Clinical Study Protocol

Protocol Title:	A Phase 2b, Randomized, Double-blind, Placebo-controlled, Multi-center Clinical Trial of Allogeneic Bone Marrow-derived Human Mesenchymal Stromal Cells for the Treatment of Acute Respiratory Distress Syndrome
Investigational Drug:	Allogeneic Bone Marrow-derived Human Mesenchymal Stromal Cells (hMSCs)
Protocol Number:	UCSF-hMSC-ARDS-P1P2-14
IND Number:	15331
Sponsor-Investigator:	Michael A. Matthay, MD University of California, San Francisco

Signature:

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Original Date:

Confidentiality Statement

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PROTOCOL Approval

Protocol: UCSF-hMSC-ARDS-P1P2-14	**Version/Date:** April 19, 2022
IND: 15331	**Protocol Chair:** Michael A. Matthay, MD
Short Title: Mesenchymal Stromal Cells For Acute Respiratory Distress Syndrome	

I have read protocol, and I approve it. As the principal investigator, I agree to conduct this protocol according to good clinical practices, which are delineated in the International Conference on Harmonization (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use "Guidance for Industry: E6 Good Clinical Practice Consolidated Guidance" (May 1996), and according to the criteria specified in this protocol.

Print Name of Principal Investigator [or Protocol Chair]:

_____

Signature:_____

Date:_____

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1 Protocol Synopsis

Name of Sponsor – Investigator	**Michael A. Matthay, MD** **University of California, San Francisco**
Investigational Product	**Allogeneic Bone Marrow-derived Human Mesenchymal Stromal Cells (hMSCs)**
Protocol Number:	UCSF-hMSC-ARD-P1P2-14
Title:	A Phase 2b, Randomized, Double-blind, Placebo-controlled, Multi-center Clinical Trial of Allogeneic Bone Marrow-Derived Human Mesenchymal Stromal Cells for the Treatment of Acute Respiratory Distress Syndrome
Short Title	Mesenchymal Stromal Cells For Acute Respiratory Distress Syndrome
Clinical Phase	Phase 2b
Methodology	Randomized, double-blind, placebo-controlled
Study duration	3-4 years
IND Sponsor-Investigator	Michael A. Matthay, MD
Funding Sponsor	Department of Defense
Study Product	Allogeneic Bone Marrow-derived Human Mesenchymal Stromal Cells (hMSCs)
IND Number	15331
Participating Sites	Intensive Care Units at seven Clinical Centers: Zuckerberg San Francisco General Hospital & Trauma Center and UCSF Moffitt-Long Hospital (San Francisco, CA), University of California Davis Medical Center (Sacramento, CA), Harborview Medical Center (Seattle, WA), Oregon Health & Science University (Portland, OR), Vanderbilt University Medical Center (Nashville, TN) and the University of Texas Health Sciences Center at Houston/Memorial Hermann-Texas Medical Center (Houston, TX)
Study Objective	To assess the efficacy of hMSCs in critically ill patients with Acute Respiratory Distress Syndrome (ARDS)
Accrual Objective	120 patients (60 patients receiving hMSCs and 60 patients receiving placebo)

Name of Sponsor – Investigator	**Michael A. Matthay, MD** **University of California, San Francisco**
Investigational Product	**Allogeneic Bone Marrow-derived Human Mesenchymal Stromal Cells (hMSCs)**
Study Design	Randomized, double-blind, placebo-controlled, multi-center. Subjects with ARDS will receive a single infusion of hMSCs or placebo.
Study Product, Dose, Route, Regimen	The cellular product is cryopreserved for long-term storage. The cryopreserved hMSCs are formulated in CryoStor (90%) and DMSO (10%). Immediately prior to administration, the product is thawed and diluted in 1:1 reconstitution media (1:1 mix of 5% human serum albumin and 10% Dextran 40). Additional reconstitution media is added to a final product volume of 300 mL. Placebo: 300 mL of reconstitution media (1:1 mix of 5% human serum albumin and 10% Dextran 40). Each dose of the hMSCs or placebo will be administered intravenously over approximately 60-80 minutes. All patients will receive one dose. × 10×10^6 cells/kg (n=60); placebo (n=60)
Primary Endpoint:	**Reduction in the severity of ARDS:** Change in the oxygenation index (OI) from baseline over 36 hours following the initiation of study product infusion (with measurements of OI at 6, 12, 18, 24, 30 and 36 hours).
Secondary Endpoints:	**Respiratory physiology endpoints**: The 4-point acute lung injury score (LIS) at days 1, 2, 3 and 7, or on the last day of positive pressure ventilation prior to day 7; pulmonary dead space at days 1, 2, 3 and 7; chest radiograph assessment of pulmonary edema (RALE score) at days 1, 2, 3 and 7; ventilatory ratio with measurements at 6, 12, 18, 24, 30, and 36 hours, and days 2, 3, and 7; incidence of barotrauma at days 1, 2, 3 and 7; ventilator free-days over 7, 14 and 28 days; duration of assisted ventilation over 28 days in the survivors; achieving pressure support ventilation equal to 5 cm H_20 with positive end-expiratory pressure (PEEP) equal to 5 cm H_20 for 2 hours. OI will be collected (as planned) also on days 2, 3, and 7 if still being ventilated with positive pressure as a secondary endpoint. **Secondary Infection endpoints:** Superficial incisional/wound infections, deep incisional wound infections, and organ/space infections, and ventilator associated pneumonia (all during the 14

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	days after enrollment). **Systemic illness endpoint**: Sequential Organ Failure Assessment (SOFA) score at 3, and 7 days; in hospital mortality at 14, 28 and 60 days; additional prognostic value of neutrophil:lymphocyte ratio; ordinal scale for clinical improvement at 7, 14 and 28 days. **Neurocognitive endpoint**: Neurocognitive function measured by the Glasgow Outcome Score at hospital discharge **Thromboembolic:** Incidence of thromboembolic events measured by ultrasound of the deep venous system or CT-angiography of the chest ordered for clinical purposes/by treating clinicians; additional prognostic value of fibrinogen at baseline. **Biomarkers**: Plasma: Change in levels of plasma, urine and genetic biomarkers from baseline compared to 6 , 24, 48, and 72 hours including: endothelial injury (angiopoietin-2), lung epithelial injury (Receptor for Advanced Glycation Endproducts [RAGE]), pro-inflammatory pathways (interleukin-6 [IL-6], interleukin-8 [IL-8], soluble tumor necrosis factor-1 [sTNF-1], intercellular adhesion molecule-1 [ICAM-1], interferon gamma-induced protein-10 [IP-10], interleukin-10 [IL-10], vascular endothelial growth factor [VEGF], matrix metalloproteinase-8 [MMP-8], thrombomodulin, surfactant protein-D [SP-D], triggering receptor expressed on myeloid cells-1 [TREM-1], interleukin-18 [IL-18], plasminogen activator inhibitor-1 [PAI-1]), impaired coagulation (protein C), pro-resolving lipids (lipoxin A4, Resolvin D_1), biomarkers that reflect the paracrine activity of the administered hMSCs (angiopoietin-1 [Ang-1] and keratinocyte growth factor [KGF]). Changes in levels of plasma SARS-CoV-2 viral antigen and antibody levels at multiple timepoints; gene expression at baseline and on day 2 and 3. Alveolar fluid: Lung protein permeability as measured by total protein in mini-bronchoalveolar lavage [mBAL] protein at day 2; the same biomarkers will also be measured in plasma to compare hMSCs versus placebo treated patients. Urine: Microalbumin will also be measured at 24 and 48 hours Others: Primary and secondary endpoints will be adjusted by SARS-CoV2 infection to better understand the hMSC effects in patients with COVID-19 disease.

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	Safety endpoints: Tolerability of the hMSCs, defined as the incidence of pre-specified infusion-associated events and unexpected severe adverse events in ARDS patients treated with hMSCs.
Inclusion Criteria	Patients will be eligible for inclusion if they meet all of the below criteria within 14 days of initial ICU admission. Criteria 1-3 must all be present within a 24-hour time period and at the time of enrollment: Acute onset (defined below) of: 1. A need for positive pressure ventilation by an endotracheal or tracheal tube with a PaO_2/FiO_2 ratio <250 mmHg and ≥5 cm H_2O positive end-expiratory airway pressure (PEEP), as per the Berlin Criteria. 2. Bilateral infiltrates consistent with pulmonary edema (defined below) on the frontal chest radiograph, or bilateral ground glass opacities on a chest CT scan. 3. No clinical evidence of left atrial hypertension as a primary explanation for the bilateral pulmonary infiltrates. 4. If the cause of ARDS is trauma, additional inclusion criteria will include ONE of the following relevant risk factors for developing ARDS: a. Hypotension (systolic blood pressure[SBP] < 90 mmHg) in the field or in the first 24 h after injury, or b. Transfusion of 3 units of blood products in the first 24 hours following injury, or c. Meets the new Critical Administration Threshold (CAT) criteria with at least 3 units of blood in one hour, or d. Blunt or penetrating torso trauma, or e. Long bone fractures, or f. The highest level of institutional trauma activation ARDS Diagnostic Criteria Defined: • Acute onset is defined as follows: The duration of the hypoxemia criterion (#1) and the chest radiograph criterion (#2) must both be present ≤ 14 days of initial ICU admission at the time of enrollment

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	• Infiltrates considered "consistent with pulmonary edema" include any infiltrates not fully explained by mass, atelectasis, or effusion or opacities known to be chronic (>14 days of initial ICU admission). Vascular redistribution, indistinct vessels, and indistinct heart borders alone are not considered "consistent with pulmonary edema" and thus would not count as qualifying opacities for this study. • If a patient meets #1 and #2 inclusion criteria but has an echocardiogram with LV ejection < 40% ordered by treating clinicians because of a clinical suspicion of acute left heart failure, the patient will be excluded. • If the patient meets #1 and #2 inclusion criteria but has a Pulmonary Arterial Occlusion Pressure (PAOP), also known as the Pulmonary Arterial Wedge Pressure (PAWP), that is initially greater than 18 mm Hg, then the inclusion criteria must persist for more than 12 hours after the PAOP has declined to ≤ 18 mm Hg.
Exclusion Criteria	1. Age less than 18 years 2. Greater than 72 hours since first meeting ARDS criteria per the Berlin definition of ARDS 3. Greater than 14 days since initial ICU admission 4. Inability to administer study product within 14 days of initial ICU admission 5. PaO2/FiO2 ≥ 250 mmHg after consent obtained and before study product is administered 6. Unable to obtain informed consent/no surrogate available 7. Pregnant or lactating 8. In custody of law enforcement officials 9. Burns > 20% of total body surface area 10. WHO Class III or IV pulmonary hypertension 11. History of cancer treatment in the last 2 years except for non-melanotic skin cancers 12. Underlying medical condition for which 6-month mortality is estimated to be > 50% 13. Moribund patient not expected to survive 24 hours

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	14. Advanced chronic liver disease (Childs-Pugh Score > 12) 15. Severe chronic respiratory disease with the use of home oxygen 16. Severe traumatic brain injury - defined as: a. A patient who has undergone intracranial neurosurgical intervention for monitoring or therapy (intracranial pressure monitoring, external ventricular drain, craniotomy), or b. Intracranial injury by head CT (does not include patients with minimal subarachnoid injury and/or minor skull fracture), or c. Post-resuscitation Glasgow Coma Score (GCS) < 9 assessed after sedation interruption, or d. Non-survivable head injury as assessed by neurosurgery 17. Evidence of anoxic brain injury 18. History of stroke within the last 3 years 19. No intent/unwillingness to follow lung protective ventilation strategy 20. Currently receiving extracorporeal life support (ECLS) or high-frequency oscillatory ventilation (HFOV) 21. Anticipated extubation within 24 hours of enrollment 22. Clinical evidence of left atrial hypertension as measured by a pulmonary arterial wedge pressure > 18mmHg or left ventricular failure measured by an echocardiogram with a left ventricular ejection fraction less than 40%. Clinical judgement will determine if either of these measurements needs to be carried out.
Statistical Consideration	The primary endpoint, Oxygenation Index (OI), will be assessed for the change from the baseline measurement (prior to infusion of the study product) over 36 hours (with measurements at 6, 12, 18, 24, 30 and 36 hours), and will be compared between the placebo and hMSCs treatment conditions using a linear mixed-effects regression model. The model will be estimated using likelihood estimation which will allow the inclusion of all collected data, regardless of the number of assessments available for variable. Terms in the model will include the two stratification

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	variables, treatment condition (active or placebo), time of assessment, condition-by-time, recruitment site, and a site-by-treatment interaction term. A test of the treatment condition will directly test the main research question. Covariates identified by preliminary analysis as potentially confounding due to baseline differences will be added to the model. Given the sample size of 60 per treatment condition, in this multisite, randomized trial using oxygenation index as the primary outcome and measures taken at 6 time points, it is estimated the study will have 80% power to detect a difference between conditions for an effect size as low as 0.43. Using the pilot data from Day 4, for example, this would translate to approximately a difference of 1.29 on the oxygenation index. The primary and secondary endpoints (including clinical and biological data) will be adjusted for SARS-CoV-2 infection as a cause of ARDS, which is adjudicated by the study physicians.

2 Glossary of Abbreviations

Abbreviation	Full Term
AE	Adverse Event
AFC	Alveolar Fluid Clearance
ALI	Acute Lung Injury
ARDS	Acute Respiratory Distress Syndrome
mBAL	Mini-Bronchoalveolar Lavage
CFR	Code of Federal Regulations
cIRB	Central Institutional Review Board
CRF	Case Report Form
DoD	United States Department of Defense
DSMB	Data and Safety Monitoring Board
EAP	Expanded Access Program
ECMO	Extracorporeal Membrane Oxygenation
EUA	Emergency Use Authorization
FDA	Food and Drug Administration
GCS	Glasgow Coma Score
HFOV	High-Frequency Oscillatory Ventilation
HIPPA	Health Insurance Portability and Accountability Act of 1996
hMSCs	Human Mesenchymal Stromal Cells
HS	Hemorrhagic Shock
HSC	Hematopoietic Stem Cells
ICH	International Conference on Harmonization
ICU	Intensive Care Unit
IND	Investigational New Drug
IRB	Institutional Review Board
IV	Intravenous(ly)
KGF	Keratinocyte Growth Factor
LIS	Lung Injury Score
mBAL	Mini Bronchoalveolar Lavage
MSCs	Mesenchymal Stromal Cells
NIH	National Institute of Health
NHLBI	National Heart, Lung and Blood Institute

Abbreviation	Full Term
NLR	Neutrophil:Lymphocyte Ratio
OI	Oxygenation Index
OSCI	Ordinal Scale for Clinical Improvement
PACT	Production Assistance for Cellular Therapy
PAOP	Pulmonary Artery Occlusion Pressure
PBW	Predicted Body Weight
PEC	Pulmonary Endothelial Cell
PEEP	Positive End-Expiratory Pressure
PHI	Protected Health Information
SAE	Serious Adverse Event
SOP	Standard Operating Procedure
VR	Ventilatory Ratio

3 BACKGROUND

3.1 *Summary*

We hypothesize that Allogeneic Bone Marrow-derived Human Mesenchymal Stromal Cells (referred to hereafter as hMSCs) administered to patients with Acute Respiratory Distress Syndrome (ARDS) will be safe and effective in reducing the severity of acute respiratory failure. A Phase 2b trial with this cell-based therapy will be used to test the safety and efficacy based on potential treatment of the major abnormalities that underlie and accompany/complicate ARDS, including oxygenation, carbon dioxide excretion, degree of pulmonary edema on the chest radiograph, systemic organ dysfunction, secondary infections, and altered lung endothelial and epithelial permeability and inflammation.

COVID-19 (coronavirus disease 2019), a disease caused by SARS-CoV-2, emerged in the United States in March 2020 and rapidly became a global pandemic. The vast majority of our enrolled patients were COVID-19 patients who developed ARDS. The main motivation for this updated protocol is to explicitly include clinical and biologic variables that are relevant to COVID-19 induced ARDS.

3.2 *Scientific Rationale*

3.2.1 Significance of this work to Acute Respiratory Distress Syndrome

Morbidity and mortality have declined only modestly in patients with ARDS in the last decade, despite extensive research into its pathophysiology. (1-3) Current treatment remains primarily supportive with lung-protective ventilation and a fluid conservative strategy. (4, 5) Pharmacologic therapies that reduce the severity of lung injury *in vivo* and *in vitro* have not yet been translated to effective clinical treatment options. At present, the mortality rate of severe ARDS remains unacceptably high, in the range of 30-40%. (6, 7) Therefore, innovative therapies are needed.

Cell-based therapy with mesenchymal stromal cells (MSCs) for the treatment of ARDS is attractive as a potential new therapy. MSCs are multi-potent and have the ability to secrete a variety of paracrine factors such as growth factors that can enhance tissue repair, anti-inflammatory cytokines and also antimicrobial peptides. All of these paracrine factors can potentially treat the major abnormalities that underlie ALI, including impaired alveolar fluid clearance, altered lung endothelial and epithelial permeability, dysregulated inflammation and ongoing infection. (8, 9)

MSCs, also called marrow stromal stem cells, were first discovered in 1968 by Friedenstein (10) who found bone marrow stromal cells that were adherent, clonogenic, and fibroblastic in appearance. Adult mesenchymal stromal cells can be isolated from a variety of human tissues, including bone marrow, adipose tissue, liver, tendons, synovial membrane, amniotic fluid, placenta, umbilical cord blood, and teeth. MSCs are presumed to reside near the sinusoids and function as support cells for hematopoietic stem cells (HSC). Although MSCs comprise less than 0.1% of all bone marrow cells, they can be isolated from whole bone marrow aspirates by their ability to adhere to plastic and form colonies. Currently, there are no cell surface markers specific

to MSCs. Consequently, in 2006, the International Society of Cellular Therapy defined MSCs by three criteria:

(1) MSCs must be adherent to plastic under standard tissue culture conditions
(2) MSCs must express certain cell surface markers such as CD105, CD90, and CD73, but must not express other markers including CD45, CD34, CD14, or CD11b; and
(3) MSCs must have the capacity to differentiate into mesenchymal lineages including osteoblasts, adipocytes, and chrondoblasts under *in vitro* conditions. (11)

Use of these cells for therapeutic purposes in a variety of diseases has attracted considerable attention due to their low immunogenicity, their immunomodulatory effects, and their ability to secrete endothelial and epithelial growth factors.

