified procedure for post-treatment consent Section 4.2 – Clarified measures to determine cardiac inclusion Section 4.3 - removed "in the supine position" from exclusion criteria Section 6.1 – Clarifications to infusion procedures Section 8.1 – Updates to clinical care procedures to reflect current standard of care, expand window of procedures, remove redundancies, and allow for virtual visits Section 8.2 – Updated window around research related procedures, updated timing of baseline research blood draw Section 9.2 – Clarification of Adverse Event reporting/documentation Section 9.5 – Edited timing of DSMB review to match DSMB charter Section 11.5 – Clarified that Acute Lung Injury Score will not be calculated in the event of no chest imaging within 48 hours window. Throughout protocol – minor updates for formatting and clarity.	No

Table of Contents

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List of Abbreviations

ABBREVIATION	DEFINITION
ACE2	ANGIOTENSIN CONVERTING ENZYME-2
AE	ADVERSE EVENT
AHC-IS	ACADEMIC HEALTH CENTER-INFORMATIONAL SYSTEMS
ALT	ALANINE AMINOTRANSFERASE
APACHE	ACUTE PHYSIOLOGY AND CHRONIC HEALTH EVALUATION SCORE
AST	ASPARTATE AMINOTRANSFERASE
ARDS	ACUTE RESPIRATORY DISTRESS SYNDROME
BMT	BLOOD AND MARROW TRANSPLANT
BNP	B-TYPE NATRIURETIC PEPTIDE
BUN	BLOOD UREA NITROGEN
CFR	CODE OF FEDERAL REGULATIONS
CI	CONFIDENCE INTERVAL
CLIA	CLINICAL LABORATORY IMPROVEMENT AMENDMENT
COPD	CHRONIC OBSTRUCTIVE LUNG DISEASE
CRF	CASE REPORT FORM
CPRC	CANCER PROTOCOL REVIEW COMMITTEE
CT	COMPUTED TOMOGRAPHY
CTCAE	COMMON TOXICITY CRITERIA ADVERSE EVENT
CTO	CLINICAL TRIALS OFFICE
CTO-CGI	CLINICAL TRIALS OFFICE FOR CELL, GENE AND IMMUNOTHERAPIES
CTSI	CLINICAL AND TRANSLATIONAL SCIENCE INSTITUTE
DSMB	DATA SAFETY MONITORING BOARD
DMSO	DIMETHYLSULFOXIDE
ECG	ELECTROCARDIOGRAM
ECHO	ECHOCARDIOGRAM
ECMO	EXTRACORPOREAL MEMBRANE OXYGENATION
FACT	FOUNDATION FOR THE ACCREDITATION OF CELLULAR THERAPY
FDA	FOOD AND DRUG ADMINISTRATION
FIO2	FRACTION OF INSPIRED OXYGEN
GCP	GOOD CLINICAL PRACTICE
GVHD	GRAFT VERSUS HOST DISEASE
HIPAA	HEALTH INSURANCE PORTABILITY AND ACCOUNTABILITY ACT
HIV	HUMAN IMMUNODEFICIENCY VIRUS
HLA	HUMAN LEUKOCYTE ANTIGEN
HTLV1/2	HUMAN T CELL LYMPHOTROPIC VIRUS 1/2
H2O	WATER
ICF	INFORMED CONSENT FORM
ICU	INTENSIVE CARE UNIT
IDMC	IDEPENDENT DATA AND SAFETY MONITORING COMMITTEE
INFγ	INTERFERON GAMMA
IQR	INTER QUARTILE RANGE
IND	INVESTIGATIONAL NEW DRUG
IRB	INSTITUTIONAL REVIEW BOARD
IV	INTRAVENOUS
LDH	LACTATE DEHYDROGENASE
LIMS	LABORATORY INFORMATION MANAGEMENT SYSTEM

LFT	LIVER FUNCTION TEST
LVEF	LEFT VENTRICULAR EJECTION FRACTION
MAP	MEAN ARTERIAL PRESSURE
MCC	[UNIVERSITY OF MINNESOTA] MASONIC CANCER CENTER
MSC	MESENCHYMAL STROMAL CELLS
PAO2	PARTIAL PRESSURE OF OXYGEN
PCR	POLYMERASE CHAIN REACTION
PEEP	POSITIVE END EXPIRATORY PRESSURE
PFT	PULMONARY FUNCTION TEST
PHI	PROTECTED HEALTH INFORMATION
PCRC	PRIMARY CLINICAL RESEARCH COORDINATOR
PNTBNP	PRO-N-TERMINAL B TYPE NATURIETIC PEPTIDE
SAE	SERIOUS ADVERSE EVENT
SCRF	SCREENING CRF
SLE	SYSTEMIC LUPUS ERYTHEMATOSIS
SOBQ	UNIVERSITY OF CALIFORNIA, SAN DIEGO SHORTNESS OF BREATH QUESTIONNAIRE
SOP	STANDARD OPERATING PROCEDURE
TMPRSS2	TRANSMEMBRANE PROTEASE, SERINE 2
TNFA	TUMOR NECROSIS FACTOR ALPHA
ULN	UPPER LIMIT OF NORMAL
UMN	UNIVERSITY OF MINNESOTA
US	UNITED STATES
WHO	WORLD HEALTH ORGANIZATION

Protocol Synopsis

Study Design: This is a multi-center, randomized, placebo controlled, interventional phase 2A safety trial to evaluate the safety profile and potential efficacy of multi-dosing of mesenchymal stromal cells (MSC) for patients with SARS-CoV-2 associated ARDS. After informed consent, treatment assignment will be made by computer-generated randomization to administer either MSC or vehicle placebo control with a 2:1 allocation to the MSC: placebo arm.

Investigational Agent: Three fixed doses of MSC (300 x 10^6 MSC) or vehicle control approximately 48 hours apart.

Key Eligibility Criteria:

- Age 18-80 years
- Meets 'Berlin Criteria' for diagnosis of moderate to severe ARDS for a minimum of 4 hours[1]
- Less than 48 hours on a ventilator after meeting criteria for diagnosis of ARDS
- SARS-CoV-2 (proven by RT-PCR assay) with radiographic infiltrates
- PaO2/FiO2 < 250
- Positive end-expiratory airway pressure (PEEP) >5 cm H20
- Elevated C-reactive protein (above laboratory upper limit of normal)
- Meets organ function requirements [detailed in protocol section 4.2]
- Off other investigational agents directed against inflammatory cytokines at least 48 hours prior to enrollment; agents directed against the replication of SARS-CoV-2 [e.g., Remdesivir] are permitted.
- Voluntary informed consent in person or virtually by the patient or patient surrogate considering the face to face limitations during the COVID-19 pandemic and, given the nature of the study population, which frequently requires mechanical ventilation with sedation, surrogate consent will likely occur in a substantial proportion of the study population. This will remain a valid consent until the patient is fully alert and consistently competent at which point the patient will be provided a second consent to continue participation in the study. In addition, a summary information sheet about this protocol will be in the patient's room and will be available for the patient and family member.

Key Exclusion Criteria:

- Ventilator support of FiO2 $\geq$0·8 or PEEP $\geq$20 cm H2O and ongoing use of more than two vasopressors for 2 or more hours with any agent at the doses shown below.
    - Norepinephrine >12 µg/min or 0.2 µg/kg per min
    - Phenylephrine >150 µg/min or 3 µg/kg per min
    - Epinephrine >10 ug/min or 0.2 µg/kg per min
    - Vasopressin >0.04 units/min
- Concurrent use of other investigational agents specifically for treatment of ARDS or inflammatory cytokines (Note: Agents, currently under investigations, shown to be efficacious will be permitted as these data emerge).
- Known ineligibility for use of a ventilator for a minimum of 7 days, as judged by the institution's Triage Team
- Known allergy to MSC components: fetal calf serum, human albumin or DMSO

- Active invasive malignant disease requiring chemotherapy/radiation
- Other concurrent life-threatening disease (life expectancy <6 months) or eligible for hospice care
- Known history of HIV infection on active treatment
- Females who are pregnant or breastfeeding
- Current mean arterial pressure (MAP) <60 mmHg while on 2 or more vasopressors at above doses for more than 2 hours
- History of any significant cardiac (myocardial infarction within 12 months of screening visit or unstable angina), chronic ongoing hepatic, or renal disease (grade 3 or higher); diagnosis of congestive heart failure with hypoxemia primarily due to decompensated heart failure; diagnosis of severe chronic obstructive pulmonary disease (COPD) or interstitial lung disease requiring supplemental oxygen at home
- Concurrent diagnosis of diffuse alveolar hemorrhage
- Inability to temporarily discontinue dialysis (i.e. unable to stop dialysis immediately prior to during infusion for 2 hours thereafter)

Primary Objective: Safety and feasibility of three sequential MSC infusions within a 7 day period

Primary Endpoint: Incidence of grade 3-5 infusional toxicities and predefined hemodynamic or respiratory adverse events related to the infusion of MSC

Secondary Endpoints: Relative to the first infused dose of study agent

- Incidence of a reduction in one or more biomarkers of inflammation by day 7
- Trend changes in PaO2:FiO2 ratio, oxygenation index, peak and plateau pressures, static compliance and PEEP from baseline on days 3, 7 and 14
- Incidence of mortality by day 28 and 100
- Number of ICU-free days alive by day 28
- Number of days alive and ventilator free composite score[3] by day 28
- Change in acute lung injury score[2] between baseline and day 28
- Incidence and severity of serious adverse events by day 28
- Number of days alive off supplemental oxygen by day 100
- Number of hospital days by day 100
- Description of lung function status over time to 1 year

Exploratory Endpoints:

- Characterize immunological changes over 28 days
- Characterize inflammatory and alveolar epithelial function/injury biomarkers changes over 28 days

Accrual Objective: 30 patients (2:1 randomization MSC:placebo) in patients with SARS-CoV-2 associated ARDS requiring ventilator support

Endpoint Footnote

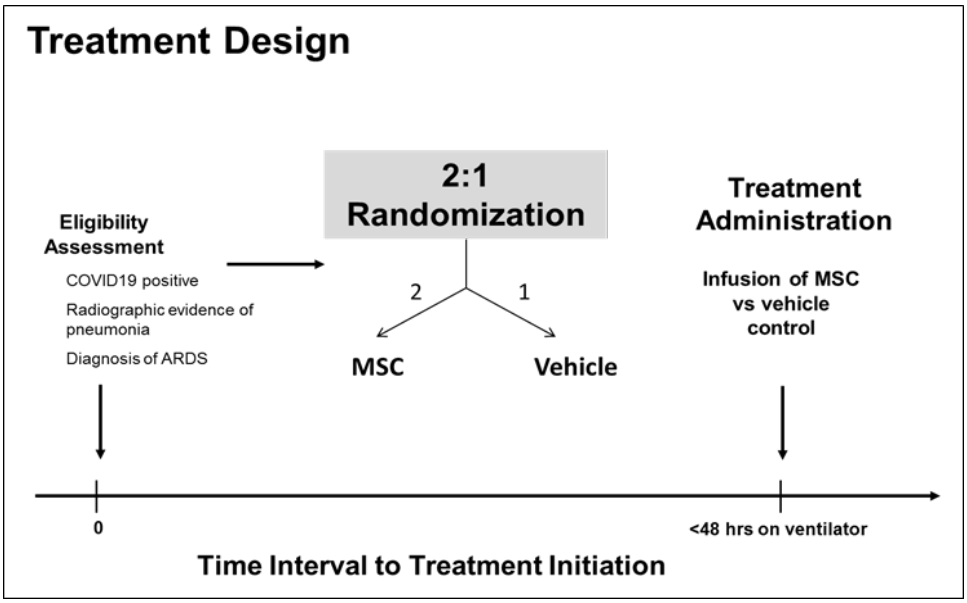
1 Berlin Criteria for ARDS: [1]