3.2.2 Significance of this work to Acute Respiratory Distress Syndrome after Trauma

ARDS is a common cause of acute respiratory failure after severe trauma that leads to considerable morbidity and mortality. (2, 12) In a recent study of patients receiving mechanical ventilation following severe trauma at the Zuckerberg San Francisco General Hospital, the incidence of ARDS was 30% (183/621) defined by: (a) bilateral infiltrates on the chest radiograph, and (b) arterial hypoxemia (PaO_2/FiO_2 < 300 mmHg). (13, 14) This incidence is higher than previously reported, most likely because prior studies have not included systematic review of serial chest radiographs for the presence of bilateral infiltrates. Another study reported an ARDS incidence of 38% (148/602) in all patients following trauma and an incidence of 50% in those who required 6 or more units of red blood cells. (15)

3.3 *Background Relevant to Mechanism of Action of Mesenchymal Stromal Cells in ARDS*

Several mechanisms of action have been established in the literature for the therapeutic effect of MSCs in lung injury models.

3.3.1 Engraftment and Trans-differentiation

Much of the initial interest in MSC therapy stemmed from the multi-potent properties of the cells. Krause et al (16) found that a single bone marrow-derived cell could give rise to cells of multiple different organs including the lung. They reported up to 20% engraftment of bone marrow-derived cells in the lung, including epithelial cells, from a single hematopoietic precursor. Kotton et al (17) reported that plate-adherent cultured bone marrow cells, when given intravenously in wild-type mice following bleomycin-induced lung injury, engrafted into the recipient lung parenchyma with a morphological and molecular phenotype of alveolar type I pneumocytes. This report gave rise to intensive investigation into the possibility that MSCs may be able to regenerate the lung epithelium and/or endothelium. (16-20) However, these results were questioned by multiple groups, who observed only engraftment of leukocyte lineages (21) and low engraftment rates in lung injury models, with observed rates of < 1%. (22-25) Despite initial interest in their multi-potent properties, engraftment in the lung does not appear to play a major beneficial role. The beneficial effect of MSCs appears to derive more from their capacity to secrete paracrine soluble factors that

modulate immune responses, as well as alter the responses of endothelium or epithelium to injury through the release of growth factors. (26-33) There may also be important cell-contact dependent effects, which may include the transfer of microvesicles and mitochondria to injured recipient host cells resulting in restoration of more normal function. (34)

3.3.2 Immunomodulatory & Anti-inflammatory Effects

A major characteristic of MSCs has been the immunomodulatory properties of the cells. Multiple studies have demonstrated that MSCs possess potent immunosuppressive effects by inhibiting the activity of both innate and adaptive immune cells. (28, 29, 35, 36) This immunosuppression has been shown to be mediated by cell contact-dependent and -independent mechanisms through the release of soluble factors. The list of candidate mediators released or induced by MSCs includes TGF-β, PGE_2, IDO, TSG, IL-10 and IL-1ra among others. In a model of sepsis following cecal ligation and puncture (CLP) in mice, Nemeth et al (26) found that MSCs, activated by LPS or TNF-α, secreted prostaglandin E_2, which reprogrammed alveolar macrophages to secrete IL-10. The beneficial effect of MSCs on mortality and improved organ function following sepsis (CLP) was eliminated by macrophage depletion or pretreatment with antibodies to IL-10 or the IL-10 receptor, suggesting a beneficial role for IL-10 in these experiments; IL-10 is a cytokine secreted predominantly by monocytes that down-regulates the expression of Th1 cytokines, MHC class II antigens, and co-stimulatory molecules on macrophages. IL-10 has also been reported to inhibit the rolling, adhesion and transepithelial migration of neutrophils. (37) In co-culture experiments, cell contact between MSCs and macrophages was required to stimulate IL-10 production following LPS stimulation; MSCs separated by a Transwell plate or MSC conditioned medium could not induce IL-10 production. (26) In a model of ALI following intratracheal E. *coli* endotoxin in mice, we found that intra-pulmonary MSCs improved survival and lung injury in association with a decrease in MIP-2 and TNF-α levels in the bronchoalveolar lavage fluid (BAL) and elevated levels of IL-10 in both the plasma and BAL fluids. (38) In bleomycin-induced lung injury and fibrosis in mice, Ortiz et al (39) found that MSCs decreased subsequent lung collagen accumulation, fibrosis and levels of matrix metalloproteinases in part by IL-1ra secretion; IL-1ra is a cytokine that competitively competes with IL-1β for IL-1 receptor binding. IL-1β is one of the major inflammatory cytokines in pulmonary edema fluid in patients with ALI/ARDS. (40) These results confirmed the anti-inflammatory effects of MSCs in multiple lung injury experiments in mice. (22, 23, 41-43) There is also new evidence that MSCs release lipoxin A4, an important lipid factor that accelerates the resolution of tissue and lung injury. (44)

Despite the well documented immunosuppressive effects of MSCs, recent literature described a dual role for MSCs as an immunostimulatory cell as well. (45) As explained above, some studies have reported that MSCs can upregulate expression of MHC II when exposed to low levels of inflammation and function as antigen presenting cells stimulating the adaptive immune system. (46, 47) Recent evidence has also shown that MSCs can secrete IL-6 and induce production of IgG by B lymphocytes in an *in vitro* setting. (48) In addition, MSCs can prevent neutrophil apoptosis and degranulation in culture without inhibiting their phagocytic or chemotactic capabilities. (49) Thus, these studies have demonstrated that MSCs have more complex effects on the immune system than their classical role as immune suppressor cells. Understanding the mechanisms

responsible for these apparently paradoxical roles that MSCs play in the immune response is important in developing cell-based therapy for clinical use.

3.3.3 Anti-Microbial Effects

Bacterial pneumonia and sepsis from a non-pulmonary cause are two of the most common etiologies of ARDS. (1) Given the preponderance of literature that describes the immunosuppressive effect of MSCs, there might be concern that this effect may impede the host's ability to clear an infection. However, new work describes a dual role for MSCs in regulating the immune system and their immunostimulatory effects. Further, a recent report has demonstrated a protective effect of systemically administered MSCs in a mouse model of bacterial sepsis (26) and data from our own group at UCSF that MSCs are associated with a reduction in the number of live bacteria in E. *coli* pneumonia in mice. The anti-microbial effect with mouse MSCs depended in part on the release of lipocalin-2, a well known anti-microbial peptide, from both the MSCs themselves and alveolar macrophages. (50) Also, we have found that the release of LL-37 by human MSCs reduces the number of bacteria in E. *coli* pneumonia in mice. (51) In recent work with gram negative peritoneal sepsis in mice, our group has also found that intravenous hMSCs improve survival and reduce the number of bacteria in the blood when given after the peritoneal sepsis was established. (52) Another study reported better survival following treatment with MSCs in a cecal-ligation model of peritonitis in mice. (53) Thus, based on preclinical evidence, treatment with MSCs has been associated with a reduction in the number of bacteria in mouse models of peritoneal sepsis and pneumonia.

3.3.4 Secretion of Paracrine Soluble Factors that Enhance Alveolar Fluid Clearance and the Resolution of Alveolar Edema

Impaired alveolar fluid clearance (AFC), or delayed resolution of pulmonary edema, is common in patients with ARDS. The level of AFC impairment has significant prognostic value in predicting morbidity and mortality. (54, 55) Experimental studies have investigated several mechanisms that reduce AFC in ALI. (56, 57) In the alveolar environment, basal AFC is determined predominately by amiloride-sensitive and insensitive sodium channels and the activity of the Na-K ATPase. (56, 58-61) Several stimuli can upregulate AFC including beta-adrenergic agonists via cAMP-dependent mechanisms. (56, 57) In the mouse and human lung, cAMP-dependent alveolar epithelial fluid transport is dependent on CFTR activity, especially in mediating β-adrenergic receptor-driven alveolar epithelial fluid transport. (62-64)

We and other investigators have reported that pulmonary edema fluid in ARDS contains high levels of several pro-inflammatory cytokines, including IL-1β, IL-8, TNF-α and TGF-β1. (65-67) Several of these pro-inflammatory cytokines have been studied in experimental fluid transport experiments. For example, TNF-α decreased the expression of ENaC (α-, β-, γ-subunits) mRNAs and protein levels, as well as the amiloride-sensitive current and ouabain-sensitive Rb^+ uptake in rat alveolar epithelial cells. (68) Similarly, IL-1β decreased dexamethasone-induced αENaC mRNA and protein levels, and the amiloride-sensitive fraction of the transepithelial current and sodium transport across rat type II cell monolayers. (69) More recently, we reported that TGF-β1 decreased the amiloride-sensitive fraction of Na^+ uptake and fluid transport across monolayers of rat and human type II cells, as well as αENaC mRNA and protein expression. (70) In chronic

inflammation associated with nasal polyposis, TGF-β1 down-regulated CFTR mRNA and protein expression as well as the cAMP-dependent current in human nasal epithelial cells. (71)

MSCs are known to produce several epithelial specific growth factors, specifically keratinocyte growth factor (KGF), the seventh member of the fibroblast growth factor family. We have been particularly interested in KGF because of work from our group, as well as from other investigators who have reported that KGF can reduce lung injury in small animal models of pulmonary edema and lung injury. Recombinant KGF pretreatment reduced mortality following intra-tracheal instillation of hydrochloric acid (72, 73), bleomycin (74, 75), hyperoxia (76, 77) and Pseudomonas *aeruginosa*. (78) In rat lungs, KGF improved alveolar fluid transport in part by up-regulating αENaC gene expression (79) and Na-KATPase activity (80).

In the *ex vivo* perfused human lung, the intra-bronchial instillation of hMSCs one hour following endotoxin-induced lung injury restored AFC in part by the secretion of KGF. (81) Several properties of KGF could explain the therapeutic effect of hMSCs on restoring AFC, including alveolar epithelial type II cell hyperplasia and differentiation, surfactant production (82), anti-apoptotic effects (83) and increased transcription and/or translation of the major sodium and chloride transport proteins. (79, 80) Because the effect of MSCs in E. *coli* endotoxin-induced lung injury in the *ex vivo* perfused human lung model occurred over a 3-hour time period, the therapeutic benefit of KGF in these experiments is less likely explained by type II cell hyperplasia or transcriptional effects. Alternatively, an increase in vectorial fluid transport across the alveolar epithelium can be mediated by an increase in trafficking of sodium transport proteins to the cell surface. (84, 85)

We have an established collaboration with the NIH sponsored Production Assistance for Cellular Therapy (PACT) Group in the University of Minnesota (Principal Investigator: David McKenna, MD) for the production of cGMP clinical grade, cryopreserved hMSCs. In additional experiments in the *ex vivo* perfused human lung, we have found that these hMSCs were effective in both endotoxin and live E. *coli* lung injury models in restoring the rate of AFC over 6 hours whether given intravenously or intratracheally. (8, 9)

We also have recently completed experiments with hMSCs in sheep with ALI over 24 hours and found evidence of safety and efficacy. We have also completed IND-enabling safety studies in rats with ALI.

3.3.5 Secretion of Paracrine Soluble Factors that Improve Lung Endothelial and Epithelial Permeability

MSCs may be potentially beneficial by therapeutic effects on the injured lung endothelium. The integrity of the lung microvascular endothelium is essential to prevent the influx of protein-rich fluid from the plasma as well as inflammatory cells, which may further aggravate the ability of the lung epithelium to reduce alveolar edema. Several paracrine soluble factors, such as angiopoietin-1 (Ang-1) and KGF, are potentially important in predicating these effects. Ang-1, a ligand for the endothelial Tie2 receptor, is a known endothelial survival (86) and vascular stabilization factor that reduces endothelial permeability and inhibits leukocyte-endothelium interactions by modifying endothelial cell adhesion molecules and cell junctions. (87-90) Regular MSCs or MSCs

(used as a vehicle for gene delivery) transfected with the human Ang-1 gene reduced both pulmonary vascular endothelial injury and the recruitment of inflammatory cells into the lungs of mice injured by LPS-induced lung injury (41, 43, 91). In a study by Mei et al (41), the transfection of Ang-1 further reduced lung inflammation and nearly completely reversed the LPS-induced increase in lung permeability. We recently found that MSCs secrete a significant quantity of Ang-1. In addition, using siRNA technology, the secretion of Ang-1 produced a therapeutic effect on lung epithelial protein permeability in primary cultures of human alveolar epithelial type II cells injured by an inflammatory insult. (92)

KGF is one of several epithelial-specific growth factors produced by MSCs. In models of acute permeability edema such as α-naphthylthiourea (93), P. *aeruginosa* (78) or ventilator-induced lung injury (94), KGF reduced lung edema and bronchoalveolar lavage protein levels. Cultured allogeneic hMSCs produced substantial quantities of KGF. The role of KGF is intriguing given the previous studies of ALI in animal models. A recent study by Murakami et al. reported that fibroblast growth factors (FGF), FGF2, FGF4 and FGF8 are specific for both FGF receptors 1IIIc and 3IIIc and are responsible for the maintenance of endothelial barrier homeostasis (95). Another epithelial-specific growth factor secreted by MSCs is hepatocyte growth factor (HGF). Previously, HGF was found to stabilize the integrity of pulmonary endothelial cells by inhibition of Rho GTPase and prevention of actin stress fiber formation and paracellular gaps among pulmonary endothelial cells injured by thrombin (96, 97). MSCs have also been reported to produce an inflammatory factor called TSG-6, which reduces the size of acute myocardial infarction in mouse studies, and also reduces the severity of acid-induced lung injury in mice (98).

3.3.6 MSCs for Treatment of Acute Lung Injury after Hemorrhagic Shock and Resuscitation

Several mechanisms contribute to the vascular barrier breakdown in multiple organs following shock and resuscitation, including inflammation, aberrant clotting, matrix metalloprotease activation, and oxidative stress, all described as part of the ischemia-reperfusion syndrome of lung, kidney, liver and cardiovascular injury. Dr. Pati's group was the first to report that MSCs regulate pulmonary endothelial cell (PEC) adherens junction stability and permeability after tissue hypoxia. (99, 100) To test the effect of intravenous MSCs in an established rat model of fixed pressure hemorrhagic shock (HS)**,** rats were treated with MSCs or lactated Ringer's (control). MSC treatment reduced leukocyte infiltration, serum pro-inflammatory cytokine levels (TNF-α, MIP-1 and MCP-1), and increased the serum anti-inflammatory cytokine IL-10. (99, 101)

In the lungs, MSC treatment reduced the HS-induced increase in lung edema, returning the gravimetric measured lung water to normal levels. Rats treated with MSCs following HS and trauma also had a significant reduction in lung vascular permeability as measured using the lung extravasation of a 70kD infrared tagged dye. Consistent with this finding, treatment with MSC preserved adherens junction integrity, as indicated by increases in the barrier proteins VE-cadherin, claudin-1, and occludin-1 .

Mechanistically, several paracrine factors (KGF, Ang-1,TSG-6, lipoxin A4, Il-1ra, LL-37) account for many of the beneficial effects of MSCs in the lung.(101) In addition to the release of these paracrine factors, there is also evidence that MSCs can transfer their mitochondria to injured lung

epithelium, (34, 102-105) which can reduce lung injury by restoring normal bioenergetics in injured alveolar epithelial cells. Also, MSCs release microvesicles that can enhance anti-microbial effects by delivery of microvesicles to macrophages that then increase monocyte phagocytosis of bacteria. (104) The evidence in laboratory studies indicates that MSCs do not engraft in the lung or other organs, but instead provide a therapeutic benefit by the mechanisms. (34, 102-106)

These mechanisms apply to the lung but may also help to explain the beneficial effects of MSCs that have been reported on non-pulmonary organ injury. In a mouse sepsis study, there was a reduction in serum creatinine as well as bilirubin in the MSC-treated mice. Another group reported the beneficial effects of MSCs on reducing acute kidney injury following ischemia-reperfusion. (106, 107) Their results indicated that organ recovery might be explained by the release of microvesicles from the MSCs that were lodged into the kidney circulation while the majority of the MSCs were lodged in the lung circulation. These preclinical studies support the hypothesis that MSCs may be beneficial in limiting multiple organ dysfunction as we propose to include in this trial since patients who suffer ARDS from medical causes such as pneumonia and aspiration and major trauma frequently develop ARDS as well as acute kidney injury and hepatic dysfunction.

The mechanisms of potential benefit have been summarized in two recent reviews by Dr. Matthay and his colleagues. (8, 9). In addition, a recent secondary study based on the 60 patient START trial demonstrated that there was a reduction in total protein and pro-inflammatory biomarkers in the bronchoalveolar lavage of patients who were treated with the MSCs compared to placebo (108). We do not have pre-clinical data demonstrating efficacy of MSCs for SARS-CoV-2 lung injury.

3.4 *Nonclinical Safety and Efficacy Studies*

In anticipation of a clinical trial, we established a collaboration with the NIH sponsored Production Assistance for Cellular Therapy (PACT) Group in the University of Minnesota (Principal Investigator: David McKenna, MD) for the production of cGMP (Current Good Manufacturing Practice) clinical grade, cryopreserved hMSCs. In experiments in the *ex vivo* perfused human lung, these clinical grade hMSCs were effective in both endotoxin and live E. *coli* lung injury models in restoring the rate of AFC over 6 h when given by intra-venous or intra-tracheal administration.

We completed safety studies in rats with ALI. Rats were injured with a standard non-infectious model of lung injury (hydrochloric acid instillation) and then randomized to placebo and three different doses of intravenous hMSCs from the University of Minnesota. The studies were carried out over 6 h. There were no respiratory or hemodynamic adverse effects in the rats treated with intravenous hMSCs (doses of 1, 5, and 10 x 10^6 cells/kg).

We have completed a study with hMSCs in sheep with ALI over 24 hours (Sheep Study 1 in 2011, n=18). The sheep were injured with inhalation of hot cottonwood smoke and instillation of live P. *aeruginosa* bacteria in both lungs. Sheep were treated with intravenous hMSCs (either 5 or 10 x 10^6 cells/kg) that had been washed to remove all of the DMSO and reconstituted in Plasma-Lyte A or with Plasma-Lyte A in the control sheep. The treatment was given 1 hour after the injury. There was a significant improvement in oxygenation (PaO_2/FiO_2 mm Hg) at 24 hours in the sheep

treated with hMSCs compared to the control sheep. Also the post-mortem extravascular lung water was lower in the sheep treated with the higher dose (10×10^6 cells/kg) compared to controls. The hMSCs were well tolerated with no infusion related changes in pulmonary or systemic hemodynamics or oxygenation in Sheep Study 1.

We also completed a second sheep study (Sheep Study 2 in 2012, n=12) in which ALI was again induced with inhaled cottonwood smoke and instillation of live P. *aeruginosa* bacteria in both lungs to test the safety of giving two doses of hMSCs (1 hour and 18 hours after the injury). In contrast to Sheep Study 1, in this second sheep study the hMSCs were administered with 2.5% DMSO (i.e., cells not washed to remove the DMSO) and the sheep were pre-treated with diphenhydramine and low dose hydrocortisone.

In the 2012 sheep experiments, the results from the first six sheep (each one paired with a control) indicated that the severity of sepsis was greater in these sheep than in the prior sheep studies from 2011, as reflected by higher levels of lactate, lower systemic blood pressure, and lower urine output. Five of these 6 sheep died before 24 hours, 2 sheep even before 18 hours. There was a protocol of euthanasia guided by a low level of systemic blood pressure and low oxygenation.

In addition to the high observed mortality rate described above, there was no evidence of improvement in the oxygenation (PaO_2/FiO_2 ratio) in the first 12 hours after the first dose of hMSCs in the first six treated sheep. This raised some concern that perhaps administering the hMSCs with DMSO decreased the efficacy of the hMSC therapy.Consultation was then obtained with the FDA after the results of the first 6 sheep. It was mutually agreed upon that we should carry out the next 6 sheep experiments with a systematic approach to determine the tolerability of the hMSCs, the possible contribution of DMSO to the reduced efficacy, and trying to reduce the bacterial injury so that it more closely resembled the experiments from 2011 with the group 1 sheep.

Thus, in the next two sheep (hMSC-treated and Plasmalyte control-treated), the dose of *Pseudomonas aeruginosa* bacteria was reduced from 2.5 to 1.8×10^{11} for instillation into the lungs. The result was a pattern of acute lung injury and sepsis that more closely resembled the sheep studies from 2011. These experiments were carried out with the same protocol of DMSO treatment and two doses of hMSCs at one and eighteen hours after onset of acute lung injury. Even with the lower dose of bacteria, the hMSC-treated sheep died at $\geq$ 20 h, suggesting again that the second dose of hMSCs at this time interval after lung injury was not tolerated in terms of systemic hypotension. Also, there was no evidence of efficacy after the first dose of hMSCs, as we had observed consistently in the 2011 group 1 sheep.

Thus, for the last four sheep, we continued to use this more moderate dose of bacteria, but returned to the original approach of administration of the hMSCs after washing them to remove all the DMSO and without any pre-treatment with Benadryl or hydrocortisone. In the final four sheep (MSC-treated and control-treated pairs), there was evidence of efficacy of improved oxygenation (PaO_2/FiO_2 ratio) as in the prior group 1 experiments from 2011. However, there was still evidence that the second dose of hMSCs given at eighteen hours was not tolerated, because one of the hMSC-treated sheep died prior to 24 hours.

We previously concluded from these studies that (1) DMSO should not be used in our clinical trial and (2) that we could not demonstrate preclinical safety for a second dose of hMSCs in this severe model of ALI when the hMSCs were given only 18 hours after the lung injury following an initial dose of hMSCs at 1 hour following the injury.

As discussed further in this clinical protocol, based on the results of the phase 2a clinical trial in 60 patients (START trial), the process of removing DMSO with an extra wash step did reduce hMSC viability. Therefore, in this phase 2b trial, we will simplify the bone marrow transplant procedures and not remove the DMSO after thawing the hMSCs product for clinical administration. The product will be thawed and diluted with 1:1 mix of 5% human serum albumin and 10% Dextran 40 (see Study Product Preparation Protocol in Appendix E).

3.5 *Description of Investigational Cellular Product and Dose Selection*

The investigational cellular product is Allogeneic Bone Marrow-derived Human Mesenchymal Stromal Cells (hMSCs). The investigational product is manufactured at University of Minnesota's Molecular & Cellular Therapeutics (MCT) Facility.

The hMSCs are formulated in CryoStor (90%) and DMSO (10%), and cryopreserved and stored in bags suspended in liquid nitrogen. Immediately prior to administration, the product is thawed and diluted in 1:1 with reconstitution media (1:1 mix of 5% human serum albumin and 10% Dextran 40). Additional reconstitution media will be added to a final product volume of 300 mL.

The placebo group will be treated with 300 mL of reconstitution media (1:1 mix of 5% human serum albumin and 10% Dextran 40).

Pre-clinical safety data has been generated from the *ex vivo* perfused human lung as well as small animals (mouse and rat) and large animal (sheep) safety studies. In the sheep safety studies, animals have been treated with intravenous hMSCs at doses of either 5×10^6 cells/kg or 10×10^6 cells/kg.

The placebo-controlled Phase 2b study will evaluate the single-dose treatment of 10×10^6 cells/kg. The number of MSCs to be administered will be based on viable cell counts at the time of MSC preparation by the PACT facility at the University of Minnesota as determined by acridine orange/propidium iodide (AO/PI), rather than based on the total number of cells prepared.

3.6 *Clinical Experience*

There are currently more than 200 clinical trials listed in clinicaltrials.gov using other preparations of human mesenchymal stromal cells for therapy for a wide variety of indications, including graft versus host disease, acute myocardial infarction, acute kidney injury, diabetes, transplant allograft rejection, lower extremity ischemia and, more recently, lung disease. The most relevant trials for this application are:

Studies Focused on Lung Disease

1) A Phase 2 trial evaluating Prochymal (Adult allogeneic mesenchymal stem cells, Osiris Therapeutics) for the treatment of moderate to severe chronic obstructive pulmonary disease (COPD, Osiris Therapeutics) involving 62 patients in a randomized, double-blinded, placebo controlled trial (NCT00683722). The six-month interim data analyses demonstrated that Prochymal significantly decreased the level of C-reactive protein in patients compared to placebo, but did not improve pulmonary function. The primary endpoint was safety, and secondary endpoints included changes in pulmonary function tests, exercise capability and quality of life. Subjects received 100 x 10^6 cells/infusion and received 4 weekly infusions (D. Weiss, personal communication). No safety concerns were identified between the placebo and treated groups.

2) Two Phase 1 trials involving the use of human umbilical cord derived mesenchymal stem cells for the treatment of severe bronchopulmonary dysplasia in neonates up to 6 months or 14 days respectively, one randomized and the other open-labeled, single-center (NCT01207869 and NCT01297205). Both trials involved the intra-tracheal administration of hMSCs (3 x 10^6/kg for NCT01207869 and 10 or 20 x 10^6/kg for NCT01297205). The primary outcomes measured included the number and severity of adverse reactions and/or the relationship between the cytokine concentrations in the BAL fluid and pulmonary artery pressure. The intratracheal transplantation of allogeneic hUCB-derived MSCs in preterm infants is safe and feasible, and warrants a larger and controlled phase II study (NCT01297205). (109)

3) A Phase 1 trial evaluating the treatment of idiopathic pulmonary fibrosis (IPF) with human placental derived mesenchymal stem cells (NCT01385644). This was an open-label, single center, non-randomized dose-escalation evaluation of the safety and feasibility of mesenchymal stem cell treatment for subjects diagnosed with IPF. A total of up to eight subjects were enrolled in the study. The first 4 patients received a dose of 1 x 10^6 placenta-derived mesenchymal stem cells/kg. The Data Safety Management Board (DSMB) carried out an interim safety analysis when these first 4 patients had all undergone their 3-month study visit. Because no serious adverse events were documented due to the infusion of mesenchymal stem cells, a subsequent 4 patients received IV infusions of 2 x 10^6 placenta-derived mesenchymal stem cells/kg. The results of this Phase 1 trial suggested that intravenous MSC administration is feasible and has a good short-term safety profile in patients with moderately severe IPF. At six months after treatment, the patients showed no worsening in their condition. (110)

4) A Phase 1 trial to test the safety and feasibility of intravenous administration of autologous human bone marrow derived mesenchymal stem cells after one-sided lung volume reduction surgery (LVRS) and prior to a second LVRS procedure for patients with severe pulmonary emphysema (NCT01306513). The study design was an open label, non-randomized, non-blinded, prospective clinical trial. Subjects would undergo two operations, initially on one lung without pre-surgical infusion of bone marrow derived hMSCs, followed by a second surgical procedure on the contralateral lung, which was preceded by two intravenous infusions of hMSCs one week apart, 4 and 3 weeks prior to the second

lung surgery. The dose was not specified in the published protocol. The primary endpoint is the safety and feasibility of the intravenous infusion of the two doses of hMSCs with 1 week interval after the first LVRS and prior to a second LVRS. Toxicity criteria were evaluated by grade according to World Health Organization (WHO). This trial suggests that autologous MSC treatment in severe emphysema is feasible and safe. The increase in CD31 expression after LVRS and MSC treatment suggests responsiveness of microvascular endothelial cells in the most severely affected parts of the lung. (111)

5) Recently, a prospective phase 1b trial of allogeneic adipose-derived MSCs for acute respiratory distress syndrome was completed in China. There were no reported serious adverse events related to MSC administration, as well as no infusion toxicities. There was not a significant difference in the overall number of adverse events between the treatment and control groups. There were also no significant differences between length of hospital stay, VFDs, and ICU-free days at day 28. Biomarker analysis showed significantly lower serum SP-D levels at day 5 in the MSCs group (p=.027). IL-6 levels showed a trend toward lower levels from day 0 to day 5, however this was not statistically significant (p=.06). Changes in IL-8 were not significant. There were no biomarker changes observed in the placebo group. The investigators concluded that the use of allogeneic adipose-derived MSCs was both safe and feasible in the treatment of ARDS. (112)

6) Dr. Matthay received this IND (#15331) from the FDA to carry out a dose escalation phase 1 clinical trial of allogeneic human MSCs for ARDS in critically ill (no-trauma) patients, with the plan to advance to a phase 2a clinical trial if the phase 1 trial demonstrated safety. Funding was obtained from the NHLBI (U01HL108713) to carry out the trial in five university medical centers. The clinical grade, allogeneic bone marrow-derived human MSCs were provided by the University of Minnesota NHLBI Production Assistance for Cellular Therapeutics (PACT) program (David McKenna, MD, Principal Investigator).

   The initial phase 1 and 2a trials were designed to test MSCs for medical causes of ARDS, with a planned exclusion of trauma-related ARDS. Dr. Matthay and his co-investigators reasoned that the trauma population should be included in a subsequent trial because the mechanisms of lung injury in trauma-related ARDS have several common pathways to medical causes of lung injury. Some trauma patients have additioInnal risk factors for ARDS that are distinct from the medical population, including hypovolemic shock from penetrating or blunt trauma, ischemia-reperfusion following fluid resuscitation, multiple blood product transfusions, pulmonary contusion and long bone fractures. However, it is not clear that the biological pathways resulting in lung injury after these specific risk factors represent novel pathogenic mechanisms of ARDS. Because the biology resulting in ARDS after trauma likely shares similar common mechanisms of ARDS with medical causes of ARDS, we reasoned that this phase 2b trial should include trauma patients. Subsequently emerging evidence suggests that the incidence of ARDS following trauma has declined, probably related to changes in resuscitation practices with the reduced use of crystalloid fluids and balanced transfusion of blood products. (113).

Therefore, the inclusion criteria for this trial have been broadened to include all causes of ARDS. This is the rationale for this requested amendment to the current IND of testing MSCs for both trauma and medical causes of ARDS.

In 2014, Dr. Matthay's group completed the phase 1 dose escalation trial of bone-marrow derived human MSCs in 9 critically ill patients with moderate to severe ARDS from non-traumatic etiologies, including pneumonia, non-pulmonary sepsis and aspiration (NCT01775774). (114) Exclusions included trauma within the prior 5 days, a history of pulmonary hypertension, thromboembolic disease, moderate to severe liver disease (Childs-Pugh > 11), more than 96 hours since the onset of ARDS, history of treatment for cancer within the last 2 years, pregnancy and age < 18 years old. The design was to test the safety of doses of 1, 5, and 10 x 10^6 hMSC/kg predicted body weight (PBW) in three patients at each dose level for a total of nine patients, and is described in detail in two publications. (114, 115) Dr. Marc Moss served as a medical monitor for the DSMB protocol. In consultation with FDA, they defined stopping criteria for the trial based on pre-specified infusion-associated adverse events.

The primary endpoint of the phase 1 trial was safety. MSCs had not been previously administered to critically ill patients, including those with acute respiratory failure from ARDS or with hemodynamic instability (shock). Because these patients were critically ill, the trial required a baseline period of 2 hours of hemodynamic and respiratory stability. Patients were monitored for pre-specified infusion-associated adverse hemodynamic or respiratory events within 6 hours of the MSC infusion. The MSCs were administered intravenously over one hour. A coordinator and physician investigator were present at the bedside during the infusion and for the subsequent monitoring period. The results were reported in 2014. (114) There were no pre-specified associated adverse hemodynamic or respiratory events. Heart rate and systemic blood pressure were no different between the two hour baseline and the six hour post-infusion monitoring period among the three groups.

The 4-point lung injury score (oxygenation, level of positive end-expiratory pressure, static respiratory compliance, and extent of infiltrates on the chest radiograph) (see **Appendix B**) was measured and calculated in all patients (116) at baseline, 6 hours, day 1, day 2 and day 3. The lung injury score declined in each of the three dosing groups with the highest dose showing the lowest lung injury score; statistical analysis of this data was not done because there were only 3 patients in each group and there was no placebo group. Overall, there were no serious adverse events attributable to the MSC treatment in this phase 1 trial of 9 patients. The DSMB then approved advancing to the phase 2a trial, which retained the primary endpoint of safety as measured by the same safety endpoints as in the phase 1 trial.

Dr. Matthay and his group then conducted a phase 2a safety trial with 60 patients with ARDS with the same inclusion and exclusion criteria as in the phase 1 trial described above. The 2a trial was carried out at the same five university medical centers as the phase 1 trial, UCSF, Stanford, Ohio State, University of Pittsburgh and Massachusetts General Hospital (NCT02097641).

The trial was reviewed by the DSMB after 20 and 40 patients, and the DSMB found no safety isssues and recommended continuing the trial and it was completed recently (NCT02097641). The primary endpoint of the trial was safety, thus randomization was 2:1 for MSC to placebo.

In terms of the primary endpoint, safety, there were no pre-specified infusion related adverse events within the 6 hours following infusion. One patient died within 24 hours (hour 22) in the hMSC group related to acute coronary ischemia and infarction during a fiberoptic bronchoscopy. This case was reviewed by the DSMB, who concluded that the death was not related to the study infusion.

There was a numerically higher mortality over 28 days in the hMSC treated group (30%) compared to the 20 patients in the placebo group (15%); this difference was not statistically significant (p=0.24). Several imbalances in the MSC vs. placebo group at baseline likely contributed to this numerical difference. For example, as an index of severity of illness, the mean of APACHE III score was numerically higher at baseline in the MSC patients compared to the placebo patients (104±31 versus 89±33, p=0.10). Also, there was a higher minute ventilation in the MSC patients compared to the placebo patients (11.1±3.2 versus 9.6±2.4 L/min, p=0.06), likely reflecting higher pulmonary dead space and potentially more severe lung disease. When mortality was adjusted by the APACHE III baseline score, the hazard ratio for 28 day mortality was 1.43 (confidence interval [CI]: 0.40 – 5.12, p =0.58).

The placebo mortality of 15% was particularly low for ARDS trials, especially considering that the entry criteria required moderate to severe hypoxemia (PaO_2/FiO_2 < 200 mmHg) which has an expected mortality of 33-49%. (14) The recent large international LUNG SAFE study of ARDS reported a 28 day mortality of 35%. (117)

In terms of signals for potential efficacy, there was a numerical, non-significant improvement in the oxygenation index at days 1, 2 and 3 in the MSC treated group compared to the placebo patients. On day 1, the median oxygenation index numerically improved in the MSC versus placebo group from the baseline (2.7 versus 1.1, p=0.32). On day 2, the median oxygenation index numerically improved in the MSC versus the placebo group (4.0 versus 2.6, p=0.29) and on day 3, the median oxygenation index numerically improved in the MSC versus placebo group (3.0 versus 1.9, p=0.29). However, this phase 2a trial of 60 patients with 2:1 MSC to placebo randomization was not powered for any efficacy endpoints, whereas this new Phase 2b trial for ARDS from medical and traumatic causes of ARDS outlined in this clinical protocol will enroll 120 patients and is powered to test for efficacy for respiratory endpoints with 1:1 randomization. In addition, further *post hoc* analysis of the earlier phase 2a trial showed that there was variability in the viability of the MSCs administered from 36 to 85%. This data was available to the investigators only after the trial was completed. By tertiles, the viability was 36-56% (n=14), 57-69% (n=13), and 71-85%, (n=13). Because of this wide range of MSC viability, we carried out a *post hoc* analysis to determine whether there were any significant differences in the levels of the biologic markers in the plasma according to the MSC

viability. There were no differences in the levels of IL-6, IL-8 or RAGE at baseline. However, after adjustment for baseline differences, angiopoietin-2, a marker of vascular leak associated with more severe lung in jury in ARDS, was significantly and progressively lower in the intermediate and the highest tertiles of MSC viability. Thus, there appeared to be a viability-dependent effect on plasma angiopoietin-2 levels based on the MSC viability. In addition, analysis of the change in oxygenation index from baseline over two days was carried out in these cell viability subgroups. This analysis showed a non-significant trend for improved oxygenation index in the intermediate and highest tertile MSC viability groups on day 2. The results of this trial have been published recently in *Lancet Resp Med.* (118)

7) Recently, a prospective phase 1 trial of MSCs for sepsis has been completed in Canada which did not show any safety issues in the treated patients compared to a historical control group. (119) Dr. Matthay has published an editorial commenting on this trial (120). Two recent reviews of MSCs co-authored by Dr. Matthay (8, 9) provide an update on MSCs in the field of ARDS and sepsis, as well as other critical care conditions.