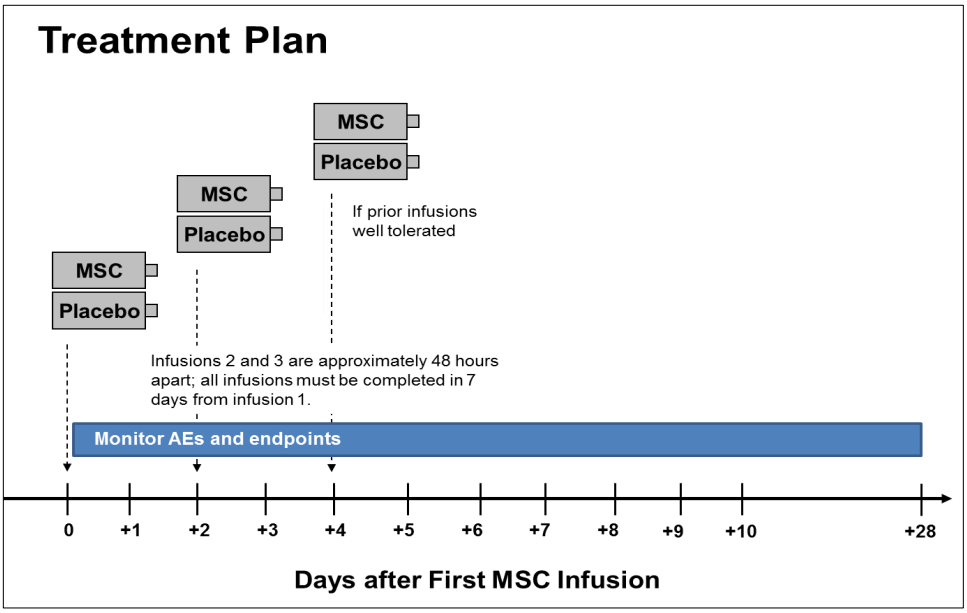
2 Acute Lung Injury Score: a composite 4 point scoring system validated by the NHLBI ARDS Network that considers PaO2/FiO2, the level of positive end-expiratory airway pressure, respiratory compliance, and the extent of pulmonary infiltrates on the chest radiograph. [2]

3 Alive and ventilator free score: Novack V, Beitler JR, Yitshak-Sade M, Thompson BT, Schoenfeld DA, Rubenfeld G, Talmor D, Brown SM. Alive and Ventilator Free: A Hierarchical, Composite Outcome for Clinical Trials in the Acute Respiratory Distress Syndrome. Crit Care Med. 2020 Feb;48(2):158-166.[3]

Treatment Design and Plan

Patients will receive study agent (MSC or placebo) within 48 hours of enrollment and within 72 hours of intubation. Three doses will be administered unless a severe infusion adverse event occurs that is related to the MSC infusion. Doses will be repeated approximately every 48 hours with the aim of completing 3 doses within 7 days of the first dose. All patients will receive standard of care treatments for ARDS. Enrollment will be staggered such that each new patient will be enrolled only after the prior patient has been followed ≥48 hours after the final dose without grade 3-5 infusional toxicities or predefined hemodynamic or respiratory adverse events.





1. Hypothesis and Objectives

1.1 Hypothesis

Three doses of mesenchymal stromal [stem] cells (MSC) with known immunomodulatory and broad anti-inflammatory properties can be safely administered and potentially effective in patients with moderate to severe COVID- 19 mediated acute respiratory distress syndrome (ARDS). If multiple doses are well-tolerated with improvements in efficacy measures (e.g. reduction in proinflammatory cytokines, ventilator days and/or acute lung injury scores), a phase 2B study will be developed with more reliable sample size estimates based on outcomes in this study.

1.2 Primary Objective

Determine safety and feasibility of three sequential MSC infusions within a 7 day period.

1.3 Primary Endpoint

Incidence of grade 3-5 infusional toxicities and hemodynamic or respiratory adverse events within 6 hours of the start of infusion probably related or related to the infusion of MSC.

1.4 Secondary Endpoints

- Incidence of a reduction in one or more biomarkers of inflammation by day 7
- Trend changes in PaO2:FiO2 ratio, oxygenation index, peak and plateau pressures and PEEP from baseline on days 3, 7 and 14
- Incidence of mortality by day 28 and 100
- Number of ICU-free days alive by day 28
- Number of days alive and ventilator free composite score[3] by day 28
- Change in acute lung injury (ALI) score[2] between baseline and day 28
- Incidence and severity of serious adverse events by day 28
- Number of days alive off supplemental oxygen by day 100
- Number of hospital days by day 100
- Description of lung function status over time to 1 year

1.5 Exploratory Endpoints

Additional analyses will be performed that may be important for the design of future trials. These include:

- Characterize the effects of study agent on host immunity over 28 days (e.g., quantitation of macrophage/monocyte, T, B, NK cell subsets, expression of interferon-γ [IFN-γ] by CD4+T, CD8+T and NK cells)
- Characterize the effects of study agent on inflammatory biomarkers over 28 days (e.g., ferritin, C reactive protein (CRP), IL-1b, IL-2, IL-6, IL-8, IL-10, IL-18, sIL-2R, sIL-6R, sTNFR1, TNFα, IFN-γ, Ang-1, Ang-2, gp130) and biomarkers of alveolar function/injury.

2 Background

As of May 16, 2020, a total of 4,530,650 persons in 185 countries and regions were reported to be infected by SARS-CoV-2, which causes coronavirus disease 2019 (COVID-19), a disease that had led to more than 307,384 deaths [https://coronavirus.jhu.edu/map.html] Although the number of new cases appears to have sharply decreased in China, there are now more than 1.5 million confirmed cases in the United States with more than 88,000 confirmed death. While New York, New Jersey, Massachusetts, have been afflicted the most, Pennsylvania and Minnesota have been hit hard with 65178 and 14249 cases confirmed respectively, and 4432 and 692 subjects confirmed death as a result of the disease to date [05/16/2020]. Mortality data discloses significant race disparities with the largest mortality identified among the black and Latino populations.

SARS-CoV-2 is causing a pandemic [4,5] because it is a new virus to which there is no underlying immunity. Based on projections, a conservatively low estimate is that 5% of the population could become infected within 3 months [6]. Preliminary data from China and Italy regarding the distribution of case severity and fatality vary widely but a recent large-scale analysis from China suggests that 80% of those infected either are asymptomatic or have mild symptoms [7]. If the 20% of the infected population requires medical services, about 15% have severe illness and 5% have critical illness. Overall mortality ranges from 0.25% to as high as 3.0% with higher case fatality rates in vulnerable populations. Similar rates and outcomes were reported from Seattle [8]. Death from the SARS-CoV-2 virus is most often due to an acute respiratory distress syndrome (ARDS). To date, there are no proven treatments for COVID- 19 associated ARDS. A recent nonrandomized observational study suggests that glucocorticoids may be associated with improved clinical outcomes in patients with COVID-19 and ARDS [9]; however, additional studies are needed to verify this conclusion. Novel strategies are needed to prevent and treat replication of the SARS-CoV-2 virus and ameliorate the severity of COVID-19 mediated ARDS [10].

2.1 ARDS: Pathophysiology and Treatment

Acute Respiratory Distress Syndrome (ARDS) is a form of acute respiratory failure with protein-rich pulmonary edema characterized by acute inflammation and injury to the lung and epithelia [11]. The nature of the underlying clinical trigger is one important determinant of outcome, e.g., sepsis-associated ARDS has a higher mortality as compared to ARDS due to trauma or pancreatitis. ARDS due to pneumonia and/or aspiration has an intermediate mortality, respectively [12]. Mortality also is highly dependent on age and, to a lesser extent, race with African-Americans and Hispanics having a higher 60-day mortality rate (33%) compared to Caucasians (27%) [13]

The acute lung injury of ARDS has rapid onset of acute inflammation with epithelial and entodothelial cell damage and disruption of the alveolar-capillary barrier. With loss of alveolar–capillary membrane integrity, alveoli are filled with a neutrophilic proteinaceous procoagulant exudate with high levels of pro- inflammatory, cytotoxic mediators, proteases and oxidants [14,15]. Biomarkers found on the epithelium and endothelium and that are involved in the inflammatory and coagulation cascades predict morbidity and mortality.

2.2 MSC for Treatment of ARDS

MSCs secrete paracrine factors that may reduce the severity of acute lung injury, including growth factors, factors that regulate barrier permeability, and anti-inflammatory cytokines [16,

17, 18, 19]. Gupta and al. reported the anti-inflammatory properties of MSC both in vivo and in vitro. In a mouse model where Escherichia coli endotoxin was instilled into the distal airspaces of the lung, followed by direct intrapulmonary administration of MSC 4 hours later. MSC was associated with a decreased extravascular lung water, alveolar–capillary permeability and mortality. Furthermore, the pro- inflammatory response was downregulated, whereas the anti-inflammatory response upregulated [20].

To date, several phase I and II clinical trials incorporating MSC in the treatment of acute lung injury and ARDS have been completed with mixed response. Two multicenter clinical trials [21,22] assessed the safety and potential efficacy of MSC in subjects afflicted by moderate to severe ARDS in whom a mortality greater than 40% was expected. The first of these was a multi-center, open-label, dose- escalation phase 1 clinical trial of a single dose of intravenous MSC in patients with moderate-to-severe ARDS [clinicaltrials.gov number NCT01775774]. The first three patients were treated with low dose MSC (1 million cells/kg predicted body weight (PBW)); the next three patients received intermediate dose MSC (5 million cells/kg PBW); and the final three patients received high dose MSC (10 million cells/kg PBW). Primary outcomes included the incidence of pre-specified infusion associated events and serious adverse events. Secondary outcomes included standard respiratory and systemic endpoints, 28- and 60-day mortality, and measurement of biologic markers of inflammation and endothelial and epithelial injury. The trial completed enrollment in January 2014. The investigators reported that there were no pre-specified infusion associated events or treatment-related adverse events in any of the nine patients in this trial. Serious adverse events (SAEs) were subsequently observed in three patients in the weeks following the infusion: two patients expired >7 days after the MSC infusion, and one patient was discovered to have multiple embolic infarcts of the spleen, kidneys, and brain that were age- indeterminate but thought to have occurred prior the MSC infusion based on MRI results. None of these SAEs were thought to be MSC-related. The investigators concluded that a single IV infusion of allogeneic, bone marrow-derived human MSCs was well-tolerated in 9 patients with moderate to severe ARDS [21].

Based on this phase I experience, a phase 2A START trial was initiated evaluating the safety of MSC (10 million/kg PBW) for moderate to severe ARDS [clinicaltrials.gov number NCT02097641]. Sixty patients were enrolled (1038 screened between 03/24/2014 and 02/09/2017). No patient experienced predefined MSC-related hemodynamic or respiratory adverse events. One patient in the MSC group died within 24 hours of MSC infusion, but death was judged to be probably unrelated. Results showed that 28-day mortality did not differ between the groups (30% in the MSC group vs 15% in the placebo group, odds ratio 2·4, 95% CI 0·5-15·1). However, at baseline, the MSC group had numerically higher mean scores than the placebo group for Acute Physiology and Chronic Health Evaluation III (APACHE III; 104 [SD 31] vs 89 [SD 33]), minute ventilation (11·1 [3·2] vs 9·6 [2·4] L/min), and PEEP (12·4 [3·7] vs 10·8 [2·6] cm H2O). After adjustment for APACHE III score, the hazard ratio for mortality at 28 days was 1·43 (95% CI 0·40-5·12, p=0·58). Viability of MSC ranged from 36% to 85% and was suspected to be at least in part related to the post-thaw wash procedure. On the basis of these results investigators concluded that a single dose of IV MSC was safe in patients with moderate to severe ARDS, larger trials were needed to assess efficacy, and viability of MSC was inconsistent and should be improved [22].