Studies Using hMSCs for Therapy for Graft versus Host Disease, Crohn's Disease, Acute Myocardial Infarction, Acute Kidney Injury and Diabetes

8) Two Phase 3 trials using Prochymal (Adult allogeneic mesenchymal stem cells, Osiris Therapeutics) in severe refractory graft vs. host disease (GvHD): one that was not steroid responsive and another that received hMSCs as part of first line therapy (NCT00366145 and NCT00562497). Both trials were placebo controlled and did not meet their endpoints. However, in the steroid refractory trial, there was a statistically significant improvement over placebo in patients with gastrointestinal and liver GvHD. The dosing regimen for the hMSCs was 2 x 10^6 cells/kg intravenously twice a week for 4 weeks. These trials were inspired by positive Phase 2 results (121), which showed an overall response rate of 94% and complete remission rate of 77% among 32 patients who received hMSCs for acute GvHD.

9) A Phase 3 trial using Prochymal (Osiris Therapeutics) for the induction of remission in subjects experiencing treatment-refractory moderate-to-severe Crohn's disease (NCT00482092). The primary outcome measured will be disease remission (Crohn's Disease Activity Index at or below 150) within a 28-day timeframe. A low dose (600 x 10^6 cells total over four intravenous infusions in two weeks) and a high dose (1200 x 10^6 cells delivered intravenously in four infusions over two weeks) will be tested.

10) A Phase 2 trial using Prochymal (Osiris Therapeutics) in patients following an acute myocardial infarction (NCT00877903). The study is currently enrolling patients (220 patients, randomized, double-blinded, placebo controlled). The primary endpoint is left ventricular systolic volume; secondary endpoints are measurements of left ventricular ejection fraction, infarct size and major adverse cardiovascular events. Subjects receive a single intravenous infusion within 7 days following an acute myocardial infarction. Note

that this group completed a double blind, placebo-controlled, dose-ranging (0.5, 1.6, and 5 million cells/kg) study of 53 patients. Left ventricular ejection fraction improved by echocardiography in patients treated with hMSCs vs. placebo. There were similar rates of adverse events (5.3/patients post hMSCs vs. 7/patient in placebo, n = 53 total). (122)

11) A Phase 1/2 trial studying the efficacy of intramyocardial injections of autologous human mesenchymal stem cells in patients undergoing cardiac surgery (Prometheus, NHLBI Sponsored, NCT00587990). This randomized, double-blinded, placebo controlled study is currently enrolling patients (planned enrollment 45 patients). Primary outcomes are incidence of serious adverse events at 6 months including the incidence of sustained ventricular arrhythmias, ectopic tissue formation or sudden unexpected death. Dosage used will be 10 to 20 intramyocardial injections of 2 to 20 million hMSCs for a total of 2×10^7 to 2×10^8 cells total.

12) A randomized, multi-center, double-blind, placebo-controlled study of AC607 (hMSCs) for the treatment of acute kidney injury in cardiac surgery subjects (AlloCure Inc, NCT01602328). Dosage was a single injection of 2×10^6 hMSCs/kg. The primary endpoint was time to kidney recovery, defined as a post-operative serum creatinine return to pre-operative baseline values within 30 days of dosing. This trial was terminated due to futility after the final DSMB meeting with 75% patient accrual. The primary analysis showed no significant difference in time to recovery of kidney function. There were no significant differences between groups in the secondary outcomes of all-cause mortality or provision of dialysis. (123)

13) There are >10 clinical trials underway for the treatment of Diabetes, both type 1 and 2, with mesenchymal stem cells. One relevant trial is a Phase 2, multicenter, randomized, double-blind, placebo controlled study to evaluate the safety and efficacy of Prochymal (Osiris Therapeutics) for the treatment of recently diagnosed Type 1 Diabetes Mellitus (NCT00690066). The dosage is not specified. The primary endpoint will be the measurement of C-peptide AUC response (mixed meal tolerance test). Secondary outcomes will be the measurements of peak C-peptide response, basal C-peptide response, total daily insulin dose, glycosylated hemoglobin (HbA1c), number of severe and documented hypoglycemic events and changes in levels of GAD or IA-2 autoantibodies.

The safety record for hMSCs prepared by conventional methods has been favorable in clinical trials to date, according to published literature and information available on clinical trials.gov. Serious infusion-related or delayed toxicities have not been associated with either autologous or allogeneic hMSCs in adult and pediatric recipients of allogeneic hematopoietic transplantation, the patient population most studied to date. (124) Osiris Therapeutics has sponsored clinical trials of Prochymal (allogeneic unrelated donor hMSCs) that have included over 2000 patients to date. In a randomized, placebo-controlled, dose-escalation safety study of Prochymal in patients with acute myocardial infarction, adverse events were comparable for the hMSC- and placebo-treated groups, and no adverse events were attributable to hMSCs administration. (122) Up to 5×10^6 cells/kg were administered in this study.

3.7 *Potential Benefits and Risks to Human Participants*

3.7.1 Potential Benefits

The benefits from hMSCs in the treatment of ARDS remain unknown. The benefit of participation in this study is the knowledge gained for the benefit of future patients. The mortality rate of moderate-to-severe ARDS (the target population for this trial) is approximately 25-40%. Administration of hMSCs may have a benefit on mortality in this patient population. Furthermore, if hMSCs have benefit in patients with ARDS, this will have a major benefit to society given the high mortality rate associated with this condition.

3.7.2 Potential Risks

3.7.2.1 **Overview**

The main potential risks of participation in this study are those associated with administration of the investigational agent. There are additional minor risks associated with study-related procedures as detailed below.

3.7.2.2 **Cardiovascular and Respiratory**

Transient occlusion of the pulmonary microcirculation with intravenously administered hMSCs could be associated with a fall in systemic blood pressure, rising vasopressor dose, a rise in heart rate, an increase in arterial carbon dioxide concentration, or a fall in oxygenation. As detailed below, patients will be monitored continuously during the infusion for these signs/symptoms and the infusion will be terminated if certain clinical criteria are met or there is concern for worsening hemodynamics or oxygenation on the part of the study investigators.

3.7.2.3 **Risks of Transfusion Reaction**

Known potential side effects of blood product transfusion include transient fever or chills. As per established regulatory requirements, bone marrow donors are prescreened for infection, reducing the risk of infection with HIV or hepatitis B or C from the hMSC cell product. Patients may experience more severe or life-threatening transfusion reactions, but these are exceedingly rare. Patients will be monitored during the infusion for signs of a transfusion reaction (urticaria or rash, bronchospasm) and medications will be readily available to treat a life-threatening transfusion reaction, should such a reaction occur.

3.7.2.4 **Risks of blood draws**

All patients will have blood drawn for research purposes. Most blood will be drawn through indwelling catheters. Risks of drawing blood percutaneously are minor and include bleeding, bruising and infection.

3.7.2.5 **Reproductive risks**

There may be an unexpected risk to an unborn or nursing child. Pregnant and breastfeeding women will be excluded from participation in the study.

3.7.2.6 **Mini-bronchoalveolar lavage (Mini-BAL)**

In the Phase 2b clinical trial, we will obtain mini-bronchoalveolar lavage (mini-BAL) on day 2 after the infusion of hMSCs. The known potential risk of mini-BAL includes transient deterioration of oxygenation or respiratory compliance, requiring an increase in ventilator settings. The exclusion of study subjects who require high levels of ventilatory support from this procedure will minimize the risk of this complication. We will also exclude patients at risk for complications of the mini-BAL procedure, including patients with significant bleeding disorders or elevated intracranial pressure using clear, predetermined criteria that are used in clinical practice. In over a decade of use at UCSF Medical Center using even less restrictive criteria for exclusion, mBAL has not resulted in any safety concerns. The mini-BAL at day 2 will allow us to test the efficacy of hMSCs within the airspaces, which is a primary proposed biological site of action.

3.8 *Study Rationale*

Pre-clinical data from the *ex vivo* perfused human lung as well as small animal (mouse and rat) and large animal (sheep) studies support the potential efficacy and safety of hMSCs administration for the treatment of ALI/ARDS. We completed phase 1 and 2a trials of allogeneic hMSCs in moderate-to-severe ARDS, using a hMSCs product derived from bone marrow by conventional methods. The trial were designed to test safety endpoints, but included secondary efficacy endpoints but the trial was not powered for these secondary efficacy endpoints. (114) (118)

In the **Phase 1 trial**, we enrolled 9 patients in a 3-dose escalation phase (1 x 10^6 cells/kg, 5 x 10^6 cells/kg and 10 x 10^6 cells/kg).

A. In Phase 1, we used an open label standard dose escalation model, with a plan for safety assessments.

B. The initial dose was 1 x 10^6 cells/kg given intravenously to 3 patients. After approval from the DSMB, we progressed to the next dose. The second dose delivered was 5 x 10^6 cells/kg given intravenously to 3 patients. The third dose level was 10 x 10^6 cells/kg given intravenously to 3 patients.

In the **Phase 2a trial,** we enrolled 60 patients in a 2:1 blinded randomized placebo-controlled design with the dose of 10 x 10^6 cells/kg (40 patients treated with hMSCs and 20 controls). Safety analyses was conducted by the independent DSMB, and the sponsor and investigators were blinded.

The initial phase 1 and 2a trials were designed to test hMSCs for medical causes of ARDS, with a plan to exclude trauma-related ARDS. Dr. Matthay and his co-investigators reasoned that the trauma population should be included in a subsequent trial because the mechanisms of lung injury in trauma-related ARDS have several common pathways to medical causes of lung injury, although some trauma patient have hypovolemic shock from penetrating or blunt trauma, ischemia-reperfusion following fluid resuscitation, multiple blood product transfusions, pulmonary contusion and long bone fractures. This is the rationale of testing hMSCs for both trauma and medical causes of ARDS.

Based on these studies, we propose a Phase 2b randomized, double-blind, placebo-controlled clinical trial of hMSCs for the treatment of moderate-to-severe ARDS after medical and traumatic causes of ARDS.

4 Study Objectives

4.1 *Primary Objective*

The primary objective of this study is to assess whether an intravenous infusion of hMSCs in patients with ARDS will lead to a reduction in the severity of acute respiratory failure from ARDS as measured by the oxygenation index**.**

4.2 *Secondary Objectives*

The secondary objectives of this study are to assess the potential efficacy of intravenous infusion of hMSCs in patients with ARDS including (1) an improvement in several physiologic and functional endpoints of respiratory failure, (2) a reduction in the incidence of secondary infections, (3) a reduction in systemic organ failure and in-hospital mortality, (4) improved neurocognitive function at hospital discharge, (5) incidence of thromboembolic events, as well as (6) to acquire mechanistic data regarding the activity of hMSCs in ARDS patients from biologic samples of plasma, urine and mBAL. In addition, safety will monitored as defined by the incidence of pre-specified infusion associated events and of unexpected severe adverse events in ARDS patients treated with hMSCs. Because the vast majority of patients enrolled in this trial will have had ARDS secondary to COVID-19, all of the objectives will be analyzed according to whether the patient developed ARDS from COVID-19 or another etiology.

5 Study Design

This clinical study design is a randomized, double-blinded, placebo-controlled Phase 2b clinical trial using a 10 x 10^6 cell/kg dose of hMSCs. Subjects will be randomized in a 1:1 randomization scheme to receive hMSCs or cell reconstitution media (1:1 mix of 5% human serum albumin and 10% Dextran 40) as the placebo; the study will enroll 120 patients who achieve a stable clinical baseline and receive study product (either hMSCs or the placebo) as described in **Section 9.4**.

The DSMB will review adverse outcomes and protocol compliance. A pre-specified interim review will occur after 60 subjects have been enrolled and received study product; enrollment will continue during DSMB review. All pre-specified clinically important events and unexpected serious adverse events including death through day 28 from study product administration 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 three subjects have pre-specified clinically important events or unexpected serious adverse events EXCEPT death since death will be common in this critically ill population due the nature of the underlying illness (e.g., ARDS).

6 Endpoints

Analyses of the primary and secondary endpoints will be conducted primarily as an intention-to-treat analysis plan and also an as-treated analysis as described in detail in **Section 11.3.** A Statisical Analysis Plan has been written according to this protocol. There is the potential for subjects to be consented for study participation but to not receive treatment because of clinical instability due to the severity of their underlying medical conditions or due to clinical improvement in ARDS.

6.1 *Primary Study Endpoint: Phase 2b*

The primary study endpoint will assess whether an intravenous infusion of hMSCs in patients with ARDS will lead to a reduction in the severity of ARDS as measured by a decrease in the oxygenation index (OI) compared to baseline OI (prior to administration of the study product) over the first 36 hours after the initiation of study product infusion (with measurements of OI at 6, 12, 18, 24, 30 and 36 hours). (125, 126)

6.2 *Secondary Endpoints: Phase 2b*

6.2.1 Efficacy Secondary Endpoints

We will test six categories of efficacy endpoints in the Phase 2b trial: respiratory physiology, secondary infection, systemic illness, neurologic, thromboembolic events, and biomarker profiles related to potential mechanism of hMSC activity in ARDS.

1. **Respiratory Physiology**: Respiratory efficacy endpoints will include:
   - Reduction in the 4-point acute lung injury (LIS) score after enrollment in the trial at days 1, 2, 3 and 7, or on the last day of positive pressure ventilation prior to day 7. The LIS is a composite scoring system including the PaO_2/FiO_2, the level of positive end-expiratory pressure, static respiratory compliance, and the quadrants of frontal chest radiograph with opacification.
   - Reduction in the pulmonary dead space on days 1, 2, 3 and 7.
   - Reduction in chest radiograph assessment of the extent of pulmonary edema (RALE score) at days 1, 2, 3, and 7.
   - OI will be collected (as planned) also on days 2, 3, and 7 if still being ventilated with positive pressure as a secondary endpoint.
   - Reduction in ventilatory ratio with measurements at 6, 12, 18, 24, 30, 36 hours, and days 2, 3 and 7.
   - Incidence of barotrauma on days 1, 2, 3, and 7.
   - Achieving pressure support ventilation with 5 cmH_20 and positive end-expiratory pressure equals to 5 cmH_20 for 2 hours
   - Ventilator free-days over 7, 14 and 28 days; duration of assisted ventilation over 28 days in the survivors. Ventilator Free Days (VFDs) to day 28 are defined as the number of days from the time of initiating unassisted breathing to day 28 after study product

administration, assuming survival for at least two consecutive calendar days after initiating unassisted breathing and continued unassisted breathing to day 28. If a patient returns to assisted breathing and subsequently achieves unassisted breathing to day 28, VFDs will be counted from the end of the last period of assisted breathing to day 28. A period of assisted breathing lasting less than 24 hours and for the purpose of a surgical procedure will not count against the calculation of VFDs. If a patient was receiving assisted breathing at day 27 or dies prior to day 28, VFDs will be zero. Patients transferred to another hospital or other health care facility will be followed to day 28 to assess this endpoint.

2. **Secondary Infection**: Infection efficacy endpoints will include:
- Superficial incisional/wound infections during the 14 days after enrollment.
- Deep incisional wound infections during the 14 days after enrollment.
- Organ (such as lung, liver) or space (such as peritoneum or pleural) infections during the 14 days after enrollment.
- Ventilator associated pneumonia (VAP) during the 14 days after enrollment.

3. **Systemic Illness**: Systemic illness efficacy endpoints will include:
- Mean SOFA score (127) at days 3, and 7.
- In hospital mortality at 14 days, 28 days, and 60 days.
- Additional prognostic value of neutrophil:lymphocyte ratio.
- Ordinal Scale for Clinical Improvement (OSCI) at days 7, 14 and 28.

To assess organ failure, we will measure serum creatinine (renal function), bilirubin (hepatic function), and platelet count (hematology) at days 0, 3, and 7.

4. **Neurologic:** Neurocognitive efficacy endpoints will include:
- Neurocognitive function at hospital discharge by the Glasgow Outcome Score (6 points)

5. **Thromboembolic:** Thromboembolic safety endpoints will include:
- Incidence of thromboembolic events: Measured by ultrasound of the deep venous system or CT-angiography of the chest when clinically indicated.
- Additional prognostic value of fibrinogen at baseline.

6. **Biologic:** Biomarker analysis will include: Changes in levels of plasma, urine, and genetic biomarkers at baseline compared to 6, 24, 48, and 72 hours, including:
    - Endothelial injury (angiopoietin-2 [Ang-2])
    - Lung epithelial injury (Receptor for Advanced Glycation Endproducts [RAGE])
    - Pro-inflammatory markers (interleukin-6, interleukin-8, soluble tumor necrosis factor-1 [sTNF-1], intercellular adhesion molecule-1 [ICAM-1], interferon gamma-induced protein-10 [IP-10], interleukin-10 [IL-10], vascular endothelial growth factor [VEGF], matrix metalloproteinase-8 [MMP-8], thrombomodulin, surfactant protein-D [SP-D], triggering receptor expressed on myeloid cells-1 [TREM-1], interleukin-18 [IL-18], plasminogen activator inhibitor-1 [PAI-1])

- Protein C (marker of impaired coagulation)
- Pro-resolving lipids (lipoxin A4, resolvin D_1)
- Biomarkers that reflect the paracrine activity of the administered hMSCs (angiopoietin-1 [Ang-1], keratinocyte growth factor [KGF])
- We will use the levels of the baseline plasma biomarkers (IL-8, Protein C, and serum bicarbonate ± vasopressor use at randomization) to classify patients as hyper- or hyper-inflammatory subphenotypes as we have described and test for higher mortality in the hyper-inflammatory group. Also, we will test for an interaction between MSC therapy and the subphenotypes for the outcomes of oxygenation index over 36 hours and 28 day mortality.

- Lung protein permeability as measured by total protein in mini-bronchoalveolar lavage [mBAL] protein at day 2; the same biomarkers will also be measured in plasma to compare MSC versus placebo treated patients.
- Measurements of urine microalbumin and urine creatinine
- Changes in levels of plasma SARS-CoV-2 viral antigen and antibody at multiple timepoints.
- Gene expression at baseline and on days 2 and 3.

6.2.2 Safety Secondary Endpoints

Because the infusion of hMSCs could theoretically, as described above, cause transient occlusion of the pulmonary microcirculation which could be associated with a fall in systemic blood pressure, rising vasopressor dose, a rise in heart rate, an increase in arterial carbon dioxide concentration, or a fall in oxygenation, patients will be monitored closely during the study infusion for changes in these parameters. These events will be considered pre-specified infusion associated events if they occur within 6 hours of the initiation of study product infusion.

Pre-specified infusion associated events will be defined as:

(a) Within 6 hours of the initiation of study product infusion:
1. An increase in vasopressor dose greater than or equal to the following:
   - Norepinephrine: 10 mcg/min
   - Phenylephrine: 100 mcg/min
   - Dopamine: 10 mcg/kg/min
   - Epinephrine: 0.1 mcg/kg/min
   - Addition of a third vasopressor
2. New ventricular tachycardia, ventricular fibrillation or asystole
3. New cardiac arrhythmia requiring cardioversion
4. Hypoxemia requiring an increase in FiO_2 of 0.2 or more and an increase in PEEP of 5 cmH_20 or more to maintain Sp02 in the target range of 88-95% that is not related to respiratory care/suctioning or ventilator dyssynchrony and persists for more than 30 minutes
5. Clinical scenario consistent with transfusion incompatibility or transfusion-related infection (e.g. urticaria, rash, new bronchospasm)

(b) Within 24 hours of the study product infusion:
1. Any cardiac arrest or death

We will also systemically collect and review the incidence and nature of serious adverse events that are different from what is expected in the clinical course of a critically ill patient with ARDS for the duration of the clinical trial (through day 28 from study product administration).

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 intensive care unit 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 drug 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. $Sp0_2$ ~85%), related to positioning or suctioning. This latter event would not be considered unexpected by nature, severity or frequency.

For this Phase 2b study, the secondary safety endpoint will be the incidence of pre-specified infusion associated events occurring within 6 hours of study product administration and any cardiac arrest or death occurring within 24 hours of study product administration, as well as any unexpected severe adverse events in ARDS patients (not including death because it is expected in ARDS) treated with hMSCs compared to patients treated with placebo through day 28 from study product administration.

6.2.3 Primary and Secondary Endpoints Adjusted for SARS-CoV-2 Infection

In addition, to better understand the hMSC effects in patients with COVID-19 disease, we will conduct the following analyses:

- The primary and secondary endpoints (specified in Section 6.1, 6.2.1, 6.2.2) as stratified in separate analyses by the following binary variables of intersest prior to randomization: SARS-CoV-2 Infection status, viral antigen level (greater or less than median value), dexamethasone (or equivalent steroid therapy) and other immunomodulatory agents.
- WHO Scale for Clinical Improvement outcome at baseline, day 7, day 14, and day 28 adjusted by COVID-19 status.
- Gene expression profiles compared by treatment arm and adjusted for or stratified by SARS-CoV-2 Infection, dexamethasone (or equivalent steroid therapy) and other immunomodulatory agents.
- Changes in levels of protein biomarker measurements by treatment arm and adjusted for or stratified by SARS-CoV-2 infection, dexamethasone (or equivalent steroid therapy), and other immunomodulatory agents.
- Analysis of primary and secondary endpoints by latent class analysis (LCA) subphenotype and adjusted for or stratified by SARS-CoV-2 Infection.

- Analyses of primary and secondary endpoints adjusted for or stratified by anticoagulation and antiplatelet treatments.
- Analyses to be adjusted for measures of MSC viability.

7 Study Population and Enrollment

7.1 *Number/Source/Screening*

This Phase 2b trial will enroll 120 patients over a 3-4 year interval. Patients with ARDS from medical causes (pneumonia, sepsis, aspiration) or trauma will be recruited from Medical and Surgical Intensive Care Units at seven medical centers, Zuckerberg San Francisco General Hospital & Trauma Center and UCSF Moffitt-Long Hospital (San Francisco, CA), University of California Davis Medical Center (Sacramento, CA), Harborview Medical Center (Seattle, WA), Oregon Health & Science University (Portland, OR), Vanderbilt University Medical Center (Nashville, TN) and the University of Texas Health Sciences Center at Houston/Memorial Hermann-Texas Medical Center (Houston, TX).

Study coordinators will screen ICUs daily to identify potential candidates for enrollment. Permission to approach patients and/or their families will be requested from the attending physicians. All patients meeting the inclusion criteria will be entered into a screening log. If the patient is not enrolled, the screening log will include information explaining why enrollment did not occur (exclusion criteria, attending physician denial, patient refusal, no surrogate available; see **Appendix C** for a listing of the de-identified data to be collected on screened, non-enrolled subjects).

7.2 *Inclusion criteria*

Patients with the presence of ARDS within 14 days of initial ICU admission. ARDS is defined by presenting the following Criteria 1-3 within a 24-hour time period and at the time of enrollment:

1. A need for positive pressure ventilation by an endotracheal or tracheal tube with a PaO_2/FiO_2 ratio < 250 mmHg with ≥5 cm H_2O positive end-expiratory airway pressure (PEEP), as per the Berlin Criteria, and
2. Bilateral infiltrates consistent with pulmonary edema on frontal chest radiograph, or bilateral ground glass opacities on a chest CT scan, and
3. No clinical evidence of left atrial hypertension as the explanation for the bilateral pulmonary infiltrates.
4. If the cause of ARDS is trauma, additional inclusion criteria will include ONE of the following relevant risk factors for developing ARDS:
    a. Hypotension (systolic blood pressure[SBP] < 90 mmHg) in the field or in the first 24 h after injury, or
    b. Transfusion of 3 units of blood products in the first 24 hours following injury, or
    c. Meets the new Critical Administration Threshold (CAT) criteria with at least 3 units of blood in one hour, or

d. Blunt or penetrating torso trauma, or
e. Long bone fractures, or
f. The highest level of institutional trauma activation

ARDS diagnostic criteria defined:

- "Acute onset" is defined as follows: the duration of the hypoxemia criterion (#1) and the chest radiograph criterion (#2) must both be present ≤ 14 days of initial ICU admission at the time of enrollment.
- Infiltrates considered "consistent with pulmonary edema" include any patchy or diffuse opacities not fully explained by mass, atelectasis, or effusion or opacities known to be chronic (>14 days of initial ICU admission). The findings of vascular redistribution, indistinct vessels, and indistinct cardiac borders alone are not considered "consistent with pulmonary edema" and thus will not count as qualifying opacities for this study.
- If a patient meets #1 and #2 inclusion criteria but has an echocardiogram with LV ejection < 40% that ordered by treating clinicians because of clinical suspicion of left heart failure, the patient will be excluded.
- If a patient meets the #1 and #2 inclusion criteria but has a PAOP (Pulmonary Arterial Occlusion Pressure, also known as the Pulmonary Arterial Wedge Pressure) that is initially greater than 18 mm Hg, then the inclusion criteria must persist for more than 12 hours after the PAOP has declined to ≤ 18 mm Hg.

7.3 *Exclusion Criteria*

1. Age less than 18 years
2. Greater than 72 hours since first meeting ARDS criteria per the Berlin definition of ARDS
3. Greater than 14 days since initial ICU admission
4. Inability to administer study product within 14 days of initial ICU admission
5. PaO_2/FiO_2 ≥250 mmHg after consent obtained and before study product is administered
6. Unable to obtain informed consent/no surrogate available
7. Pregnant or lactating
8. In custody of law enforcement officials
9. Burns > 20% of total body surface area
10. WHO Class III or IV pulmonary hypertension
11. History of cancer treatment in the last 2 years except for non-melanotic skin cancers
12. Underlying medical condition for which 6-month mortality is estimated to be > 50%
13. Moribund patient not expected to survive 24 hours
14. Advanced chronic liver disease (Childs-Pugh Score > 12) (See Appendix J)
15. Severe chronic respiratory disease with the use of home oxygen
16. Severe traumatic brain injury - defined as a patient who:
    a. Has undergone intracranial neurosurgical intervention for monitoring or therapy (intracranial pressure monitoring, external ventricular drain, craniotomy), or

b. Has an intracranial injury by head CT (does not include patients with minimal subarachnoid injury and/or minor skull fracture) , or
c. Has a post-resuscitation Glasgow Coma Score (GCS) < 9 assessed after sedation interruption, or
d. Has non-survivable head injury as assessed by neurosurgery

17. Evidence of anoxic brain injury
18. History of stroke within the last 3 years
19. No intent/unwillingness to follow lung protective ventilation strategy
20. Currently receiving extracorporeal life support (ECLS) or high-frequency oscillatory ventilation (HFOV)
21. Anticipated extubation with 24 hours of enrollment
22. Clinical evidence of left atrial hypertension as measured by a pulmonary arterial wedge pressure > 18mmHg or left ventricular failure measured by an echocardiogram with a left ventricular ejection fraction less than 40%. Clinical judgement will determine if either of these measurements need to be carried out. (If the pulmonary arterial wedge pressure declines to less than 18 mmHg, then the patient would qualify if ARDS criteria persist for at least 12 hours.)

Rationale for Exclusions

Criteria 1: Patients less than 18 years old are excluded because of limited clinical trial data with hMSCs in subjects younger than 18 years.
Criteria 2: ARDS that develops more than 72 hours not likely to be related primarily to the acute insult, but rather to a nosocomial complication. The efficacy of hMSCs is likely to be greater if given in the early phase of ARDS.
Criteria 3 and 4: By increasing the enrollment window from within 120 hours to within 14 days of initial ICU admission, we want to increase the opportunity to recruit more medical and surgical patients into this trial.
Criteria 5: By increasing the PaO_2/FiO_2 from 200 to 250 mmHg, we want to increase the opportunity to recruit more COVID-19 patients into this trial.
Criteria 6: Informed consent required for enrollment in this trial.
Criteria 7 and 8: Standard exclusion of vulnerable patient populations.
Criteria 9: Burns > 20% constitute a different clinical entity than major trauma alone.
Criteria 10: Intended to exclude patients at increased risk in case pulmonary arterial pressures increase after administration of hMSCs and hypoxemia from pulmonary hypertension could attenuate efficacy signal.
Criteria 11: Those with active malignancies within the past 2 years are excluded because of uncertainty regarding the possibility that hMSCs might enhance the growth of tumors (though no evidence for this from any clinical trial to date).
Criteria 12, 13 and 14: Intended to exclude patients unlikely to survive because of their underlying medical conditions that have a greater impact on clinical outcomes than ARDS.
Criteria 15: Chronic severe lung disease causing baseline hypoxemia may attenuate assessments of efficacy of hMSCs in ARDS
Criteria 16: Patients with severe traumatic head injury are not ideal candidates for testing hMSCs therapy, in part because severe traumatic head injury is a more important factor in poor clinical

outcomes in the setting of ARDS, and also because lung protective ventilation may not match well with the need for hyperventilation and maintaining hypocapnia in some patients with traumatic brain injury.
Criteria 17: Anoxic brain injury will have an overriding influence on secondary outcomes including death and multiple organ dysfunction and thus these subjects would not be good candidates for testing hMSCs therapy.
Criteria 18: Stroke in the last years could make assessment of hMSCs therapy for ARDS difficult to interpret, especially since acute stroke is another target for hMSCs therapy that is being tested in clinical trials currently.
Criteria 19: Intended to exclude patients with the use of ventilation strategies other than lung protective ventilation delivered by assist control because these alternative ventilation strategies would complicate assessment of the primary and secondary endpoints.
Criteria 20: Those on ECLS are excluded because the effect of the ECLS circuit on hMSCs half-life is unknown. HFOV is a ventilator strategy shown not to be effective in adults with ARDS.
Criteria 21: Anticipated extubation within 24 hours would make it difficult to assess the impact of the hMSCs therapy given the primary endpoint of oxygenation index.
Criteria 22: The presence of an elevated pulmonary arterial wedge pressure > 18mmHg or an echocardiogram that show decreased left ventricular function less than 40% would mean that the patient most likely had a cardiogenic mechanism for the pulmonary edema and should not be included in this trial.

7.4 *Study Initiation Time Window*

All ARDS criteria must occur within the same 24-hour period. The onset of ARDS is when the last criterion is met. See **Section 9.4** for further clarification on the timelines.

Information for determining when these time window criteria were met may come from either the recruitment hospital or reports from a referring hospital. Following randomization, the low tidal volume protocol for mechanical ventilation must be initiated within one hour (if not already being utilized).

7.5 *Subject Recruitment and Informed Consent*

Study subjects will be recruited in the ICU by a study investigator or study coordinator. Because these study subjects may be unable to consent for themselves, written informed consent will be obtained from a surrogate when appropriate. Written informed consent will be obtained by one of the study investigators or study coordinators before enrollment in the trial. No study procedures will be conducted before obtaining informed consent. If surrogates are not available at bedside, study investigators will reach them by phone to seek study recruitment, and a signed electronic consent form will be obtained through HIPAA compliant REDCap platform prior to conducting study procedures. The signed consent forms can also be scanned and sent to secured fax if surrogates prefer. If consent is obtained from a surrogate, the study participant will be asked to re-consent to study participation when they regain the ability to consent for themselves.

7.6 *Randomization and stratification*

For this Phase 2b trial, after informed consent is given, an assignment will be made by computer-generated randomization to administer either hMSCs therapy or placebo with a 1:1 allocation to the hMSCs:placebo arms. Randomization will occur during the 2 hour planned baseline stability period and must occur in time to allow the study team to initiate the study product infusion within 14 days of initial ICU admission. The study participant must have an arterial blood gas with a PaO_2/FiO_2 ratio < 250 with PEEP ≥ 5 cm H_2O within 6 hours of randomization.

Since oxygenation index is the primary endpoint, we would like to minimize imbalances in the hMSC and placebo groups in their initial levels of arterial hypoxemia. Therefore, the randomization plan at each site will be designed to randomize patients by those with a PaO_2/FiO_2 < 150 mmHg into equal numbers into the hMSC versus placebo arms. The randomization will also be stratified by the presence or absence of trauma.

7.7 *Early Withdrawal of Subjects*

7.7.1 When and How to Withdraw Subjects

Since the study treatment is a single infusion of study product, a subject is unlikely to be withdrawn by investigators due to safety concerns because there is only a single treatment to be administered. However, a surrogate or subject may withdraw consent for study participation either before, during or after study product administration. As described below, if a subject or surrogate withdraws consent for study participation after study product administration, we will work with them to allow collection of follow-up data to ensure safety.

7.7.2 Data Collection and Follow-up for Withdrawn Subjects

If a subject or surrogate withdraws consent for the study, we will discuss with the subject or surrogate the importance of complete data follow-up for the purposes of our safety analyses and determine if they are at least willing to participate in a follow up telephone call to ascertain survival.

8 CHEMISTRY, MANUFACTURING AND CONTROLS

Allogeneic, human bone marrow-derived mesenchymal stem cells (hMSCs) are isolated from fresh bone marrow of healthy donors procured by the AllCells, LLC (Alameda, CA). Following a density gradient separation, mononuclear cells containing a population of hMSCs are placed into culture. The cells are continued through a single passage. At this point, the hMSCs are harvested, washed in 5% human serum albumin (HSA), and cryopreserved using a controlled rate freezer [40% fetal bovine serum, 50% HSA (5%), and 10% DMSO] and stored at ≤ -150°C. hMSCs frozen at passage 1 are subsequently thawed and expanded through a second passage. Following harvest, wash, sampling for lot release testing, and cryopreservation in an optimized cryopreservation solution [CryoStor, BioLife Solutions, Bothell, WA; 10% DMSO final concentration], the final hMSC product is stored at ≤ -150°C for eventual shipment in a liquid nitrogen dry shipper to the clinical site for patient administration.

Immediately prior to administration, the product is thawed and diluted in 1:1 reconstitution media (1:1 mix of 5% human serum albumin and 10% Dextran 40). Additional reconstitution media is added to a final volume of 300 mL. The product is not further cultured or propagated prior to administration.

hMSCs Manufacturer:
University of Minnesota Medical Center (UMMC)
Clinical Cell Therapy Laboratory (AABB-accredited, FACT-accredited, CAP #18060-01, CLIA #24D0688128)
Molecular and Cellular Therapeutics (MCT)
1900 Fitch Avenue
Saint Paul, MN 55108

MCT Facility Description:
Cross reference is made to CBER Type V Drug Master File #12975.
See **Appendix A1** of CMC (Chemistry, Manufacturing and Controls) for cross-reference letter from MCT authorizing FDA to cross-reference DMF 12975 on behalf of this IND.