2.3 SARS-CoV-2 Mediated ARDS

Both clinical and epidemiological features of patients with COVID-19 have recently been reported, demonstrating that the SARS-CoV-2 infection can cause clusters of severe respiratory illness with clinical presentations of rapid progression of ARDS, leading to intensive care unit (ICU) admission and high mortality [23]. Clinical manifestations have included fever, fatigue, dry

cough, shortness of breath, and ARDS. Acute respiratory distress syndrome (ARDS) is defined as acute-onset hypoxemia (the ratio of the partial pressure of arterial oxygen to the fraction of inspired oxygen [Pao2:Fio2], <300) with bilateral pulmonary opacities on chest imaging that are not fully explained by congestive heart failure or other forms of volume overload.

Using the Berlin Definition, mild, moderate, and severe ARDS were associated with increasing mortalities (27%; 95% CI, 24%-30%; 32%; 95% CI, 29%-34%; and 45%; 95% CI, 42%-48%,respectively; P < .001) and increasing median durations of mechanical ventilation in survivors (5 days; interquartile [IQR], 2-11; 7 days; IQR, 4-14; and 9 days; IQR, 5-17, respectively; P < .001). While these outcomes predate COVID-19 mediated ARDS, limited data suggest that outcomes may be similar, although some data suggest that COVID-10 ARDS patients who recover require long periods of ventilator support, with a mean of approximately 14 days in the Wuhan, China experience [23].

Most patients with severe COVID-19 exhibit substantially elevated serum levels of pro-inflammatory cytokines including IL-6 and IL-1β, as well as IL-2, IL-8, IL-17, G-CSF, GM-CSF, IP10, MCP1, MIP1α (also known as CCL3) and TNF, characterized as cytokine storm [23, 24, 25, 26]. Also, C-reactive protein and D-dimer are found to be abnormally high. High levels of pro-inflammatory cytokines may lead to shock and tissue damage in the heart, liver and kidney, as well as respiratory failure or multiple organ failure. They also mediate extensive pulmonary pathology, leading to massive infiltration of neutrophils and macrophages, diffuse alveolar damage with the formation of hyaline membranes and a diffuse thickening of the alveolar wall. Spleen atrophy and lymph node necrosis were also observed, indicative of immune-mediated damage in deceased patients. In addition to the cytokine-based pathology in patients with severe COVID-19, complement activation has also been observed, indicating that complement inhibitors, if used at an early stage of the infection, may attenuate the inflammatory damage. Hopefully these approaches will be approved into clinical trials to benefit the patients.

2.4 Rationale for MSC in the Treatment of SARS-CoV-2 Associated ARDS

MSCs are adult, non-hematopoietic precursor cells derived from a variety of tissues (e.g., bone marrow, adipose tissue, and placenta) and have been used as therapy in multiple conditions, especially in immune-mediated inflammatory diseases, such as graft versus-host disease (GVHD) and systemic lupus erythematosus (SLE) with evidence of benefit. In preclinical models, MSC are effective in ameliorating acute lung injury due to their ability to secrete paracrine factors that regulate lung endothelial and epithelial permeability, including growth factors, anti-inflammatory cytokines, and antimicrobial peptides [27, 28, 29]. As infused MSC home to the areas of inflammation, it is possible that these cells will limit the severity and duration of ARDS and potentially improve survival. Even in the setting of ARDS, MSC infusions are safe [22]. Of 40 patients treated with MSC in this study, no MSC- related hemodynamic or respiratory adverse events were observed.

Multiple clinical trials using stem cell therapy to treat the SARS-CoV-2 from China have been registered at www.clinicaltrials.gov . Leng et al. reported on the results of MSC in 7 subjects with COVID-19 pneumonia treated at a single institution between 01/23/2020 and 02/16/2020 [30]. Signs and symptoms were severe in 5 and moderate in 2 with an additional 3 patients with severe disease treated with placebo. The pulmonary function and symptoms in all MSC treated patients significantly improved in 2 days after MSC infusion. No infusion related events were observed. After treatment, the peripheral lymphocytes increased, C-reactive protein decreased, with changes

in the cytokine profile suggesting a switch away from the underlying proinflammatory state. While not conclusive, these data support further study of MSC in the treatment of SARS-CoV-2 associated pneumonia and ARDS.

2.5 Rationale for the Proposed Study

Currently, there is no pharmacologic agent or other approach, such as a cell therapy with proven impact on the risk of mortality of ARDS. Treatment remains primarily supportive, with lung-protective ventilation and a fluid conservative strategy, as well as prone positioning in more severe cases. Despite improved management, mortality of ARDS generally remains high between 30-40% in clinical studies. [31,32]

Seven patients with COVID-19 pneumonia were treated with MSC with promising preliminary results [30]. The pulmonary function and symptoms significantly improved in 2 days after MSC transplantation, potentially improving resource allocations as well as survival. Furthermore, the gene expression profile demonstrated that MSC were ACE2neg and TMPRSS2neg which suggests that MSC should be free from COVID-19 infection. In summary, the intravenous infusion of MSC in patients with COVID-19 pneumonia was safe and potentially effective.

In a prospective, double-blind, multicenter, randomized trial, Matthay et al. [22] compared a single dose of MSC compared to placebo in 60 patients with ARDS. No patient experienced any of the MSC-related hemodynamic or respiratory adverse events. While MSC were found to be safe in ARDS, 28-day mortality did not differ between the groups (30% in the MSC group vs 15% in the placebo group, odds ratio 2·4, 95% CI 0·5–15·1). While this outcome might be due to higher risk patients being assigned to the MSC arm, (i.e. higher baseline APACHE III score; minute ventilation and PEEP compared to the control arm) and variable MSC viability, the lack of an efficacy signal caused us to consider alternative approaches with MSC.

Based on the promising preliminary data that MSC can block the expression of inflammatory induced lung injury in animal models, and intriguing results in patients with COVID19 associated pneumonia and ARDS as well as an established safety profile of MSC generally and in ARDS in particular, the proposed study will evaluate the safety of multiple doses of MSC rather than a single large dose and their efficacy and biological activity in suppressing proinflammatory cytokines, and utilizing a uniform, optimized MSC thaw procedure to reduce the variability of MSC viability upon thawing.

Additionally, since the phase 2A trial [22] the Cell Therapy Lab of the University of Minnesota, which supported MSC manufacture for all sites, optimized manufacturing and harvest to improve on both pre- and post-thaw viability.

3 Study Design

This is a multi-center, randomized, placebo controlled, interventional phase 2A safety trial to evaluate the safety profile of multidosing of MSC and the potential efficacy and biological activity in patients with SARS-CoV-2 associated ARDS. There will be a 2:1 randomization for MSC and vehicle placebo control, respectively.

4 Patient Selection

Study entry is open to persons regardless of gender or ethnic background. There will be every effort to seek out and include females and minority patients.

4.1 Inclusion Criteria

- Age 18-80 years
- Meets 'Berlin Criteria' for diagnosis of moderate orsevere ARDS for a minimum of 4 hours [1]
- Less than 48 hours on a ventilator after meeting criteria for diagnosis of ARDS
- SARS-CoV-2 (proven by RT-PCR assay) with radiographic infiltrates
- PaO2/FiO2 ratio ≤ 250
- Positive end-expiratory airway pressure (PEEP) >5 cm H20
- Elevated C-reactive protein (above laboratory upper limit of normal)
- Meets organ function requirements[detailed in protocol section 4.2]
- Off other investigational agents directed against inflammatory cytokines 48 hours prior to enrollment; agents directed against the replication of SARS-CoV-2 [e.g., Remdesivir] are permitted
- Voluntary informed consent in person or virtually by the patient or patient surrogate considering the face to face limitations during the COVID-19 pandemic and, given the nature of the study population, which frequently requires mechanical ventilation with sedation, surrogate consent will likely occur in a substantial proportion of the study population (this will remain a valid consent until the patient is fully alert, and consistently competent, and can provide a second consent to continue participation in the study).

4.2 Organ Specific Inclusion Criteria

- Adequate organ function is defined as:
  - Renal: Calculated estimated glomerular filtration rate ≥30 mL/min/1.73 m2 (on chemistry panel)
  - Hepatic: Bilirubin <3x upper limit of normal (ULN) and AST, ALT and alkaline phosphatase <5x ULN
  - Cardiac: Absence of uncontrolled arrhythmia and heart failure (i.e., normal B-type natriuretic peptide [BNP] or LVEF ≥35% by echocardiogram)

4.3 Exclusion Criteria

- Ventilator support of FiO2 >0·8 or PEEP >20 cm H2O and ongoing use of more than two vasopressors for 2 or more hours with any agent at doses shown below.
  - Norepinephrine >12 µg/min or 0.2 µg/kg per min
  - Phenylephrine >150 µg/min or 3 µg/kg per min
  - Epinephrine >10 ug/min or 0.2 µg/kg per min
  - Vasopressin >0.04 units/min
- Concurrent use of other investigational agents specifically for treatment of ARDS or inflammatory cytokines. (Note: Agents, currently under investigations, shown to be efficacious will be permitted as these data emerge).

- Known ineligibility for use of a ventilator for a minimum of 7 days, as judged by the institution's Triage Team
- Known allergy to MSC components: fetal calf serum, human albumin or DMSO
- Active invasive malignant disease requiring chemotherapy/radiation
- Other concurrent life-threatening disease (life expectancy <6 months) or eligible for hospice care
- Known history of HIV infection on active treatment
- Females who are pregnant or breastfeeding
- Current mean arterial pressure (MAP) <60 mmHg while on 2 or more vasopressors at above doses for more than 2 hours
- History of any significant cardiac (myocardial infarction within 12 months of screening visit or unstable angina), chronic ongoing hepatic, or renal disease (grade 3 or higher); diagnosis of congestive heart failure with hypoxemia primarily due to decompensated heart failure; diagnosis of severe chronic obstructive pulmonary disease (COPD) or interstitial lung disease requiring supplemental oxygen at home
- Concurrent diagnosis of diffuse alveolar hemorrhage
- Requiring continuous dialysis (unable to stop dialysis during study agent infusion)

5 Patient Registration and Randomization

Enrollment will be staggered. Specifically, patients potentially meeting eligibility cannot be enrolled until 48 hours after the last infusion in the most recently treated patient. Registration will occur after the patient or patient's surrogate has signed the subject consent but before any treatment has been administered. Patients who were not able to sign consent originally, will be re- consented if they are able to subsequently. To be eligible for registration to this study, the patient must meet each criterion listed on the eligibility checklist (found in ONCORE) based on the eligibility assessment documented in the patient's medical record.

5.1 Registration with the University of Minnesota Masonic Cancer Center's CTO-CGI

Any patient who has been consented is to be registered in ONCORE by the Primary Study Coordinator or designee. If a patient is consented, but not enrolled, the patient's record is updated in ONCORE as a screen failure and reason for exclusion recorded. Complete registration information is found in the study's Procedures Manual for Participating Sites.

Participating sites only: At the time of registration, the signed consents will be uploaded into ONCORE as an attachment under the appropriate record.

Participating sites are responsible for fulfilling any local registration requirements.

5.2 Study Enrollment with the University of Minnesota CTO-CGI

To be eligible for study enrollment, the patient or patient's surrogate must sign the treatment consent and the patient must meet each of the inclusion criteria and none of the exclusion on the eligibility checklist (ONCORE) based on the eligibility assessment documented in the patient's medical record.

The PCRC or designee will assign the study treatment arm and add the on-treatment date to complete enrollment. Additional information can be found in the study's Procedures Manual for Participating Sites.

5.3 Enrolled Patients Not Receiving Study Treatment

If a patient is enrolled onto the study and is subsequently not able to begin the planned study treatment for whatever reason, the patient will be removed from study and treated at the physician's discretion. The study coordinator or designee will update ONCORE of the patient's non-treatment status and notify the Principal Investigator. The reason for removal from study prior to starting study treatment will be clearly indicated in ONCORE. The patient may be replaced.