8.1 *Product Manufacturing Components*

8.1.1 Source Material Procurement

For hMSCs derived from allogeneic, unrelated donors, the source bone marrow is collected through AllCells (Alameda, CA).

Bone Marrow Procurement:

AllCells, LLC
1301 Harbor Bay Pkwy #200
Alameda, CA 94502

Allogeneic bone marrow will be harvested using standard bone marrow harvest procedures at AllCells.

The typical volume of bone marrow collected is approximately 100 mL. Cells are not mobilized or activated *in vivo*. Marrow is transported in a validated shipping container by a designated courier to the Clinical Cell Therapy Laboratory at the MCT facility, University of Minnesota.

8.1.2 Donor Screening

The standard health screen questionnaire and tests for donor infectious diseases are performed by AllCells on each marrow donor per 21 CFR 1271. Testing includes assays for the detection of:

- Antibody HIV-1, HIV-2, group O
- HBsAg
- Antibody HBcore

- Antibody HCV
- HIV-1 NAT
- HBV NAT
- HCV NAT
- Antibody HTLV-I/II
- Antibody T. pallidum (syphilis serology)
- Antibody CMV
- West Nile Virus NAT

Testing is performed by certified Clinical Laboratory Improvement Ammendment (CLIA) laboratory using FDA-approved/cleared test kits for donor infectious disease screening.

Additional testing and screening deemed necessary by regulations and/or institutional policy including screening for Zika Virus and Trypanosoma cruzi will be performed. Standard (universal) precautions are practiced throughout processing. Standard operating procedures for the prevention of cross-contamination have been established.

8.1.3 Reagents

See **Table 8.1-1** and **Table 8.1-2** for summary of reagents used. All reagents with the exception of human serum albumin (HSA), and dextran 40 are not approved by the FDA for infusion into humans. Information regarding the source, safety, and performance of these reagents can be found in the included sample certificates of analysis (**Appendix A2** of CMC).

Standard operating procedures are in place at University of Minnesota Medical Center (UMMC) to assure that no recalled lots of HSA are used in the production of the hMSC. HSA is FDA-approved and is only obtained from suppliers producing product derived from plasma collected in countries considered free of variant Creutzfeldt-Jakob disease (vCJD) risk.

Animal- or human-derived raw materials include fetal bovine serum (FBS) and human serum albumin (HSA). FBS is obtained from qualified herds from USDA inspected abattoirs located in the United States of America. HSA is FDA approved for human use.

Table 8.1-3 lists the excipients used to formulate the final frozen product. Prior to infusion of the hMSC product, the cells are thawed, diluted (dextran 40/5% HSA) and sent to the patient for administration.

Table 8.1-1 Summary of Animal/Human-Derived Reagents

Reagent/Excipient	Description	COA[1] Requirements	Supplier	Concentration in Final (Frozen) Product	Justification
Fetal bovine serum (FBS)	Sterile, nonpyrogenic	Viral (9CFR113.53) Mycoplasma, Endotoxin, Sterility	Hyclone	Minimal (washed)	Supplier Review, specifications, MD approved for use under IND
Human serum albumin (HSA), 5%	Sterile, nonpyrogenic	Sterility, Endotoxin, HBsAg, HIV-1/2 HCV	Grifols	Minimal (washed)	Supplier Review, specifications, FDA approved for human use

[1]COA (Certificate of Analysis): samples provided in **Appendix A2.**

Table 8.1-2 Summary of Reagents

Reagent	Description	COA[1] Requirements	Supplier	Concentration in Final (Frozen) Product	Justification
alpha-MEM	Sterile, nonpyrogenic	Osmolality, pH, Endotoxin, Sterility	Gibco/ Invitrogen	Minimal (washed)	Supplier Review, specifications, MD approved for use under IND
DPBS	Sterile, nonpyrogenic	Endotoxin, Sterility	Life Technologies	Minimal (washed)	Supplier Review, specifications, MD approved for use under IND
GlutaMax	Sterile, nonpyrogenic	Osmolality, pH, Sterility	Life Technologies	Minimal (washed)	Supplier Review, specifications, MD approved for use under IND
Hanks's Balanced Salt Solution	Sterile, nonpyrogenic	Osmolality, pH, Toxicity, Endotoxin, Sterility	Lonza	Minimal (washed)	Supplier Review, specifications, MD approved for use under IND
Ficoll Paque Premium	Sterile, nonpyrogenic	Density, osmolality,	GE-Healthcare	Minimal (washed)	Supplier Review, specifications,

Reagent	Description	COA[1] Requirements	Supplier	Concentration in Final (Frozen) Product	Justification
		endotoxin, sterility			MD approved for use under IND
TrypLE Select	Sterile, nonpyrogenic	Sterility, Endotoxin	Gibco/ Invitrogen	Minimal (washed)	Supplier Review, specifications, MD approved for use under IND
Human serum albumin (HSA), 5%	Sterile, nonpyrogenic	Sterility, Endotoxin, HBsAg, HIV-1/2 HCV	Grifols	Mininal (washed)	Supplier Review, specifications, FDA approved for human use

[1]COA (Certificate of Analysis): samples provided in **Appendix A2.**

Table 8.1-3 Summary of Excipients

Excipient	Description	COA[1] Requirements	Supplier	Concentration in Final (Frozen) Product	Justification
CryoStor10/CryoStor Buffer	Sterile, nonpyrogenic	Sterility, Endotoxin, Osmolality	BioLife Solutions	Primary excipient	Supplier Review, specifications, MD approved for use under IND
DMSO (additional amount added to CryoStor10 to attain final concentration of 10%)	Sterile, nonpyrogenic	Specific gravity, Sterility, Endotoxin	Bioniche Pharma	10%	Supplier Review, specifications, MD approved for use under IND
Human serum albumin (HSA), 5%	Sterile, nonpyrogenic	Sterility, Endotoxin, HBsAg, HIV-1/2 HCV	Grifols	Minimal (washed)	Supplier Review, specifications, FDA approved for human use

[1]COA (Certificate of Analysis): samples provided in **Appendix A2.**

8.1.4 Qualification Program

Studies performed during the validation demonstrated sufficient removal of media components. Measurement of bovine transferrin pre-wash and post-wash (prior to freezing) served as the marker for media component removal in the validation studies performed at UMMC.

8.2 *Product Manufacturing Procedures*

8.2.1 Cell Processing

A summary of the cell processing is provided in **Figure 8.2-1**.

Bone marrow aspirates from AllCells are tested for nucleated cells (NC), differential, viability, and sterility, and a sample is saved for cytogenetics testing should the final hMSCs product be found to have a chromosomal abnormality.

Enrichment of the mononuclear cell (MNC) fraction of the marrow is accomplished using an automated separation method involving ficoll hypaque density gradient medium, specific gravity 1.077 g/dl (Ficoll Paque Premium, GE Healthcare). A wash with Hank's Balanced Salt Solution (without phenol red, calcium or magnesium) follows.

Enriched samples are tested for NC, differential, viability, flow cytometry (CD105, CD73, CD90, CD45, CD34, CD14, CD19, HLA-DR).

For cell culture, cells are seeded at 1.0-1.5 x 10^5 viable cells/cm^2 at a media depth of 1.6 mm in an appropriately sized T-flask and placed in a 5% CO_2/37°C incubator. Media consists of αMEM, 16.5% fetal bovine serum (FBS) and GlutaMax (1% 200 mM). On days one and two, non-adherent cells are removed, and media is changed. Fresh media is exchanged every 2-4 days until 70-90% confluence.

Cells are washed with Dulbecco's phosphate buffered saline (DPBS) and detached. Cells are cryopreserved at passage one and are held at ≤ -150°C for subsequent expansion. hMSCs frozen at passage one are inoculated into a cell factory at 40-50 viable cells/cm^2. Media is exchanged every 2-4 days.

At 70-90% confluence, cells are washed, harvested and resuspended in CryoStor Buffer before they are cryopreserved (controlled rate) in freezer bags. This is performed by adding an equal volume of CryoStor10 supplemented with additional DMSO to attain 20% DMSO to the resuspended cells. The final formulation hMSC product will contain 10% DMSO in CryoStor Buffer.

The frozen hMSC final cellular product is shipped to the clinical site in a validated liquid nitrogen dry shipper with continuous temperature monitoring device to assure shipping temperature ≤ -150° C. On receipt, the hMSC cellular product is inspected and placed in a controlled, continuously monitored liquid nitrogen storage tank at the clinical site.

See flow diagram in **Figure 8.2-1** for summary of processing and testing.

Figure 8.2-1 Summary of hMSC Processing at UMMC Cell Therapy Laboratory

Process Step	Testing
Bone Marrow Aspirates from AllCells	Cell count, Differential, Viability, Sterility, Cytogenetics (hold sample)
↓	
MNC Enrichment Automated Density Gradient Separation	Cell count, Differential, Viability, Flow Cytometry (CD105, CD73, CD90, CD45, CD34, CD14, CD19, HLA-DR)
↓	
Culture	

Process Step	Testing
Media = MSC CM (16.5% FBS) Initial seeding density (passage 1) = 1.0E+05 VNC/cm^2; ↓ Cells cryopreserved after passage 1 ↓ Thaw single vial and seed for Passage 2 Passage 2 seeding density = 40 -50 VNC/cm^2	
↓	
Passage 2	
↓	
Harvest 70 – 90% Confluent with Tryple Select	**Final Product Release Testing:** Cell count, Differential, Viability (AO/PI), Sterility, Mycoplasma, Flow Cytometry (CD105, CD73, CD90, CD45, CD34, CD14, CD19, HLA-DR), Endotoxin, Cytogenetics (send T75flask), KGF (for information only)
↓	
Freeze 1 – 10E+06 NC/mL, CryoStor10, DMSO final (10%)	
↓	
Store frozen at 1-10 million cells/mL	
↓	
Ship frozen to clinical site	
↓	
On day of infusion: Thaw/dilute (dextran 40/5% HSA) and infuse at bedside	Sample for: Cell count, Viability

8.3 *Final Infusion Formulation*

On the day of infusion, the hMSC final cellular product is thawed, diluted with dextran 40/5% HSA and infused into the patient.

Samples will be taken for cell count and viability.

See **Appendix E** for the Study Product Preparation Protocol to be performed at the clinical sites.

8.4 *Product Testing*

8.4.1 In-process Testing and Criteria

The in-process testing performed is described in **Table 8.4-1**.

Table 8.4-1 In-process Testing

Test	Manufacturing Step Where Performed	Method	Criteria
Sterility (14-day)	Bone marrow aspirate	Bactec (UMMC Clinical Microbiology Laboratory)	No growth
Identity Immunophenotype	MNC Enrichment	UMMC Clinical Flow Cytometry Lab	Report: CD90+, CD105+ CD45+, HLA-DR+ CD73+, CD14+, CD19+, CD34+
Other: Cell count	Bone marrow aspirate; MNC Enrichment	UMMC Cell Therapy Lab	Report Results
Other: Viability	Bone marrow aspirates; MNC Enrichment	UMMC Cell Therapy Lab (AO/PI)	Report Results

8.4.2 Description of Assays

See **Section 8.5**.

8.5 *hMSC Final Cellular Product Release Criteria/Specifications*

The hMSCs final cellular product testing (see **Table 8.5-1**) and release will be performed prior to the hMSCs being transported to the clinical site for administration. All samples will be drawn from the pre-freeze final product.

Table 8.5-1 hMSCs Final Cellular Product Specifications

Test	Method	Criteria	Results Available Prior to Release
Sterility (14-day)	Bactec (UMMC Clinical Microbiology Laboratory)	No growth	yes
Mycoplasma	PCR; Apptec protocol	Negative	yes
Endotoxin	LAL, chromogenic	<5.0 EU/kg/dose	yes
Identity/Purity Immunophenotype	UMMC Clinical Flow Cytometry Lab	≥ 85.0%: CD90+, CD105+ ≤ 15.0%: CD45+, HLA-DR+ Report Results: CD73+, CD14+, CD19+, CD34+	yes
Potency	KGF Expression by ELISA	Report Results	no
Other: Karyotype	UMMC Clinical Cytogenetics Lab	No clinically significant abnormality	yes
Other: Cell count	Manual enumeration	Report Results	yes
Other: Viability	UMMC Cell Therapy Lab (AO/PI)	≥ 70%	yes

8.5.1 Description of Assays

An aliquot for release testing will be removed upon completion of the final product prior to freezing.

8.5.1.1 **Sterility**

Sterility testing will be performed using the Bactec System (Becton, Dickinson and Company, 1 Becton Drive, Franklin Lakes, NJ). Final sterility testing results will be available prior to release of the product for patient infusion. The lot release criterion will be 'no growth'.

8.5.1.2 **Mycoplasma**

Mycoplasma testing will be performed by a contract laboratory (AppTec) for the PCR based assay.

8.5.1.3 **Endotoxin**

Endotoxin testing is performed using the LAL chromogenic-based EndoSafe-PTS (Charles River Laboratories International, Inc., Wilmington, MA). Endotoxin value will be determined for each bag and will be included on the certificate of analysis. Clinical centers will determine total endotoxin level based upon cell dose/total bags needed. The lot release criterion will be <5.0 EU/kg/total product.

8.5.1.4 **Identity/Purity**

Analysis by flow cytometry will be used to characterize the cell types within the final product, as hMSCs have a unique phenotype signature. Positive markers include CD73 (report), CD90 (≥85%), and CD105 (≥85%); negative markers include CD14 (report), CD19 (report), CD34 (report), CD45 (≤15%), and HLA-DR (≤15%).

Identity will also be assured through established standard operating procedures outlining labeling and segregation of product practices (i.e., dedicated processing space and medical technologist) within the laboratory. Donor HLA type will be known, and this may serve as an additional means of identity.

8.5.1.5 **Potency**

KGF content will be used as the early stage potency assay. A sample of each lot will be lysed and the lysate tested for KGF content (ELISA, R& D system, Minneapolis, MN, Cat: DKG00).

Additional testing will be performed and analyzed to determine relevance as a potency assay. This will include immunophenotype/flow cytometry (see above) and G-banded karyotype analysis on 20 metaphase cells. The cytogenetic testing will be performed to rule out presence of any numerical or structural chromosomal abnormality. If one cell is abnormal, additional cells will be analyzed to rule out the presence of a clonal abnormality. Documented benign heritable heteromorphisms, such as 9ph, would not be considered as an abnormality. Pre-culture samples of marrow will be stored and available for cytogenetic analysis should such a comparison be needed.

8.5.1.6 **Viability**

Viability testing (AO/PI) will be performed on a sample drawn from the final product. A pre-freeze viability of ≥70% will serve as a lot release criterion. Viability by AO/PI will be used to freeze cells in a viable dosing strategy.

8.5.1.7 **Cell Count**

Cell number (i.e., NC count) will be determined on a sample of the hMSCs prior to cryopreservation using a manual enumeration method (hemacytometer) or automated hematology analyzer (Sysmex XS1000i, Lincolnshire, IL) as per standard operating procedure.

8.5.2 Final Cellular Product Certificate of Analysis

See **Appendix A4** of CMC for a sample of the certificate of analysis.

8.6 *Product Stability*

Following processing and successful lot release testing, frozen hMSCs will be transported in a validated container (liquid nitrogen dry shipper) with continuous temperature monitoring to the clinical site where the cells will be stored in validated, continuously monitored liquid nitrogen storage tanks. A small aliquot will be cryopreserved and stored for potential further testing. Limited post-thaw stability studies were performed to support use of the cells.

8.7 *Placebo product*

The placebo is treated with a reconstitution media (1:1 mix of 5% human serum albumin and 10% Dextran 40). Both are FDA approved for human use.

8.8 *Other Issues*

8.8.1 Product Tracking

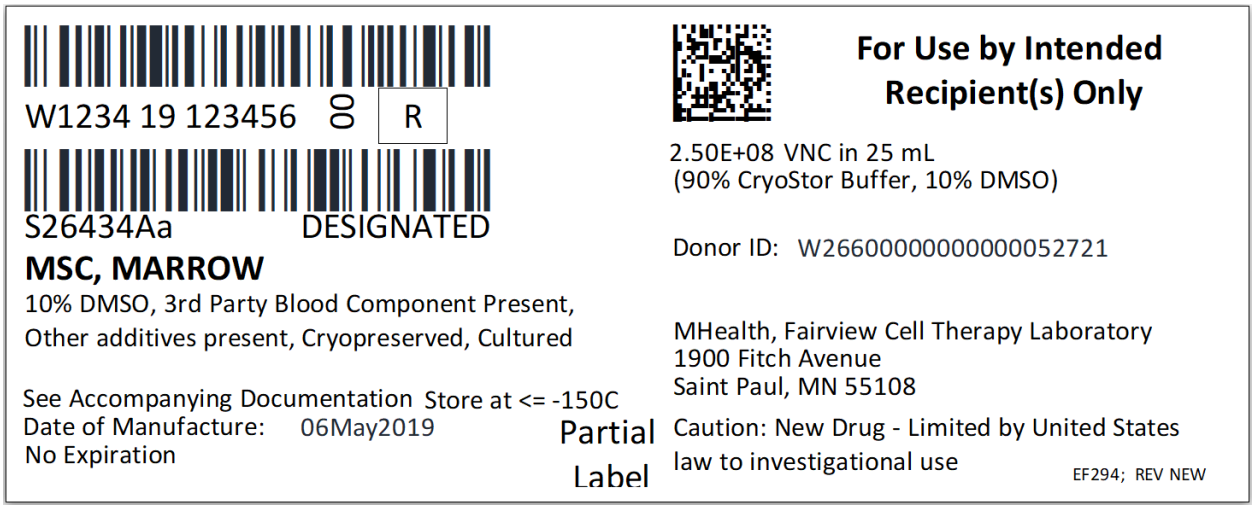
A standard operating procedure for product labeling per Foundation for the Accreditation of Cellular Therapies (FACT) standards has been established at UMMC. Labels with appropriate identifiers (unique identification number, etc.) will be provided to the collection staff. In-processing labels and final product labels will have these same identifiers to assure product identity throughout production and administration. Confirmation of identity of product and intended patient by two parties will take place prior to administration by review of product labeling and patient identification.