5.4 Randomization

Patients will be randomly assigned (2:1) to receive either 300 × 10^6 MSC or vehicle placebo control. Randomization will be stratified by risk (high versus standard risk detailed in Section 5.5.), using permuted block sizes of 3. The allocation sequence will be accessed by each cell processing laboratory through ONCORE. Personnel in the cell processing laboratories are not masked to the treatment group, but patients, clinical staff, and investigators will be unaware of treatment assignment. To maintain masking of the investigators and clinicians, bags containing the study products and intravenous tubing had opaque coverings applied in the cell laboratories.

5.5 Risk Group Assignment

Presence of preexisting co-morbidities appears to be associated with risk of death from COVID-19 [30]. For this study patients will be stratified as high risk or standard risk (Table 1) based on the presence of specific co-morbidities based on the judgement of the care team.

Table 1:Risk Definitions

High Risk Definition	Standard Risk Definition
History of any of the following on medical history review: • Coronary artery disease or other chronic cardiovascular disease (h/o myocardial infarction (MI); heart failure; stent placement) • Hypertension (excluding well-controlled hypertension on a single agent) • Chronic pulmonary disease (moderate or severe as defined by either multiple exacerbations requiring treatment and/or outpatient FEV1 ≤ 70% predicted when free of exacerbation) • Insulin dependent diabetes (type 1 or type 2) • Cerebrovascular disease (h/o ischemic or hemorrhagic stroke) • Chronic renal disease – GFR <30% • Age >65 years	Absence of any high risk co-morbidities

Note: It is recognized that the standard of care for COVID-19 ARDS is rapidly changing. In the event that drugs

like Tocilizumab are approved during the study period.

6 Treatment Plan

All patients with ARDS will be managed with standard of care treatments, including lung protective ventilation as implemented by the NIH ARDS Network (typically with a tidal volume ≤6 ml/kg/ideal body weight and a plateau airway pressure <30 cm H_2O). Patients may be proned and, if necessary, may be placed on extracorporeal membrane oxygenation (ECMO).

6.1 Investigational Intervention

In addition to standard aggressive medical management, patients enrolled in this clinical trial will receive the investigational product, MSC-derived from allogeneic bone marrow, or vehicle placebo control based on a 2:1 randomization scheme.

Table 2: Study Agents

Agent	Route and Time of Administration	Preparation
MSC	IV, masked bag and tubing, delivered within 30 minutes of start of infusion	Thawed product containing MSC in DMSO resuspended 1:1 with Dextran 40 + 5% human serum albumin [total volume 60 mL]
Vehicle Placebo Control	IV, masked bag and tubing, delivered over 30 minutes	Dextran 40 + 5% human serum albumin + [total volume 60 mL]

Prior to each Infusion: Study agent may be infused (Table 2) after a patient is off hemodialysis (if applicable) and considered to be sufficiently stable, as defined by a transcutaneous oxygen saturation of ≥88% with ventilator support of FiO2 ≤0·8 or PEEP ≤20 cm H2O and use of no more than two vasopressors at doses shown below in the supine position.

- Norepinephrine <12 µg/min or 0.2 µg/kg per min
- Phenylephrine <150 µg/min or 3.0 µg/kg per min
- Epinephrine <10 µg/kg per min or 0.2 µg/kg per min
- Vasopressin <0.04 units/min

Administration of Investigational Agent: MSC or the vehicle placebo control will be administered intravenously with a standard blood filter tubing set with a pore size of 170 µm. The study product will be infused by gravity over approximately 30 minutes.

Pre-medications and hydration 30 minutes before administration of the investigational product will include:

- Benadryl 25 mg IV
- Tylenol 500 mg, PO or rectal

The product before infusion will not be irradiated and in-line leukocyte filters will not be used for product infusion. In addition, no medications will be infused in the same line as investigational product during the product infusion. Potential toxicities of MSC and vehicle control are detailed in section 7.1.

6.2 Management of Adverse Events During Study Agent Infusion

During the infusion of study agent, patients will have monitoring of continuous arterial blood pressure, heart rate, rhythm as well as oxygen saturation. Study personnel will be available for the duration of the infusion to monitor the patient. Body temperature will be monitored at a minimum at the start, midway through, and at the end of the infusion. During study agent infusion, patients will be monitored closely for signs of allergic reactions, e.g. rash, urticarial, angioedema, wheezing as well as worsening hypotension or new arrhythmias. Emergency drugs for hypersensitivity reactions, worsening hypotension or arrhythmias will be available and administered according to institutional guidelines. If there are any signs of a transfusion related reactions, the infusion will be stopped immediately. The infusion can also be stopped at the discretion of the study investigator or care team if there is concern about the patient's status. If a patient is deteriorating or not improving after the third MSC infusion, they may enter another study 7 days after last infusion.

Appropriate medical care should be instituted as per standard institutional practices. If possible, additional systemic corticosteroids should be avoided unless absolutely required for management of acute allergic/infusion reactions (Table 3), as determined by the Investigator. Study agent infusion may be interrupted temporarily upon resolution of the event at the discretion of the care team and Investigator, based on the management guidelines below.

Table 3 CTCAE Term: Infusion Related Reaction

Grade 1	Grade 2	Grade 3	Grade 4	Grade 5
Mild transient reaction; infusion interruption not indicated; intervention not indicated	Therapy or infusion interruption indicated but responds promptly to symptomatic treatment (e.g., antihistamines, suctioning, IV Fluids or other supportive care measure typical in the management of COVID-19 related ARDS	Prolonged (e.g., not rapidly responsive to symptomatic medication and/or brief interruption).	Life-threatening consequences; urgent intervention indicated	Death
Common Toxicity Criteria for Adverse Events version 5.0 (CTCAE v5) are detailed at: https://ctep.cancer.gov/protocoldevelopment/electronic_applications/docs/CTCAE_v5_QuickReference_5x7.pdf for documenting severity of observed events				

The study agent infusion may be reinitiated if the reaction is grade 2 as defined in Table 3. If the grade 2 event recurs after infusion re-initiation or if the event is considered to be grade 3 or greater, the infusion will be stopped and the uninfused product will be retained for further evaluation (a Transfusion Medicine consultation will be obtained and the product will be evaluated for sterility).

Antihistamines, such as diphenhydramine 50 mg IV, should be considered for treatment of allergic reactions, any grade reaction. Systemic corticosteroids may be used for grade 3-4 allergic reactions but should be avoided for grade 1 and 2 reactions. Fluids and.or increases in dose or addition of vasopressors will be used for treatment of hypotension. Anti-arrhythmia agents will be used to treat new arrhythmias.

Concomitant Therapies

All patients will be treated with standard of care based on Society or CDC guidelines. All other pharmacologic agents used in the conventional treatment of ARDS will be recorded. Patients may be treated with anti-viral agents; however, all concomitant drugs with potential immunomodulatory

activities should be reviewed by the PI or co-investigators prior to enrollment. Chloroquine or hydroxychloroquine is permitted.

Note: Every effort should be made not to initiate glucocorticoids if the patient is not receiving glucocorticoids after treatment or increase the dose of steroids if the patient is receiving low to moderate dose steroids on study initiation. If the treating physician feels it is in the best interest to initiate high dose steroids, e.g. due to new onset of diffuse alveolar hemorrhage, lack of response after 7 days or worsening respiratory status after 4 days from the first infusion of the investigational product (MSC vs vehicle placebo control), the reason should be documented in the medical record.

6.3 Duration of Study Participation

Patients will be followed for 1 year after treatment.

7 Expected Treatment Related Toxicities

7.1 Potential MSC and Vehicle Placebo Control Toxicities

MSC, like other blood products, may cause infusional reactions, including hemodynamic effects or acute hypersensitivity reactions. Furthermore, the cryoprotectant, DMSO, can cause side effects as shown below (Table 4).

Table 4: Side Effects of MSC

Relatively Common	Less Common	Unknown Risks in MSC Group
• DMSO toxicities (e.g., headache, hypertension, cardiac toxicity, nausea and vomiting)	• Allergic reaction (including itching, hives, flushing [red face], skin rash, fever, chills, wheezing, stiff muscles. While shortness of breath and chest tightness are common symptoms in those who are not on the ventilator, these symptoms might not be obvious. Rapid increase in FIO2 requirement and other ventilator settings may occur.	• Hemodynamic instability • Increased pulmonary vascular resistance

DMSO side effects and symptoms are generally associated with histamine release and include coughing, flushing, rash, chest tightness and wheezing, nausea and vomiting, and cardiovascular instability [33]. However, due to the small MSC cryobag volume (~30 ml), a reaction to DMSO is not expected. Signs of allergic reaction and any changes in hemodynamic stability or respiratory changes will be treated with appropriate medications per institutional practice for the signs and symptoms observed.

Note: Vehicle placebo control will not contain DMSO. While the MSC product will contain DMSO, for sedated, ventilated patients, caregivers will not be able to detect the associated DMSO odor

(e.g. cabbage or wild radish odor), important for maintaining the blinding of caregivers and investigators.

Emergency drugs should be available and administered per institutional guidelines.

8 Schedule of Patient Activities

Scheduled evaluations after screening and until day 100 are shown in Table 5A (clinical) and Table 5B (research). For assessments after discharge from the hospital, evaluations may be performed +/-3 days from the listed target date through day 28 and +/-7 days for the month 2 and month 3 evaluations and +/-1 month for the one year evaluation.

8.1 Required Clinical Care Examinations

Table 5A: Clinical Care												
Study Phase	**Screening**	**First MSC or Placebo Infusion[7]**	**Follow up Days [unless otherwise noted] relative to first Study Agent (MSC/placebo) Infusion**									
Study Time Interval	**Days -2 to Day 0 prior to infusion**	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**14**	**21[11]**	**Day 28[11], month 2[11], month 3[11], Month 6[11] and 1 year[11]**
Informed* Consent	X											
Medical history	X											
Current medical conditions#	X	X	X	X	X	X	X	X	X	X	X	X
Body weight	X								X	X	X	X
Height	X											
Vital signs#	X	X[8]	X	X	X	X	X	X	X	X	X	X
Physical examination#	X	X	X	X	X	X	X	X	X	X	X	X[12]
BNP	X[10]											Day 28[9,10]
EKG	X											Day 28[9]
Chest x-ray or chest CT	X											Day 28 chest imaging X
Lung function status[1]												Month 2, Month 3, Month 6 and 1 year
Dyspnea Index Questionnaire[1]												Day 28, month 2, month 3, month 6 and 1 year
Full PFTs Including Lung Volumes & DLCO1												3 month, 6 month, 1 year
6 minute walk test (O2 assessment)[1], unless clinically contraindicated												Day 28, month 2, month 3, month 6 and 1 year
Non-contrast High Resolution Chest CT with												3 month, 1 year

Table 5A: Clinical Care												
Study Phase	**Screening**	**First MSC or Placebo Infusion[7]**	**Follow up Days [unless otherwise noted] relative to first Study Agent (MSC/placebo) Infusion**									
Study Time Interval	**Days -2 to Day 0 prior to infusion**	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**14**	**21[11]**	**Day 28[11], month 2[11], month 3[11], Month 6[11] and 1 year[11]**
inspiratory & expiratory views[1]												
SARS-CoV-2 pcr	X											Day 28 only
Chemistries[2]	X	X	X	X	X	X	X	X	X	X	X	X
Hematology[3]	X	X	X	X	X	X	X	X	X	X	X	X
Immuno-globulin levels (IgG, IgA, IgM)	X											Day 28[9]
Coagulation panel[4]	X								X			
Clinical Covid Cytokine Panel[5]	X		X	X	X	X	X	X	X	X	X	X
Pregnancy test (females)	X											
SOFA and APACHE II Score[6]	X								X	X	X	X

Abbreviations: ECHO=echocardiogram; ECG=electrocardiogram; PFT=pulmonary function tests; SOFA=sequential organ failure assessment; APACHE=acute physiology and chronic health evaluation; AE=adverse event; ICRF=result recorded in infusion case report form; pcr=polymerase chain reaction; HLA=histocompatibility locus antigens.

*Consent should be re-obtained when the patient's condition permits, if the patient's surrogate provided the original consent.

#Medical monitoring will be conducted daily at least for 7 days but will continue daily during the length of the hospitalization. e-Visits using iPads and visualization are permitted to reduce infectious disease risks and minimize PPE use.'

1. Recommended pulmonary clinic follow up visits (virtual only if patient is unable to return to clinic)
2. Chemistries include: Na, K, Cl, CO2, BUN, Cr, glucose, Ca, P, ALT, AST, Alk P, TB, Alb, TP, LDH, C-reactive protein (CRP).
3. Hematology includes: white count with differential, hemoglobin, MCV, platelets.
4. Coagulation panel: PT, PTT, fibrinogen, protein C, D-dimer (add lupus anticoagulant, if PTT elevated [34])
5. Clinical COVID-19 cytokine panel: IL-1, IL-6, TNFa, and IL-8.

6. SOFA and APACHE II scores (Appendix I) are done only for patients remaining in the ICU.
7. MSC or vehicle placebo control will be administered three times within 7 days of the first infusion, aiming for 48 hour intervals. Vital signs will be checked before and every 15 minutes during the infusion, at the completion of the infusion, and one, 3 and 6 hours post study agent infusion. More frequent vital signs may be required depending on reactions to the product infusion.
8. Vital signs include body weight, body temperature, blood pressure, pulse, and pulse oximetry. The highest temperature recorded within 24 hr period will be reported.
9. If abnormal on d28, additional tests will be done until normal at 2, 3 and 12 months
10 Echocardiogram is only required if BNP is abnormal
11. If patient cannot return to clinic for a follow-up visit; every attempt will be made to obtain laboratory tests along with a virtual clinic visit
12. 21 day, 28 day and 2 month assessments can include either virtual visit and/or use of data from clinical provider visits.

8.2 Patient Research Related Examinations

Table 5B: Research Related Examinations												
Study Phase	**Screening**	**First MSC or Placebo Infusion[5]**	**Follow up Days [unless otherwise noted] relative to first Study Agent (MSC/placebo) Infusion**									
Study Time Interval	**Days -2 to Day 0 prior to infusion**	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**14**	**21**	**Day 28, month 2, month 3, Month 6 and 1 year**
Randomization	X											
MSC or Placebo Infusion		X		X		X						
Arterial blood gas		X [30 min before and once 1-4 hours post infusion]										
Immune profile[1] 10 ml green top	X								X	X	X	X
Cytokine profile[2] 5 ml red top	X		X	X	X	X	X	X	X	X	X	X
Serum, plasma, cell storage[3] 5 ml red top 10 ml green top	X		X	X	X	X	X	X	X	X	X	X
Arterial blood gasses[4]		X										
Adverse events	X	X ICRF	X	X	X	X	X	X	X	X	X	X

1. All samples are collected and handled per current University of Minnesota guidelines for SARS-CoV-2 infected patients. All research samples (unless otherwise noted) are transferred to the BSL-2 Lab on the 2nd floor of Microbiology Research Facility for processing and storage. This lab is certified for COVID-19 samples. Immune profile (green top) includes: T, B, NK, macrophage, dendritic cell subsets and expression of interferon-γ [IFN-γ] by CD4+T, CD8+T and NK cells (samples will be cryopreserved, stored in liquid nitrogen or -120oC freezers and evaluated retrospectively). All samples collected and handled at the University of Minnesota will follow local guidelines for SARS- CoV-2 infected patients. All research samples (unless otherwise noted), including those from University of Pittsburgh shipped after cryopreservation, are transferred to the BSL-2 Lab on the 2nd floor of Microbiology Research Facility for processing stored following Institutional guidelines.
2. Extended cytokine profile (red top) includes: IL-2, IL-10, IL-18, sIL-2R, sIL-6R, sTNFR1, IFN-γ, Ang-1, Ang-2, gp130. Samples will be processed and stored following Institutional guidelines. Serum will be stored at - 80oC for planned batch analysis of all samples at the end of study. Evaluations will be performed at the University of Minnesota Cytokine Laboratory a BSL2 Laboratory.
3. Serum (red top) and mononuclear cell preps (green top) will be stored in liquid nitrogen at each site for planned batch analysis of all samples at the end of study (e.g.,

research will include development of antibodies to SARS-CoV-2). All samples collected and handled at the University of Minnesota will follow local guidelines for SARS-CoV-2 infected patients. All research samples (unless otherwise noted) are transferred to the BSL-2 Lab on the 2nd floor of Microbiology Research Facility for processing and storage. This lab is certified for COVID-19 samples.

4. Prior to and sometime between 1 and 4 hours after the first study agent infusion
5. MSC or vehicle placebo control will be administered three times within 7 days of the first infusion, aiming for 48 hour intervals. Vital signs will be checked before and every 15 minutes during the infusion, at the completion of the infusion, and one, 3 and 6 hours post study agent infusion. More frequent vital signs may be required depending on reactions to the product infusion. An arterial blood gas will be obtained within 30 minutes prior and once between 1 and 4 hours after the first study agent infusion.

9 Adverse Event Monitoring, Recording and Reporting

Toxicity and adverse events will be classified according to NCI's Common Terminology Criteria for Adverse Events V 5.0 (CTCAE) and reported on the schedule below. A copy of the CTCAE events can be downloaded from http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm#ctc_50

9.1 Definitions

The following definitions are based on the Code of Federal Regulations Title 21 Part 312.32 (21CFR312.32(a)).

Adverse Event: Any untoward medical occurrence associated with the use of a study agent in humans, whether or not considered study agent related.

Suspected Adverse Reaction: Any adverse event for which there is a reasonable possibility that the study agent caused the adverse event.

Treatment-Emergent Adverse Event: Any event not present prior to the initiation of the treatment or any event already present that worsens in either intensity or frequency following exposure to the treatment. A treatment emergent AE refers to an event temporally related to the study agent regardless of the causality assessment by the investigator.

Life-Threatening Adverse Event or Life-Threatening Suspected Adverse Reaction: An adverse event or suspected adverse reaction is considered “life-threatening” if, in the view of either the investigator or sponsor, its occurrence places the patient or subject at immediate risk of death. Note: a life-threatening event does not necessarily equate to a CTCAE grade 4.

Serious Adverse Event or Serious Suspected Adverse Reaction: An adverse event or suspected adverse reaction to the study agent is considered “serious” if, in the view of either the investigator or sponsor, it results in any of the following outcomes:

- Death
- A life-threatening adverse event
- Inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly/birth defect.

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

Unexpected adverse event or unexpected suspected adverse reaction: An adverse event or suspected adverse reaction is considered "unexpected" if it is not listed in the investigator brochure or is not listed at the specificity or severity that has been observed; or, if an investigator brochure is not required or available, is not consistent with the risk information described in the general investigational plan or elsewhere in the current application, as amended. Thus, as defined by the FDA adverse events that occur as part of the disease process or underlying medical conditions are considered unexpected; however, for the purposes of this study they will not be documented or reported.

Major Deviation: A deviation or violation that impacts the risks and benefits of the research; may impact subject safety, affect the integrity of research data and/or affect a subject's willingness to participate in the research. Deviations that place a subject at risk, but do not result in harm are considered to be major deviations.

Minor Deviation: A deviation or violation that does not impact subject safety, compromise the integrity of research data and/or affect a subject's willingness to participate in the research.

9.2 Adverse Event Monitoring, Recording and Reporting

9.2.1 Event Monitoring

Monitoring for adverse events will begin on the day of the first study agent infusion and continue through 28 days.

9.2.2 Event Recording/Documentation

Due to the seriousness of SARS-CoV-2 mediated ARDS, numerous adverse events are anticipated in this patient population regardless of the infusion of the study agent.

Use of specific supportive interventions, including prone positioning (days of proning), use of paralytics, pulmonary vasodilators, tocilizumab, steroids, and use of alternative modes of ventilation (APRV, HFOV, etc.) and ECMO will be collected.

Other events to be documented will include:

1) Changes in heart rate, systolic and diastolic blood pressure, temperature, oxygen saturation, ventilator parameters, urine output, and vasopressor dose at baseline and every 15 minutes during the one hour of study agent infusion, then hourly for six hours. every 8 hours until the next study agent infusion.

2) Oxygenation Indices including: PaO2:FiO2 ratio, peak and plateau pressures, PEEP at baseline, day of study agent infusion and each day thereafter while ventilated. In addition, at specified time points (see Table 5A), lung function status, dyspnea index, pulmonary function tests, 6 minute walk test, high resolution chest CT, will be assessed to determine the short and long term effects on COVID-19 ARDS and the impact of treatment.

3) Number of ventilator days

4) Number of ICU Days

For this trial, a reportable adverse event is defined as:

1) Sustained decline (>1 hour) in cardiorespiratory status within 6 hours from the start of study product infusion as defined by:
   - An increase in vasopressor requirement such that the for any agent doses are:
     - Norepinephrine ≥12 µg/min or 0.2 µg/kg per min
     - Phenylephrine ≥150 µg/min or 3.0 µg/kg per min
     - Epinephrine ≥10 µg/kg per min or 0.2 µg/kg per min
     - Vasopressin ≥0.04 units/min
   - Addition of a third vasopressor

- New cardiac arrhythmia requiring cardioversion or ventricular tachycardia, ventricular fibrillation or asystole
- Hypoxemia requiring an increase in FiO2 of 0.2 or more and an increase in PEEP of 5 or more to maintain Sp02 in the target range of 88-95%
- Clinical scenario consistent with transfusion incompatibility or transfusion-related infection (e.g., urticaria, new bronchospasm)

2) Any cardiac arrest or death occurs within 24 hours from the start of study product will be reported as a pre-specified infusion-associated significant event. Any cardiac arrest or death occurs during the study period will be a reportable event that will be reviewed in detail.

As patient safety is an essential component of this protocol, each participating investigator has primary responsibility for the safety of study participants under his or her care. The Principal Investigator will evaluate all clinically important adverse events.

Expected events for ARDS are untoward clinical occurrences that are perceived by the investigator to occur with reasonable frequency in the day to day care of patients with ARDS treated in an ICU with mechanical ventilation. Examples of adverse events that are expected in the course of ARDS include transient hypoxemia, agitation, delirium, nosocomial infections, skin breakdown, and gastrointestinal bleeding. Such events, which are often the focus of prevention efforts as part of usual ICU care, will not be considered reportable adverse events unless the event is considered by the investigator to be associated with the study cell product or procedures, or unexpectedly severe or frequent for an individual patient with ARDS. Examples of unexpectedly frequent adverse events would be repeated episodes of unexplained hypoxemia. This would be in contrast to an isolated episode of transient hypoxemia (e.g., Sp02 ~85%), related to positioning or suctioning. This latter event would not be considered unexpected by nature, severity or frequency.

Organ failure related to ARDS or the patient's underlying condition will be systematically captured by the protocol but should not be reported as adverse events unless they are considered to be study related.

All adverse events occurring during the study period must be recorded in the patient's case report forms (CRFs). The clinical course of each event should be followed until resolution, stabilization, or until it has been determined that the study treatment or participation is not the cause. SAEs that are still ongoing at the end of the study period must be followed up to determine the final outcome. Any SAE that occurs after the study period and is considered to be possibly related to the study treatment or study participation should be recorded and reported immediately to the study sponsor (see Section 9.2.3.).

9.2.3 Site Investigator's Reporting Requirements

Investigators will report all serious AND unexpected, AND study-related adverse events to the study sponsor and CTO-CGI at the University of Minnesota within 24 hours by fax, phone or email.

Investigators must also report Unanticipated Problems, regardless of severity, associated with the study drug or study procedures within 24 hours.

The IRB must also be notified in a timely manner, as per the IRB"s requirement. The investigator will then submit a detailed written report to the study sponsor and the IRB (as required) no later than 5 calendar days after the investigator discovers the event.