8.8.2 Labeling

Product labeling follows American Associatoin of Blood Banks (AABB) and FACT standards and includes unique patient identifiers, product name, storage conditions, and expiration date/time (if applicable), as well as collection and manufacture date/time, product volume, identification and address of processing lab.

Warning labels, as required per 1271.90 are applied for those donors determined to be ineligible. The label also contains the following statement: “Caution: New Drug – Limited by Federal Law to Investigational Use” per 21 CFR 312.6.

Sample Label



8.8.3 Container/Closure

Marrow will be collected and processed using syringes, anticoagulant, transfer bags, tubing sets, and vials and freezing bags (for samples and products being cryopreserved). Compatibility of all containers and sets with the product has been established with practice and validation runs.

8.8.4 Environmental Impact

The sponsor claims categorical exclusion [under 21 CFR 25.31(e)] for the study under this IND. To the sponsor's knowledge, no extraordinary circumstances exist.

8.8.5 Validation and Qualification of the Manufacturing Process

See Type V Drug Master File (#12975) describing the MCT Facility.

8.8.6 Cellular Product Preparation

Just prior to use, the cellular product will be thawed and diluted in in 1:1 reconstitution media (1:1 mix of 5% human serum albumin and 10% Dextran 40) for intravenous administration in the clinical site's clinical bone marrow transplant (BMT) facility (or equivalent) by facility technicians. See **Appendix E** for cellular product preparation instructions.

8.8.7 Placebo Product Preparation

The placebo will be the same volume of reconstitution media (1:1 mix of 5% human serum albumin and 10% Dextran 40). See **Appendix E** for study product preparation instructions.

8.9 *Subject Compliance Monitoring*

The study product will be administered as a single intravenous dose over 60-80 minutes within 14 days of initial ICU admission. Study personnel will be on site for safety and compliance monitoring for the 2 hour baseline stability period, as well as for 6 full hours starting from the time the study product infusion is initiated.

8.10 *Prior and Concomitant Therapy*

Rescue therapies for severe ARDS, including prone ventilation, corticosteroids, inhaled vasodilators, and neuromuscular blockade will be permitted. We will record whether these therapies were administered prior to or concomitantly with hMSCs. Patients on ECLS or HFOV will be excluded from this trial. Co-enrollment in randomized clinical trials of supportive therapies such as proning will be allowed after approval from the Scientific Review Committee (includes the Principal Investigator and two other study investigators) and the Chair of the DSMB. With respect to patients with SARS-CoV-2 infection, co-enrollment in randomized clinical trials of other interventions such as pharmacologic therapies and convalescent plasma will be considered on a trial by trial basis in concert with the Scientific Review Committee and the Chair of the DSMB. The use of treatments through Emergency Use Authorization (EUA) and Expanded Access Program (EAP) studies shall not be considered an exclusion. The consideration of co-enrollment with other experimental therapy trials that target patients requiring ICU level of care must be evaluated by the Scientific Review Committee and request approval from the Chair of the DSMB. Patients who are enrolled in clinical trials targeting outpatients or patients admitted to the acute care floor with trial endpoints assessed before admission to the Intensive Care Unit will be eligible for participation.

8.11 *Blinding of Cellular Product*

As in prior studies of hMSCs (122), the infusion bag will be covered with aluminum foil at the time of product preparation in the clinical site's bone marrow transplant laboratory so that the product is not visible to the investigators or to the clinicians who are administering the product. Personnel in the bone marrow transplant laboratory who will be preparing the study infusion will not be blinded to treatment allocation.

8.12 *Receiving, Storage, Dispensing and Return*

8.12.1 Receipt of Cellular Product

The hMSCs will be shipped using temperature monitored shippers to the clinical site's clinical bone marrow transplant laboratory (or equivalent) from UMMC. Upon receipt of the study cellular product, an inventory must be performed and a receipt log filled out and signed by the person accepting the shipment. Any damaged or unusable study product in a given shipment will be documented in the study files. The investigator must notify the study sponsor of any damaged or unusable study treatments that were supplied to the investigator's site.

8.12.2 Storage

The hMSCs will be stored under controlled conditions in liquid nitrogen tanks in the clinical bone marrow transplant laboratory.

8.12.3 Dispensing of Cellular Product

The study product will be administered as a single dose following randomization; the clinical bone marrow transplant laboratory will be responsible for preparing the cellular product or placebo and for dispensing the product to study investigators for administration (**Appendix E**).

The study log for each patient will include treatment assignment, assigned dose of hMSCs, lot and bag number for each frozen bag of cellular product used.

8.12.4 Return or Destruction of Cellular Product

At the completion of the study, there will be a final reconciliation of cellular product shipped, cellular product consumed, and cellular product remaining. This reconciliation will be logged on the cellular product log, signed and dated. Any discrepancies noted will be investigated, resolved, and documented prior to return or destruction of unused study cell product. Cellular product destroyed on site will be documented in the study files.

APPENDICES FOR CMC

A1. Cross reference authorization letter: UMMC DMF 12975

A2. Sample certificates of analysis for raw materials/reagents

A3. Study Product Preparation Protocol for the Clinical Site

A4. hMSCs product certificate of analysis

9 Study Procedures

See **Appendix A** for the Time-Events Schedule.

9.1 *Pre-Screening*

All patients in the Intensive Care Unit will be screened for potential study eligibility by trained study personnel under a waiver of consent for screening purposes approved by the Institutional Review Board. When eligible study subjects are identified, the attending physician caring for the patient will be asked if we have permission to approach the patient or their surrogate for potential study participation.

9.2 *Informed Consent and Randomization*

Informed consent will be obtained from each surrogate before enrollment in the trial. No study procedures will be conducted before obtaining informed consent. Randomization will occur after informed consent as described in **Sections 7.5 and 7.6**. An arterial blood gas must be obtained to confirm a $PaO_2/FiO_2 < 250$ mmHg on PEEP ≥ 5 cm H_2O within 6 hours of randomization. The randomization plan at each site will be designed to randomize patients by those with a $PaO_2/FiO_2 < 150$ mmHg and by those with trauma into equal numbers into the hMSCs versus placebo arms in order to minimize the possibility of imbalance at baseline.

If a pregnancy test is not available before informed consent, a blood or urine pregnancy test will be obtained in women of childbearing age (50 or younger) after informed consent but before

randomization to ensure eligibility. Patients excluded on the basis of tests obtained in this manner will not be included in the as-treated population.

9.3 *Baseline Study Procedures*

If not obtained within the past 24 hours as part of clinical care, patients will have serum creatinine, platelet count, total bilirubin and alanine aminotransferase (ALT) measured before the infusion of the study product. Blood and urine samples for biomarker measurements will be obtained before the infusion of study product as well.

We will measure pulmonary dead space at baseline, as well as on days 1, 2, 3 and 7. This is a non-invasive test that measures exhaled carbon dioxide from the endotracheal tube.

9.4 *Investigational Product Administration*

The investigational product will be administered intravenously according to the following dose schedule. The investigational product (h MSCs or placebo) will be administered intravenously over approximately 60-80 minutes.

We will enroll 120 patients in a 1:1 blinded, randomized, placebo-controlled design with the dose of 10 x 10^6 viable cells/kg (60 patients treated with hMSCs and 60 controls), with viability determined at the time of cell preparation at UMMC. Safety analyses will be conducted by the independent DSMB, and the sponsor and investigators will remain blinded. The safety reviews will be conducted after treatment of 60 patients, and the trial will continue during these analyses.

Critically ill patients often experience minute-to-minute changes in vital signs. Investigators will begin the infusion of hMSCs after a stable baseline for 2 hours has been observed.

A. Stable baseline will be defined as:

    a. Transcutaneous oxygen saturation in the target range of 88-95% without any increase in ventilator settings **AND**
    b. Stable vasopressor use if the patient requires vasopressors for blood pressure support. The dose of vasopressor may be increased a small amount during this 2-hour period, predefined as: no more than a 5 mcg/min increase in norepinephrine dose; no more than a 50 mcg/min increase in phenylephrine dose; no more than a 5 mcg/kg/min increase in dopamine dose; and no more than a 0.05 mcg/kg/min increase in epinephrine dose.

B. The following patients will be considered clinically unstable and will **NOT** receive the study product:

    a. Patients 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%
    OR
    b. Patients who require 3 vasopressors for blood pressure support and/or the use of > 0.1 mcg/kg/min of epinephrine for blood pressure support,

OR

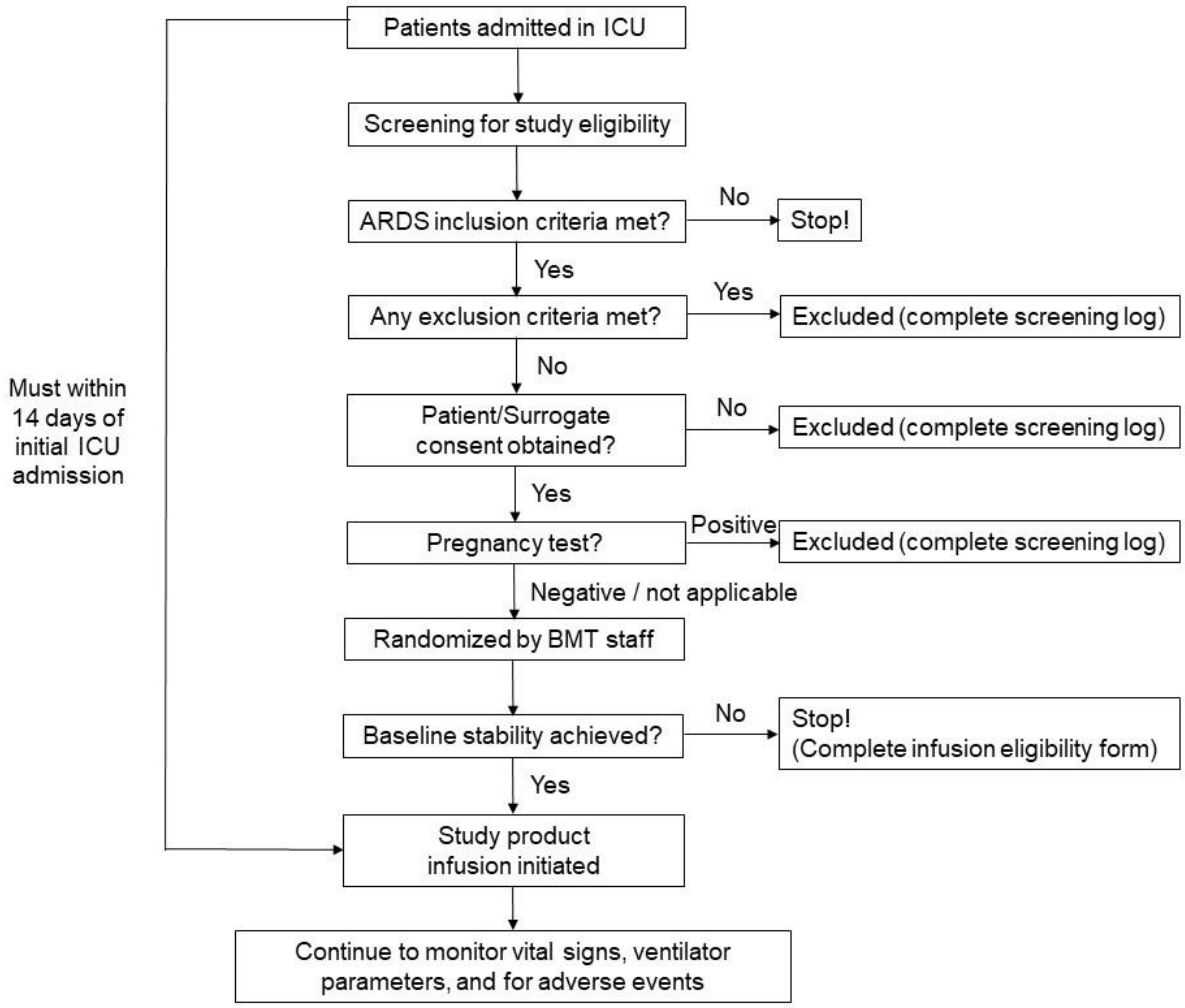
c. Patients who do not meet stability criteria in the supine or prone position,
OR
d. Patients who have evidence of ongoing bleeding by the requirement of more than 3 units of packed red blood cells during the prior 24 hours prior to randomization.

C. Patients who are clinically unstable will be monitored closely; if they achieve a stable baseline within 14 days of meeting the entry criteria for the study and, at the time of the stable baseline, still meet criteria for ARDS ($PaO_2/FiO_2 < 250$ with bilateral infiltrates and no evidence of left atrial hypertension) with a PEEP of ≥ 5, they can receive the study product if the study product can be initiated within 14 days of initial ICU admission.

D. To confirm study product eligibility, an arterial blood gas will be obtained within 90 minutes prior to beginning the baseline stability period. Patients will not receive the study product infusion if the PaO_2/FiO_2 ratio is equal or greater than 250 mmHg on this pre-infusion arterial blood gas. Subjects who do not initiate the infusion of study product (e.g., not obtain 2 hours of stable baseline, increased PaO2/FiO2 ≥250, study withdrawn, et al.) will be excluded from patient accruals and from the data analyses. They will not be included in an intention to treat analysis. Arterial blood gases will also be obtained at the end of the study product infusion (~1 hour) and at 6, 12, 18, 24, 30 and 36 hours (+/- 1 hour for all timepoints) after the initiation of study product infusion.

E. To summarize the timelines for eligibility for the trial, see these key criteria and the timeline:

- Eligibility for the trial - ARDS develops less than 72 hours since first meeting ARDS criteria per the Berlin definition of ARDS.
- Eligibility for the trial - Must begin to administer study product within 14 days of initial ICU admission
- Eligibility - The PaO_2/FiO_2 remains less than 250 mmHg after consent obtained and within 90 minutes prior to beginning the baseline stability period.



F. During infusion of study product, patients will have continuous monitoring of arterial blood pressure, heart rate, rhythm, and 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. Patients will be monitored closely for other signs of transfusion reaction, e.g., rash, urticaria or wheezing. If there are any signs of a transfusion reaction, the infusion of study product will be stopped immediately. Similarly, if a patient has a pre-specified infusion associated event, the infusion will be stopped. The infusion can also be stopped at the discretion of the study investigator if there is any concern about the patient's status.

9.5 *Ventilator Management*

Ventilator management, including weaning, will follow the modified ARDS Network lower tidal volume (6 mL/kg PBW) protocol (**Appendix D**) which will require assist volume control (not

pressure control) as the mode of ventilation. Using this ventilator management protocol will standardize the application of PEEP, which is a component of the primary endpoint, oxygenation index, thus reducing the potential for bias. If not already being used, this low tidal volume protocol for mechanical ventilation must be instituted with assist control mode (not pressure control or any other ventilator modality) within one hour of randomization. Because recent evidence-based consensus recommendations have identified a best practice for weaning, weaning strategy will also be controlled by protocol rules in accordance with these evidence-based recommendations. This will assure similar weaning methods and provide potential benefit to both study groups. This newer weaning strategy is a simplified version of the weaning strategy protocol used in ARDS Network studies (see **Appendix D**).

9.6 *Concomitant Therapy*

We will record the use of other pharmacologic agents that are sometimes used by clinicians in the treatment of ARDS, including glucocorticoids (dexamethasone or equivalent steroids therapy), and inhaled vasodilators or neuromuscular blockade, anticoagulant and antiplatelet treatment, immunomodulatory agents and any COVID-19 related therapies. We will also record if patients are in a prone position, receive recruitment maneuvers or extracorporeal membrane oxygenation (ECMO), or if patients are enrolled in other experimental therapy trials or treatment.

9.7 *Radiographic Assessment of Lung Edema (RALE) Score*

Radiographic Assessment of Lung Edema (RALE) score (128) will be used to evaluate the extent and density of alveolar opacities on chest radiographs at baseline, and days 1, 2, 3 and 7. To determine the RALE score, each radiograph will be divided into quadrants, defined vertically by the vertebral column and horizontally by the first branch of the left main bronchus. Each quadrant will be assigned a consolidation score from 0 to 4 to quantify the extent of alveolar opacities, based on the percentage of the quadrant with opacification and a density score from 1 to 3 to quantify the overall density of alveolar opacities, unless the consolidation score for that quadrant is 0. The density score allows for more quantitative assessment of the density of opacification by quadrant (see Appendix L). We will obtain the de-identified chest x-ray graphs at baseline and on days 1, 2, 3, 7, and evaluate the RALE scores. We will concurrently systematically evaluate for and record evidence of barotrauma including pneumothorax, pneumomediastinum, subcutaneous emphysema, and the presence of tube thoracostomy on the same set of radiographs.

9.8 *Biospecimens for Biological Endpoint Measurements*

Blood samples will be obtained at 6 timepoints (before the infusion of study product, and then at 6 hours, 12 hours, 24 hours, and on days 2, 3 after the initiation of the study product infusion) for biomarker measurements, which include measurements of epithelial injury, inflammation and hMSC activity. Plasma obtained from two 6.5 ml EDTA anti-coagulated blood samples will be divided immediately after centrifugation into 0.5 mL and 1 mL aliquots and frozen at –80°C.

Urine samples from the patients will be obtained at 3 timepoints (before the infusion of study product, then at 24 hours and on day 2 after the initiation of study product infusion). Before the infusion of study product, urine will be collected in a 40 ml sterile cup; 30 mL will be frozen immediately, and 8 mL will be centrifuged prior to dividing into 4 equal aliquots and freezing at

–80°C. For 24 hours and day 2, only 8 mL of urine samples will be collected and centrifuged and divided into 4 equal aliquots and freezing at –80°C.