The minimum necessary information (Table 6) to be provided at the time of the initial written report includes:

Table 6: Essential Information	
○ Study identifier ○ Study Center ○ Subject number ○ Date of event onset ○ A description of the event	○ Whether study treatment was discontinued ○ The reason why the event is classified as serious ○ Investigator assessment of the association between the event and study treatment ○ Current status

9.2.4 Event Reporting to the Study Sponsor, IRB and FDA

The University of Minnesota has the following expedited reporting responsibilities (Table 7):

Table 7: Expedited Reporting

Agency reporting to	Criteria for reporting	Timeframe	Form to Use	Submission address/email address
University of Minnesota IRB	Events requiring prompt reporting include, but are not limited to unanticipated death of a locally enrolled subject(s); new or increased risk, any adverse event that requires a change to the protocol or consent form, or any protocol deviation that results in harm. Deviations from the protocol that do not result in harm may also need to be reported to the IRB, per the IRB's current reporting requirements. For a complete list, refer to http://www.research.umn.edu/irb/guidance/ae.html#.VC7xral0-sh.	Within 5 business days of event discovery	RNI form	http://www.ethos.umn.edu
FDA	Unexpected and fatal or unexpected and life threatening suspected adverse reaction	no later than 7 Calendar Days	SAE Report Form	Submit to FDA as an amendment to IND, with a copy to each participating site
	1) Serious and unexpected suspected adverse reaction or 2) increased occurrence of serious suspected adverse reactions over that listed in the protocol or investigator brochure or 3) findings from other sources (other studies, animal or in vitro testing)	no later than 15 Calendar-Days		
	Note: Events due to the disease under treatment or an preexisting medical condition will not require expedited reporting to the FDA for the purposes of this study			

The MCC SAE Coordinator or designee is responsible for reviewing each report and determines if the event is reported in real time to the Independent Data Safety Monitoring Board (DSMB) in real time or as part of quarterly summary reports required for any high risk trial.

At the time of continuing review/IND annual report, relevant events recorded in ONCORE will be reported in summary format by those persons responsible for such reporting.

9.2.5 Institutional Event Reporting Table

Individual institutional sites will be responsible for reporting any event meeting local reporting requirements to their institutional IRB and/or other research oversight committees.

Institutional sites will be responsible for reporting events within the following time frames to the MCC Multisite Program Manager.

Table 8: Institutional Reporting

Event Type	Reporting Timeframe	Form to Use	Report to
Any event meeting the definition of an SAE	Within 24 hours of knowledge	For UMN: ONCORE SAE Report Form For Participating Sites: Paper SAE Report Form	For Participating Sites: Masonic Cancer Center (MCC) Multisite Program Manager affilates@umn.edu Local institutional IRB or other entities per institutional policies and guidelines Independent DSMB
Stopping Rule Events as per section 9.2.2 and section 12.4	Within 24 hours of knowledge	Stopping Rule Event Form in ONCORE	
Major Deviations, as defined in Section 9.1.	Within 5 working days of knowledge	Deviation Report Form in ONCORE	
Minor Deviations, as defined in Section 9.1.	Per Institutional Policy	n/a (record in Deviations Tab)	For UMN MCC: Report to the study's regulatory specialist For Participating Sites: minor deviations are not reportable to the MCC Multisite Manager. Report to local institutional IRB or other entities per institutional policies and guidelines.

Sponsor Reporting: Notifying the DSMB

The study sponsor will report all serious, unexpected, and study-related adverse events to the DSMB, by email, or telephone, within 7 calendar days of the study sponsor being notified of the event. A written report will be sent to the DSMB within 15 calendar days, and these reports will be sent to investigators for submission to their respective IRBs, as required. The DSMB will also review all adverse events during scheduled interim analyses. Sponsor Reporting: Notifying All Participating Investigators.

It is the responsibility of the study sponsor to notify all participating investigators, in a written IND safety report, of any adverse event associated with the use of the drug that is both serious and

unexpected. Additionally, sponsors are also required to identify in IND safety reports all previous reports concerning similar adverse events and to analyze the significance of the current event in light of the previous reports. IND safety reports will be sent to the participating investigators immediately after submission to the IND.

The study sponsor will distribute the written summary of the DSMB"s periodic review of adverse events to investigators for submission to their respective IRBs in accordance with NIH guidelines.

9.3 Unblinding Procedures

The study will have an external Medical Monitor separate from the Investigator/Sponsor and who will not be affiliated with any of the study sites or participate in any study procedures (e.g., screening of subjects, consenting of subjects or surrogates, administration of MSCs or study follow-up). If unblinding the study therapy is necessary to ensure a subject's safety, this will be done by the external medical monitor after review of the clinical events. If this is done in conjunction with the site Principal Investigator, the study sponsor must be informed within 24 hours by phone, fax or email of the unblinding event, followed by a detailed written narrative within 48 hours of the event by the Principal Investigator at the study site.

In other circumstances, the medical monitor may choose to unblind him/herself if there is concern based on the safety data that a type of adverse event may be associated with MSCs treatment; in this case, the medical monitor will inform the study sponsor that he/she has unblended him/herself, but not of the treatment assignments.

9.4 External Medical Monitoring

An external expert in pulmonary medicine and critical care will serve as the Medical Monitor. The Medical monitoring will include a regular assessment of each adverse event, as well as the number and type of SAEs. The Medical Monitor will be blinded to the treatment but will provide an assessment of the relationship to study agent. If an SAE is considered to be related to the study agent infusion, the DSMB will be notified.

9.5 Monitoring for Stopping Rule Events

Early stopping rules will be in place for infusion related toxicity in recipients of MSC. The stopping rules are generated using an adaptation of Pocock stopping boundaries [35]. In the event that a stopping boundary is triggered, study enrollment will be suspended and the PI, IRB, DSMB will be notified.

For this study, excess grade 3-5 infusional toxicities (using Common Toxicity Criteria for Adverse Events version 5.0 (CTCAE v5, section 6.2, Table 3) or predefined hemodynamic or respiratory AEs within 6 and 24 hours of the start of infusion of study agent will be used for triggering early stopping. As patients must have been had a transcutaneous oxygen saturation 88–95% without the need for an FiO2 >0.8 or PEEP >20 cm H2O) or use of more than two vasopressors in the supine position, worsening as defined in table 9 below within 6 hours after MSC infusion will be considered an adverse event(s) that contributes to the cumulative stopping boundary.

It is expected that the rate of events will be <10%. The goal is to construct a boundary based on toxicity such that the probability of early stopping is at most 10% if the rate is equal to 9.5%and our sample size is 20. With these stipulations, the trial will be stopped and reviewed if 2 events out of 3 patients, 3 events out of 9 patients, 4 events out of 15 patients or 5 events occur. The probability of hitting the stopping boundary if the rate is 30% is 82%.

Predefined hemodynamic and respiratory SAEs (based on the START Study parameters [22]):

Table 9: Hemodynamic and Respiratory SAEs	
Event within 6 hours of study agent infusion	
Definition of hemodynamic deterioration at 6 hours	Sustained decline (>1 hour) in cardiorespiratory status within 6 hours from the start of study product infusion as defined by: 1. An increase in vasopressor requirement such that the doses are: • Norepinephrine ≥12 µg/min or 0.2 µg/kg per min • Phenylephrine ≥150 µg/min or 3.0 µg/kg per min • Epinephrine ≥10 µg/kg per min or 0.2 µg/kg per min • Vasopressin ≥0.04 units/min 2. Addition of a third vasopressor 3. New cardiac arrhythmia requiring cardioversion or ventricular tachycardia, ventricular fibrillation or asystole 4. Hypoxemia requiring an increase in FiO2 of 0.2 or more and an increase in PEEP of 5 or more to maintain Sp02 in the target range of 88-95%
Event within 24 hours of study agent infusion	
Definition of cardiopulmonary deterioration at 24 hours	Cardiac arrest or death

For this study, a DSMB will review day 28 safety data after 5 and 15 patients have been enrolled and received study agent. Enrollment will not be suspended pending DSMB review. All pre-specified and other clinically important events and unexpected SAEs, including death, will be reported to the DSMB on an ongoing basis. The study will be stopped for a safety evaluation by the DSMB if the DSMB members have any concerns or if three subjects have pre-specified clinically important events or unexpected SAEs except death since death will be common in this critically ill population due to the nature of the underlying illness, COVID-19 ARDS.

ONCORE

For participating sites, a copy of the completed Event Form must be sent to the MCC Multisite Program Manager at the time it is submitted to the study statistician.

As these events are outcomes of the treatment, not toxicity as found in CTCAE, they should only be recorded/reported as an AE, if they meet the criteria found in section 9.2.

10 Study Data Collection and Monitoring

10.1 Data Management

This study will collect regulatory and clinical data using University of Minnesota CTSI's ONCORE® (Online Enterprise Research Management Environment).

The ONCORE database resides on dedicated secure and PHI compliant hardware consisting of 3 physical servers: dev, DR, and production. The dev server is located in the University of Minnesota datacenter and houses six database instances (test, train, sandbox, mcc reports, oncdm, and vendor) that are backed up locally because the data is refreshed from ONCORE production data. The production server is located in the UMN datacenter. All the data servers are managed by the Academic Health Center – Information Systems (AHC-IS) virtual servers which utilize clustered infrastructure to provide real-time failover of virtual servers. This real- time clustering is physically limited to the UMN data center. All relevant AHC IS procedures related for PHI compliant servers (as required by the Center of Excellence for HIPAA Data) apply to ONCORE databases.

The integrated data will be stored in PHI compliant servers managed by AHC IS with access given to those authorized users in the Clinical and Translation Science Institute Informatics team. The data will be integrated and extracted to researchers through the CTSI Informatics team and will be delivered through secure and compliant mechanisms (e.g. AHC IE data shelter, BOX, sftp, etc). If data de-identification is needed, then compliant AHC IE data de- identification tools will be used. The informatics team will grant the IRB approved study team members access to data.

10.2 Case Report Forms

Participant data will be collected using protocol specific CRFs developed within ONCORE, based on its library of standardized forms. The CRFs will be approved by the study's Principal Investigator and the Biostatistician prior to release for use. There is a screening case report form (SCRF) which the Study Coordinator or designee will use to register the patient into ONCORE at time of study entry. An infusion case report form (ICRF) will be used to document adverse events during the infusion of study agent (MSC or vehicle placebo control) and outcomes case report form (CRF) will record specific outcomes data for submission to the BMT Database. As medications, blood product administration, provider examinations and laboratory/radiographic data are accessible to the BMT / Cell Therapy Database through a Data Sharing Agreement, CRFs will only be needed for specific data collection.

10.3 Data and Safety Monitoring Board

This Phase 2A study will be monitored by an independent DSMB. The DSMB will contain at least 3 members, 2 intensivists familiar with the care of patients with ARDS, expert in cell therapies. A DSMB Charter will define the DSMB's responsibilities, summarized below.

The DSMB will meet by teleconference prior to the start of the trial, and at the planned interim safety analyses after 5 and 15 patients. At these meetings, the DSMB will review post infusion safety data through day 28, all pre-specified clinically important events and unexpected serious

adverse events, including deaths. All safety data will be reviewed in aggregate and will include individual adverse event narratives. During the Phase 2A trial, the DSMB will review the safety data in an unblinded fashion.

SAEs and deaths will be reported to the DSMB on an ongoing basis; the study will be stopped for a safety evaluation by the DSMB if they have any concerns, or if a stopping rule is activated.

The external Medical Monitor will serve as the liaison between the DSMB and the study sponsor and Principal Investigators. The actual analyses will be conducted by the Study Biostatistician who will ensure that the unblinded analyses are not available to study investigators.

The DSMB chair will be responsible for recording the summary of its various meetings and for reporting findings and/or recommendations to the study sponsor and to the funding agency.

10.4 IND Annual Reports

In accordance with regulation 21 CFR § 312.33, the sponsor-investigator with assistance from the University of Minnesota Masonic Cancer Center's Clinical Trials Office for Cell, Gene and Immunotherapies (CTO-CGI) will submit a progress report annually. The report will be submitted within 60 days of the anniversary date that the IND went into effect.

10.5 Data Sharing with Participating Sites

All data including identifiers will be submitted to the University of Minnesota BMT / Cell Therapy Database. Only the study sponsor and MCC investigators will have access to the complete data set including identifiers.

10.6 Monitoring

The investigator will permit study-related monitoring, audits, and inspections by the IRB, government regulatory bodies, and University of Minnesota compliance groups. The investigator will make available all study related documents (e.g. source documents, regulatory documents, data collection instruments, study data, etc.). The investigator will ensure the capability for inspections of applicable study-related facilities (e.g. pharmacy, diagnostic laboratory, etc.) will be available for trial related monitoring, audits, or regulatory inspections.

10.7 Teleconferences – Lead Site and Participating Site

Regular teleconferences to facilitate communication between participating sites regarding the study's progress, patient updates, summary of safety reports, case report form completion, and other issues for discussion. The University of Minnesota Multisite Program Manager will be responsible for arranging these teleconferences and preparing the agenda. Teleconferences will be scheduled at a regular interval determined by the lead institution. Participation of a minimum of one representative from each participating site will be required. These teleconferences are in addition to other previously described site interactions including centralized patient registration, institutional and MCC required reporting of safety related issues, case report form completion in the study's central database (ONCORE) and participating site oversight through self-monitoring in compliance with the Masonic Cancer Center's Data and Safety Monitoring plan.

10.8 Participating Site Monitoring

The Sponsor (Dr. Wagner) with the CTO has oversight responsibility for trial monitoring at participating sites. Participating sites must self-monitor following the University of Minnesota Cancer Center Data and Safety Monitoring Plan (DSMP - http://z.umn.edu/dmsp) and the CTO Affiliate and Satellite Site Monitoring SOPs. Alternately, participating sites that are an NCI designated Cancer Center may self-monitor using their NCI approved Data and Safety Monitoring Plan.

See section 10.6 for additional information.

10.9 Study Record Retention

The sponsor and principal investigators will retain study records including source data, copies of case report form, consent forms, HIPAA authorizations, and all study correspondence in a secured facility for at 6 years after the study file is closed with the IRB and FDA.

In addition, the CTO-CGI will keep a master log of all patients participating in the study with sufficient information to allow retrieval of the medical records for that patient.

Please contact the CTO-CGI before destroying any study related records.

11 Definition of Study Endpoints

This phase 2A clinical trial is intended to study the safety and effectiveness of three doses of MSCs on inflammatory biomarkers commonly observed in patients with COVID-19 ARDS. Safety will be measured by the absence of severe adverse pulmonary and hemodynamic changes related to MSC infusion. Efficacy will be measured by a trend toward greater reduction in inflammatory cytokines, CRP or ferritin during the first 7 days after the initial MSC infusion as compared to the vehicle placebo control. For assessing the safety outcome, systematically collected hemodynamic, respiratory, and laboratory data along with physical findings during and after MSC administration versus vehicle placebo control will be compared. Safety parameters will include changes in heart rate, systolic and diastolic blood pressure, temperature, oxygen saturation, ventilator parameters, urine output, and vasopressor dose will be recorded at baseline and every 15 minutes during the one hour of MSC or placebo infusion, then hourly for three hours, then every 8 hours until the next MSC/Placebo infusion.

Secondary endpoints include the following that require further definition:

11.1 Change in Oxygenation Indices

All patients on a ventilator will have daily evaluations including: PaO2:FiO2 ratio, peak and plateau pressures, PEEP. The peak values for baseline, day of study agent infusion and each day thereafter will be recorded.

11.2 Ventilator Free Days

Days alive off the ventilator during the first 28 days after the initial dose of study agent was infused.

11.3 ICU Free Days

Days alive discharged from the ICU during the first 28 days after the initial dose of study agent was infused

11.4 Mortality

Mortality is death from any cause. Primary, secondary and tertiary causes of death will be recorded. Pulmonary failure and hypotension may be the proximate causes of death but only the underlying etiology will be recorded as the primary cause of death.

11.5 Acute Lung Injury Score

The Acute Lung Injury Score (adapted from Murray et al. [2] erratum 1989:139:1065) is based on the maximum score at baseline and day 28 focusing on PaO2/FiO2, the level of positive end-expiratory airway pressure, respiratory compliance, and the extent of pulmonary infiltrates on the chest radiograph, using the scoring below. Note: If no chest imaging is available from within 48 hours of the specified time, then the Acute Lung Injury Score for that time cannot be calculated and this will be a 'missing' data point.

	Value
1. Chest radiograph score	
No alveolar consolidation	0
Alveolar consolidation confined to 1 quadrant	1
Alveolar consolidation confined to 2 quadrants	2
Alveolar consolidation confined to 3 quadrants	3
Alveolar consolidation in all 4 quadrants	4
2. Hypoxemia score [a]	
$PaO_2/FIO_2 \geq 300$	0
PaO_2/FIO_2 225 to 299	1
PaO_2/FIO_2 175 to 224	2
PaO_2/FIO_2 100 to 174	3
$PaO_2/FIO_2 < 100$	4
3. PEEP score (when ventilated)	
PEEP ≤ 5 cm H_2O	0
PEEP 6 to 8 cm H_2O	1
PEEP 9 to 11 cm H_2O	2
PEEP 12 to 14 cm H_2O	3
PEEP ≥ 15 cm H_2O	4
4. Respiratory system compliance score (when available)	
Compliance ≥ 80 ml/cm H_2O	0
Compliance 60 to 79 ml/cm H_2O	1
Compliance 40 to 59 ml/cm H_2O	2
Compliance 20 to 39 ml/cm H_2O	3
Compliance ≤ 19 ml/cm H^2O	4

12 Statistical Considerations

12.1 Study Design, Objectives and Endpoints

This is a Phase 2A safety study that will verify safety of and help guide estimates of efficacy for the design of future larger scale study for both efficacy and safety. Patients will be randomized in a 2:1 fashion (MSC: placebo).

12.1.1 Primary Endpoint

- Incidence of predefined grade 3-5 infusional toxicities and hemodynamic or respiratory adverse events related to the infusion of MSC.

12.1.2 Secondary Endpoints

- Incidence of a reduction in one or more biomarkers of inflammation by day 7
- Trend changes in PaO2:FiO2 ratio, oxygenation index, peak and plateau pressures and PEEP from baseline on days 3, 7 and 14Incidence of mortality by day 28 and 100
- Number of ICU-free days alive by day 28
- Number of days alive and ventilator free composite score3 by day 28
- Change in acute lung injury (ALI) score2 between baseline and day 28
- Incidence and severity of serious adverse events by day 28
- Number of days alive off supplemental oxygen by day 100
- Number of hospital days by day 100
- Description of lung function status over time to 1 year

12.1.3 Exploratory Endpoints

- Characterization of immunological changes over 28 days
- Characterization of inflammatory and alveolar epithelial function/injury biomarker changes over 28 days

12.2 Statistical Analysis

Our primary hypothesis is that 3 doses of MSC with known immunomodulatory and broad anti-inflammatory properties can be safely administered to patients with moderate to severe COVID-19 mediated ARDS. If multiple doses are well-tolerated with improvements in efficacy measures (e.g. in terms of trends in mortality rates, ventilator days and/or acute lung injury scores), a phase 2B study will be developed with more reliable sample size estimates based on outcomes in this study. The analysis will primarily be descriptive. The primary endpoint will be estimated with a simple proportion and a 95% confidence interval along with its standard deviation at day 28.

Comparison of groups may be performed by a Chi-square test or Fisher's exact test. Other categorical endpoints will be measured in a similar manner. Overall survival at 100 days post MSC infusion will be estimated by Kaplan-Meier curves. Comparison of groups may be performed by a log-rank test. Continuous endpoints such as days on oxygen will be estimated with means and standard deviations or if skewed, then medians and ranges. A t-test, Wilcoxon may be used to compare continuous measures. Feasibility will simply be assessed by the ability to run a smooth study without missing data and providing estimates of the proportion of patients able to receive 3 MSC doses. Descriptive endpoints may also be summarized with boxplots and dot plots.

12.3 Rationale for Sample Size

Due to the nature of the early phase safety study, the study accrual will stop when a total of 20 treated patients and 10 control patients have been enrolled. In general, however, the MSC and control arms will be used to help determine safety and whether there is a signal of benefit in each arm as well as to help us develop more accurate projections of the numbers needed to prove that the infusion of MSC is associated with improved survival.

12.4 Monitoring Guidelines

Early stopping rules will be in place for infusion related toxicity in recipients of MSC. The stopping rules are generated using an adaptation of Pocock stopping boundaries [35]. In the event that a stopping boundary is triggered, study enrollment will be suspended and the PI, IRB, DSMB will be notified.

For this study, predefined hemodynamic or respiratory AEs within 6 and 24 hours of the start of infusion of study agent will be used for triggering early stopping. As patients must have been had a transcutaneous oxygen saturation 88–95% without the need for an FiO2 ≥0.8 or PEEP ≥20 cm H2O) or use of more than two vasopressors in the supine position, worsening as defined in table 9 below within 6 hours after MSC infusion will be considered an adverse event(s) that contributes to the cumulative stopping boundary.

It is expected that the rate of events will be <10%. The goal is to construct a boundary based on toxicity such that the probability of early stopping is at most 10% if the rate is equal to 9.5%and our sample size is 20. With these stipulations, the trial will be stopped and reviewed if 2 events out of 3 patients, 3 events out of 9 patients, 4 events out of 15 patients or 5 events occur. The probability of hitting the stopping boundary if the rate is 30% is 82%.

Predefined hemodynamic and respiratory SAEs (based on the START Study parameters [22]):

Table 9: Hemodynamic and Respiratory SAEs	
Event within 6 hours of study agent infusion	
Definition of hemodynamic deterioration at 6 hours	Sustained decline (>1 hour) in cardiorespiratory status within 6 hours from the start of study product infusion as defined by: 1) An increase in vasopressor requirement such that the doses are: ○ Norepinephrine ≥12 µg/min or 0.2 µg/kg per min ○ Phenylephrine ≥150 µg/min or 3.0 µg/kg per min ○ Epinephrine ≥10 µg/kg per min or 0.2 µg/kg per min ○ Vasopressin ≥0.04 units/min 2) Addition of a third vasopressor

	3) New cardiac arrhythmia requiring cardioversion or ventricular tachycardia, ventricular fibrillation or asystole 4) Hypoxemia requiring an increase in FiO2 of 0.2 or more and an increase in PEEP of 5 or more to maintain Sp02 in the target range of 88-95%
Event within 24 hours of study agent infusion	
Definition of cardiopulmonary deterioration at 24 hours	Cardiac arrest or death

Causality: Adverse events with a reasonable possibility of a causal relationship will be considered "related". Causality will be assigned by the blinded independent Medical Monitor who is expert in critical care medicine. The unblinded DSMB will provide the final assessment and determine whether the study can continue based on the defined stopping rules.

12.5 Gender and Ethnicities Statement

This study is open to both males and females and to all racial/ethnic groups. It is not anticipated that the outcome will be affected by either race or gender. The study will not have separate accrual targets for different subgroups.

13 Conduct of the Study

13.1 Good Clinical Practice

The study will be conducted in accordance with the appropriate regulatory requirement(s). Essential clinical documents will be maintained to demonstrate the validity of the study and the integrity of the data collected. Master files will be established at the beginning of the study, maintained for the duration of the study and retained in a centralized file within the Clinical Trials Office of the Masonic Cancer Center according to the appropriate regulations.

13.2 Ethical Considerations

The study will be conducted in accordance with ethical principles founded in the Declaration of Helsinki. The IRB will review all appropriate study documentation in order to safeguard the rights, safety and well-being of the patients. The study will only be conducted at sites where IRB approval has been obtained. The protocol, consent, written information given to the patients, safety updates, annual progress reports, and any revisions to these documents will be provided to the IRB by the investigator.

13.3 Informed Consent

All potential study participants or their surrogates will be given a copy of the IRB-approved consent to review. The investigator or designee will explain all aspects of the study in lay language and answer all questions regarding the study either in person or by virtual or telephone meeting. If the participant or surrogate decides to participate or allow participation in the study, he/she will be asked to sign and date the consent document. Patients who refuse to participate or who withdraw

from the study will be treated without prejudice and will receive standard medical care.

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Appendix I – Apache II and Sofa Scoring

MULTI-CENTER, RANDOMIZED, PLACEBO CONTROLLED, INTERVENTIONAL PHASE 2A CLINICAL TRIAL EVALUATING THE SAFETY OF MULTIPLE DOSING OF MESENCHYMAL STROMAL CELLS IN PATIENTS WITH SEVERE ACUTE RESPIRATORY SYNDROME CORONAVIRUS 2 (SARS-Cov-2)

APACHE II SCALE

Sample ID	_____
Temperature - rectal (degrees)	_____
Celsius or Fahrenheit?	◯ Celsius ◯ Fahrenheit
Degrees Celsius	_____
Temperature Score Temperatures are in Degrees Celsius	◯ 0 if: 36-38.4 ◯ 1 if: 34-35.9 or 38.5-38.9 ◯ 2 if: 32-33.9 ◯ 3 if: 30-31.9 or 39-40.9 ◯ 4 if: less than or equal to 29.9 or greater than or equal to 41
Mean Arterial Pressure - mm Hg	_____
Mean Arterial Pressure Score	◯ 0 if: 70-109 ◯ 2 if: 50-69 or 110-129 ◯ 3 if: 130-159 ◯ 4 if: less than or equal to 49 or greater than or equal to 160
Heart Rate (ventricular response)	_____
Heart Rate Score	◯ 0 if: 70-109 ◯ 2 if: 55-69 or 110-139 ◯ 3 if: 40-54 or 140-179 ◯ 4 if: less than or equal to 39 or greater than or equal to 180
Respiratory Rate (non-ventilated or ventilated)	_____
Respiratory Rate Score	◯ 0 if: 12-24 ◯ 1 if: 10-11 or 25-34 ◯ 2 if: 6-9 ◯ 3 if: 35-49 ◯ 4 if: less than or equal to 5 or greater than or equal to 50
Oxygenation: FIO2 level	◯ Greater than or equal to 0.5 ◯ Less than 0.5
FIO2 >=0.5 record A-aDO2 mm Hg	_____

A-aDO2 Score	◯ 0 if: Less than 200 ◯ 2 if: 200-349 ◯ 3 if: 350-499 ◯ 4 if: Greater than or equal to 500
FIO2 < 0.5 record PaO2 (mm Hg)	_____
FIO2 Score	◯ 0 if: Greater than 70 ◯ 1 if: 61-70 ◯ 3 if: 55-60 ◯ 4 if: Less than 55
Arterial pH (preferred) or Serum HCO3 (venous mEq/l) (not preferred, but may use if no ABGs)	◯ Arterial pH ◯ Serum HCO3 (venous mEq/l)
Arterial pH	_____
Arterial pH Score	◯ 0 if: 7.33-7.49 ◯ 1 if: 7.5-7.59 ◯ 2 if: 725-7.32 ◯ 3 if: 7.15-7.24 or 7.6-7.69 ◯ 4 if: Less than 7.15 or Greater than or equal to 7.7
Serum HCO3 (venous mEq/l)	_____
Serum HCO3 Score	◯ 0 if: 22-31.9 ◯ 1 if: 32-40.9 ◯ 2 if: 18-21.9 ◯ 3 if: 15-17.9 or 41-51.9 ◯ 4 if: Less than 15 or Greater than or equal to 52
Serum Sodium (mEq/l)	_____
Serum Sodium Score	◯ 0 if: 130-149 ◯ 1 if: 150-154 ◯ 2 if: 120-129 or 155-159 ◯ 3 if: 111-119 or 160-179 ◯ 4 if: Less than or equal to 110 or Greater than or equal to 180
Serum Potassium (mEq/l)	_____
Serum Potassium Score	◯ 0 if: 3.5-5.4 ◯ 1 if: 3-3.4 or 5.5-5.9 ◯ 2 if: 2.5-2.9 ◯ 3 if: 6-6.9 ◯ 4 if: Less than 2.5 or Greater than or equal to 7
Serum Creatinine (mg/dl)	_____

A. Total Acute Physiology Score (sum of 12 above points)	_____
B. Age points (years)	◯ 0 if Less than or equal to 44 ◯ 2 if 45-54 ◯ 3 if 55-64 ◯ 5 if 65-74 ◯ 6 if Greater than or equal to 75
Serum Creatinine Score (Point score is doubled for acute renal failure)	◯ 0 if: 0.6-1.4 ◯ 2 if: Less than 0.6 or 1.5-1.9 ◯ 3 if: 2-3.4 ◯ 4 if: Greater than or equal to 3.5 or in ARF Less than 0.6 or 1.5 - 1.9 ◯ 6 if: in ARF 2-3.4 ◯ 8 if: in ARF Greater than or equal to 3.5
Hematocrit (%)	_____
Hematocrit Score	◯ 0 if: 30-45.9 ◯ 1 if: 46-49.9 ◯ 2 if: 20-29.9 or 50-59.9 ◯ 4 if: Less than 20 or Greater than or equal to 60
White Blood Count (total/mm3) (in 1000s)	_____
White Blood Count Score	◯ 0 if: 3-14.9 ◯ 1 if: 15-19.9 ◯ 2 if: 1-2.9 or 20-39.9 ◯ 4 if: Less than 1 or Greater than or equal to 40
Glasgow Coma Score (GCS) Score = 15 minus actual GCS	_____

C. Chronic Health Points Does the patient have a history of severe organ system insufficiency or is immunocompromised as defined below? Definitions: organ insufficiency or immunocompromised state must have been evident prior to this hospital admission and conform to the following criteria: Liver - biopsy proven cirrhosis and documented portal hypertension; episodes of past upper GI bleeding attributed to portal hypertension; or prior episodes of hepatic failure/encephalopathy/coma. Cardiovascular - New York Heart Association Class IV. Respiratory - Chronic restrictive, obstructive, or vascular disease resulting in severe exercise restriction (i.e., unable to climb stairs or perform household duties; or documented chronic hypoxia, hypercapnia, secondary polycythemia, severe pulmonary hypertension (>40 mmHg), or respirator dependency. Renal - receiving chronic dialysis. Immunocompromised - the patient has received therapy that suppresses resistance to infection (e.g., immunosuppression, chemotherapy, radiation, long term or recent high dose steroids, or has a disease that is sufficiently advanced to suppress resistance to infection, e.g., leukemia, lymphoma, AIDS).	◯ Yes ◯ No
Assign points as follows:	◯ 5 points - nonoperative or emergency postoperative patients ◯ 2 points - elective postoperative patients
---	---
Total APACHE II Score (add together the points from A+B+C)	_____
---	---

Interpretation of Score:

0 to 4 = ~4% death rate 20 to 24 = ~40% death rate

5 to 9 = ~8% death rate 25 to 29 = ~55% death rate

10 to 14 = ~15% death rate 30 to 34 = ~75% death rate

15 to 19 = ~25% death rate Over 34 = ~85% death rate

MULTI-CENTER, RANDOMIZED, PLACEBO CONTROLLED, INTERVENTIONAL PHASE 2A CLINICAL TRIAL EVALUATING THE SAFETY OF MULTIPLE DOSING OF MESENCHYMAL STROMAL CELLS IN PATIENTS WITH SEVERE ACUTE RESPIRATORY SYNDROME CORONAVIRUS 2 (SARS-Cov-2)

SOFA SCORE

Sample ID _____

Partial Pressure of Oxygen (mm Hg) if ABG available, substitute Sp02 if no ABG available _____ (mm Hg)

Fraction of Inhaled O2 (%), if on room air enter 21%. See conversion table for supplemental 02. _____ (%)

PaO2/FiO2 (mmHg). Substitue Sp02 for Pa02 if no ABG available. _____

Mechanically ventilated ◯ Yes ◯ No

Platelet Count (x10^9L) _____ (x10^9L)

Glasgow Coma Scale (points) _____ (points)

Bilirubin (mmol/L) _____ (mmol/L)

Level of Hypotension (Vasopressor Status For ? 1 Hr)

◯ No Hypotension: 0
◯ MAP < 70: +1
◯ On vasopressors, dopamine < 5 g/kg/min or dobutamine (any dose): +2
◯ Dopamine > 5 g/kg/min or Epi/Norepi < 0.1 g/kg/min: +3
◯ Dopamine > 15 g/kg/min or Epi/Norepi > 0.1 g/kg/min: +4

Creatinine (or Urine Output, Use Worst Value)

◯ Cr < 1.2 mg/dL (< 106 mol/L): 0
◯ Cr 1.2-1.9 mg/dL (106-168 mol/L): +1
◯ Cr 2.0-3.4 mg/dL (177-301 mol/L): +2
◯ Cr 3.5-4.9 mg/dL (309-433 mol/L) or Urine Output < 500ml/day: +3
◯ Cr > 5.0 mg/dL (> 442 mol/L)+4

Score _____

Appendix II – Dyspnea Index Questionnaire

Below are the questions used in the University of California, San Diego Shortness of Breath Questionnaire (SOBQ).

Please rate the breathlessness you experience when you do, or if you were to do, each of the following tasks. **Do not skip any items.** If you've never performed a task or no longer perform it, give your best estimate of the breathlessness you would experience while doing that activity. Please review the two sample questions below before turning the page to begin the questionnaire.

When I do, or if I were to do, the following tasks, I would rate my breathlessness as:

0	None at all
1	
2	
3	
4	Severe
5	Maximal or unable to do because of breathlessness

1. Brushing teeth ..... 0 1 2 ③ 4 5

Harry has felt moderately short of breath during the past week while brushing his teeth and so circles a three for this activity.

2. Mowing the lawn ..... 0 1 2 3 4 ⑤

Anne has never mowed the lawn before but estimates that she would have been too breathless to do this activity during the past week. She circles a five for this activity.

When I do, or if I were to do, the following tasks, I would rate my breathlessness as:

0	None at all
1	
2	
3	
4	Severe
5	Maximal or unable to do because of breathlessness

1.	At rest	0	1	2	3	4	5
2.	Walking on a level at your own pace	0	1	2	3	4	5
3.	Walking on a level with others your age	0	1	2	3	4	5
4.	Walking up a hill	0	1	2	3	4	5
5.	Walking up stairs	0	1	2	3	4	5
6.	While eating	0	1	2	3	4	5
7.	Standing up from a chair	0	1	2	3	4	5
8.	Brushing teeth	0	1	2	3	4	5
9.	Shaving and/or brushing hair	0	1	2	3	4	5
10.	Showering/bathing	0	1	2	3	4	5

When I do, or if I were to do, the following tasks, I would rate my breathlessness as:

0	None at all
1	
2	
3	
4	Severe
5	Maximal or unable to do because of breathlessness

11.	Dressing	0	1	2	3	4	5
12.	Picking up and straightening	0	1	2	3	4	5
13.	Doing dishes	0	1	2	3	4	5
14.	Sweeping /vacuuming	0	1	2	3	4	5
15.	Making bed	0	1	2	3	4	5
16.	Shopping	0	1	2	3	4	5
17.	Doing laundry	0	1	2	3	4	5
18.	Washing car	0	1	2	3	4	5
19.	Mowing lawn	0	1	2	3	4	5
20.	Watering lawn	0	1	2	3	4	5
21.	Sexual activities	0	1	2	3	4	5

0	None at all
1	
2	
3	
4	Severe
5	Maximal or unable to do because of breathlessness

How much do these limit you in your daily life?

22.	Shortness of breath	0	1	2	3	4	5
23.	Fear of "hurting myself" by overexerting	0	1	2	3	4	5
24.	Fear of shortness of breath	0	1	2	3	4	5