A mini-BAL will be performed 2 days (48 hours +/- 4 hours) after the initiation of the study product infusion (See **Appendix A**, Time-Events Schedule) for total protein measurement, a marker of lung epithelial permeability, as well as the same biomarkers measured in the plasma.

Finally, 5 mL of blood specimen prior to study product infusion and 5 mL of blood specimen on day 2 will be collected in Paxgene tubes for future RNA and DNA extraction.

Biospecimens collected at 6 hours, 12 hours, and 24 hours after the initiation of study product infusion can be obtained +/- 1 hour of the indicated timepoint. Day 2 biospecimens will be collected at 48 hours (+/- 4 hours) after the initiation of study product infusion and day 3 biospecimens can be collected anytime.

9.9 *Days 1, 2, and 3 Measurements*

If not obtained as part of clinical care, patients will have platelets, serum creatinine, total bilirubin, and alanine aminotransferase (ALT) measured on day 3 (+/- 1 day) after administration of the study product.

Arterial blood gases will be obtained at baseline and at hours 6, 12, 18, 24, 30, and 36 (+/- 1 hour for all timepoints) after the initiation of the study product infusion in order to assess the primary endpoint of oxygenation index. Subsequently, arterial blood gases will be obtained on days 2 (48 hours +/- 4 hours) and day 3 (anytime). In the unusual circumstance that a patient does not have an arterial line for ABG monitoring on day 3, the ABG will not be obtained for study purposes alone. If an arterial blood gas is collected +/- 1 hour of an indicated timepoint for clinical purposes, another arterial blood gas does not need to be collected for research.

9.10 *Day 7 Measurements*

Arterial blood gases will be obtained anytime on days 7 if patient still has arterial line. If not obtained within the past 24 hours as part of clinical care, patients will have platelets, serum creatinine, total bilirubin and alanine aminotransferase (ALT) measured on day 7 (+/- 1 day) after administration of the study product if they are still hospitalized.

9.11 *Day 28 Measurements*

Patients will be followed daily for adverse events through day 28 after study product administration, death or hospital discharge, whichever occurs first. If a patient is discharged from hospital before day 28, investigators will follow-up by phone interview with the patient, family member or caregiver after day 28 to ensure that no adverse events have occurred through day 28. Mechanical ventilation history (on or off ventilator support), ICU history and the need for dialysis through day 28 after study product administration will also be collected. Decisions about hospital discharge will not be made by study personnel; rather such decisions will be made by clinicians caring for the study subject based on the underlying condition that led to the hospitalization. Also, we will assess neurologic status by the 6 point Glasgow Outcome Score at hospital discharge.

9.12 *Day 60 Measurements*

Vital status and the need for dialysis will be collected at day 60 after study product infusion. If patients are discharged from the hospital prior to day 60, we will collect this data through telephone interviews with care-givers at the outside facility, or family members, or the patient.

9.13 *Post-hospitalization Follow-up*

Hospital mortality and discharge location will be collected if patient is discharged from hospital alive after study enrollment. Also, vital status at 6 month after study enrollment will be collected via a structured telephone interview.

10 Data Collection

10.1 *Medical History*

To be collected from patient charts and patient/surrogate, where applicable.

1. Demographic and admission data
2. Pertinent medical history with comorbidities and physical examination
3. Height, gender, measured body weight, calculated predicted body weight
4. Pre-hospital times and interventions
5. Time on ventilator prior to enrollment
6. High flow nasal oxygen (HFNO) and non-invasive ventilation (NIV) use for the 3 calendar days prior to meeting ARDS criteria
7. Mechanisms of Injury if the primary cause of ARDS due to trauma:
    a. Abbreviated injury score by region
    b. Injury severity score
    c. Presence of hypotension on admission (SBP < 90 within 24 hours of admission)
    d. Need for blood transfusion, as well as volume of blood and components transfused during the 1st 24 hours after injury.
    e. Volume of crystalloid resuscitation transfused during the 1st 24 hours after injury.
    f. Procedures performed prior to randomization
    g. Intubated on arrival or time to intubation after admission
    h. Base deficit (lowest level in each 24 hour period)
8. Acute or chronic renal failure and use of dialysis
9. History of lung disease
10. History of solid organ transplant
11. History of bone marrow transplant
12. COVID-19 vaccination status prior to enrollment (number, type, and date of doses)
13. Etiology of ARDS
14. Outpatient medications

10.2 *Baseline Assessments*

The following information will be recorded during the 24-hour interval preceding randomization. If more than one value is available for this 24-hour period, the value closest to the time of randomization will be recorded. If no values are available from the 24 hours prior to randomization, then values will be measured post randomization but prior to initiation of study drug.

10.2.1 To Be Collected From Patient Charts

1. APACHE III Score
2. Vital signs: Heart rate, systolic and diastolic blood pressure, body temperature, mean arterial pressure, central venous pressure (if available)
3. Ventilator mode, tidal volume, FiO_2 and PEEP, inspiratory plateau pressure, and mean airway pressures. If on a pressure-targeted mode, peak pressure during inspiration will be assumed to be the plateau pressure
4. Arterial PaO_2, $PaCO_2$, pH and transcutaneous oxygen saturation and base deficit
5. Date and time of all creatinine determinations in the 96 hours prior to enrollment.
6. Frontal chest radiograph – radiographic lung injury score (# of quadrants)
7. Vasopressors or inotropes (epinephrine, norepinephrine, phenylephrine, vasopressin, dopamine > 5 μg/kg/min, dobutamine, phosphodiesterase inhibitors)
8. Suspected or known site of infection
9. Baseline platelet count and kidney/liver function tests: creatinine, total bilirubin, alanine aminotransferase. Make laboratory measurements as specified in Study Procedures **Section 9.3** if not available from testing obtained as part of clinical care
10. Baseline assessments must include all data needed for the 4-point acute lung injury score, SOFA score, Ordinal Scale of Clinical Assessment (OSCI) and trauma registry index (if applicable)
11. COVID-19 test results (if applicable)

10.3 *Assessment after Enrollment: Determination of Stable Baseline for Study Product Administration and Monitoring During and After Infusion Protocol*

The following parameters will be measured and recorded every 15 minutes for the two hour period used to establish the stable baseline prior to the study product infusion:

1. Respiratory: FiO_2, PEEP, transcutaneous oxygen saturation; additional ventilator parameters will be recorded if clinically available.
2. Cardiovascular: Heart rate, systolic and diastolic blood pressure, vasopressor doses.

The same clinical parameters will be recorded every 15 minutes for the duration of the infusion and every hour for the next 5 hours.

An arterial blood gas will be obtained within 90 minutes prior to beginning the baseline stability period, at the end of the study product infusion (~1 hour), and 6 hours (+/- 1 hour) after the initiation of the study product infusion; additional blood gases will be recorded at 12, 18, 24, 30

and 36 hours (+/- 1 hour for all timepoints) after the initiation of the study product infusion. If an arterial blood gas is collected +/- 1 hour of an indicated timepoint for clinical purposes, another arterial blood gas does not need to be collected for research.

10.4 *Assessment after Enrollment: Reference Measurements*

The following data will provide the basis for assessing protocol compliance and safety as well as between-group differences in several efficacy variables. Data for each of the variables will be recorded on the days shown in the Time-Events Schedule (**Appendix A**) or until death, discharge from the ICU, or unassisted ventilation for 48 hours.

10.4.1 Reference Measurements (to be collected from patient charts)

The following parameters will be measured and recorded at the time of Randomization as well as on subsequent dates using values closest in time to 8:00 A.M. on the days specified in the Time-Events Schedule (**Appendix A**). The following conditions will be ensured prior to measurements: no endobronchial suctioning for 10 minutes; no invasive procedures or ventilator changes for 30 minutes. All vascular pressures will be zero-referenced to the mid-axillary line..

1. If receiving assisted ventilation, record daily up to day 7:
    a. Tidal volume, FiO_2, PEEP, inspiratory plateau pressure, and mean airway pressures
    b. Pressure during inspiration if on a pressure targeted mode (PSV, PCV, etc)
    c. Arterial PaO_2, $PaCO_2$, pH and transcutaneous oxygen saturation
2. Fluid intake and output
3. Vital signs: Heart rate, systolic and diastolic blood pressure, body temperature, CVP
4. Modified Brussels Score data on days 0 through 14
    a. Vasopressor use (Y/N), lowest systolic blood pressure, creatinine, bilirubin, and platelet count for the day
5. The date and value of the highest creatinine between days 15 and 28
6. Safety laboratory studies: all creatinine and bilirubin measurements will be recorded as above. Creatinine, bilirubin and ALT will be measured and recorded as safety labs on day 7 if not obtained as part of clinical care.
7. Data regarding infections, including:
    a. Surgical site and wound infections by CDC criteria
    b. Ventilator associated or nosocomial pneumonia as diagnosed by CDC criteria
    c. Culture-proven bacteremia
    d. SARS-CoV-2 virus infection
8. Blood product transfusions
9. On-study procedures and surgical interventions
10. Co-enrollment in randomized clinical trials of other interventions and treatments related to SARS-CoV-2 infection
11. Inpatient medications (selected)
12. Must include all data needed for the 4-point acute lung injury score (including frontal chest radiograph), SOFA score, OSCI score and trauma registry index (if applicable)
13. D-dimer, lymphocyte count, neutrophil count, fibrinogen, ferritin and serum C-reactive protein (if applicable)

10.4.2 Specimen Collection

1. Date and time of specimen collection
2. Time of specimen processing and storage

10.5 *Endpoint Determinations*

1. Vital status at 60 days
2. Brussels Organ dysfunction failures at days 0-14
3. SOFA score at 3 and 7 days
4. Oxygenation index (PaO_2/FiO_2 and mean airway pressure) at 6, 12, 18, 24, 30 and 36 hours after the initiation of study product infusion as well as on days 2, 3, and 7 if still being ventilated with positive pressure (cannot be calculated if patient is on pressure support, as the mean airway pressure is not reliable)
5. Ventilatory ratio at 6, 12, 18, 24, 30, 36 hours after the initiation of study product infusion, and on days 2, 3, 7
6. Incidence of barotrauma on days 1, 2, 3 and 7
7. Time when patient achieves pressure support ventilation with 5 cmH_20 and 5 cmH_20 positive end-expiratory pressure for 2 hours
8. Time of initiation of unassisted breathing (assuming a patient achieves 48 consecutive hours of unassisted breathing)
9. Need for re-instituting assisted or mechanical ventilation after achieving 48 consecutive hours of unassisted breathing
10. Status 48 hours after initiation of unassisted breathing
11. Need for, timing, and duration of dialysis; change in renal function as measured by creatinine
12. ICU length of stay in calendar days including ICU days after readmission to ICU
13. Hospital length of stay in calendar days
14. Discharge diagnoses and discharge disposition (home, other facility, with or without assisted ventilation)
15. WHO Ordinal Scale of Clinical Assessment at days 7, 14 and 28

10.6 *Assessments After Hospitalization*

A health status questionnaire will be conducted at 28 days, 60 days and 6 months through telephone interviews.

11 Statistical Plan

The Statistical Analysis Plan has been written along with this Protocol and provides the details of the statistical methodologies applied to this Phase 2b trial.

11.1 *Sample Size Determination*

The sample size for this trial will be 60 patients in the hMSCs arm and 60 patients in the placebo arm, for a total of 120 patients with a balanced randomization of patients with PaO_2:FiO_2 <150 mmHg and patients with trauma. This sample size was determined on the basis of feasibility.

Given the sample size of 60 per treatment condition, in this multisite, randomized trial using oxygenation index as the primary outcome and measures taken at 6 time points, it is estimated the study will have 80% power to detect a difference between conditions for an effect size as low as 0.43. Using the pilot data from Day 4, for example, this would translate to approximately a difference of 1.29 in the oxygenation index.

11.2 *Statistical Methods for Primary and Secondary Endpoints*

Descriptive summary statistics (e.g. means and standard deviations, or median with inter-quartile, as appropriate) will be used to summarize the primary and secondary endpoints by study treatment. The primary and secondary efficacy endpoints will also be analyzed using appropriate models.

The primary endpoint, change in the oxygenation index over time, will be compared between treatment conditions using a linear mixed-effects regression model. The model will be estimated using likelihood estimation which will allow the inclusion of all collected data, regardless of the number of assessments available for assessment. Terms in the model will include the two stratification variables, treatment condition (active or placebo), time of assessment, condition-by-time, recruitment site and a site-by-treatment interaction term. A test of the treatment condition will directly test the main research question. Covariates identified by preliminary analysis as potentially confounding due to baseline differences will be added to the model.

The data analysis plan for secondary efficacy and safety endpoints has been provided in the Statistical Analysis Plan.

The primary and secondary endpoints (including clinical and biological data) will be adjusted for COVID-19 infection as a cause of ARDS, which is adjudicated by the study physicians.

11.3 *Subject Population(s) for Analysis*

A total of three populations will be used for all summaries and analyses. Subjects who have satisfied the population criteria will be classified in the designated population and will only be included in analyses for which they have available data.

Intent-to-Treat (ITT) Population
The primary analysis for this trial will be an intention-to-treat (ITT) analysis based on randomization to either hMSCs or placebo.

Since the study treatment is a single infusion of study product, a subject is unlikely to be withdrawn by investigators due to safety concerns because there is only a single treatment to be administered. However, a surrogate or subject may withdraw consent for study participation either before, during

or after study product administration. For subjects who are withdrawn prior to the initiation of study product infusion, they will not be included in ITT population. For subjects who are withdrawn during or after study product administration, they will be included in ITT population.

The ITT population will be used to present primary efficacy endpoint and secondary efficacy endpoints by allocated randomization. Subjects will be summarized according to the treatment to which they were randomized, regardless of which treatment they actually received.

Per Protocol Population

It is possible that there will be a number of deviations from the trial protocol (e.g. non-compliance with the allocated intervention, non-adherence to other elements of the protocol, et al.) The Per Protocol (PP) population is defined as all patients who will complete the study without major protocol deviations. The PP population will be fixed following clinical review of all protocol deviations at the end of the study and prior to unblinding and data locking. Due to the blinding feature of the study, the analyses of endpoints using PP population will not feasible in the interim report to DSMB. However, as a sensitivity analysis, the analysis of efficacy and safety endpoints using PP population will be included in the final study report.

Safety Population

The Safety population will be used for the analysis of safety, including adverse events, toxicity and safety laboratory evaluation. In this study, the Safety population is defined as all randomized who started the study product administration, regardless of infusion completion.

Due to the blinding feature of the trial, the safety and adverse events will be summarized using ITT population in the interim report for 60 patients to the DSMB. However, in the final report, the safety summaries and adverse events listings will be grouped by actual treatment received.

12 Safety and Adverse Events

12.1 *Definitions*

Investigators will determine daily if any clinical adverse experiences occur during the period from enrollment through study day 28 after investigational product administration. The investigator will evaluate any changes in laboratory values or physical signs, and will determine if the change is clinically important and different from what is expected in the course of treatment of patients with ARDS.

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

Any clinically important untoward medical occurrence in a patient receiving study product which is different from what is expected in the clinical course of a patient with ARDS, or:

1. Any clinically important, untoward medical occurrence that is thought to be associated with the study product, regardless of the "expectedness" of the event for a patient with ALI, or
2. The following pre-specified infusion associated events occurring within 6 hours from the start of study product will always be reported as adverse events:

- If on a vasopressor, an increase in vasopressor dose greater than or equal to the following:
  - Norepinephrine: 10 mcg/min
  - Phenylephrine: 100 mcg/min
  - Dopamine: 10 mcg/kg/min
  - Epinephrine: 0.1 mcg/kg/min
  - Addition of a third vasopressor
- New cardiac arrhythmia requiring cardioversion OR ventricular tachycardia, ventricular fibrillation or asystole
- Hypoxemia requiring an increase in FiO_2 of 0.2 or more and an increase in PEEP of 5 cmH_20 or more to maintain Sp02 in the target range of 88-95% that is not related to respiratory care/suctioning or ventilator dyssynchrony and persists for more than 30 minutes
- Clinical scenario consistent with transfusion incompatibility or transfusion-related infection (e.g., urticaria, new bronchospasm)

3. Any cardiac arrest or death that 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 that occurs during the study period will be a reportable event that will be reviewed in detail.

A ***serious adverse event*** is any event that is fatal or immediately life threatening, is permanently disabling, or severely incapacitating, or requires or prolongs inpatient hospitalization. Important medical events that may not result in death, be life threatening, or require hospitalization may be considered serious adverse events 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 above.

1. 'Life-threatening' means that the patient was, in the view of the investigator, at immediate risk of death from the reaction as it occurred. This definition does not include a reaction that, had it occurred in a more serious form, might have caused death. Assessment of the cause of the event has no bearing on the assessment of the event's severity.
2. Adverse events will be considered to be study-related if the event follows a reasonable temporal sequence from a study procedure and could readily have been produced by the study procedure.

All adverse events that do not meet any of the criteria for serious should be regarded as ***non-serious adverse events***.

An ***unanticipated problem (UP)*** is any incident, experience, or outcome that meets all of the following criteria (129):

- Unexpected, in terms of nature, severity, or frequency, given the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document, as well as the characteristics of the subject population being studied;
- Related or possibly related to participation in the research. In this guidance document, possibly related means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research;

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

Adverse Event Reporting Period
Reportable adverse events that occur during the 28 days following study product administration must be reported to the Investigator-Sponsor. Patients who are discharged home or outside facilities prior to study day 28 will be contacted after day 28 to determine vital status and for the occurrence of adverse events.

Prolonged Hospitalization or Surgery
All study subjects will be hospitalized at the time of study enrollment, by virtue of the disease being studied. Any adverse event that results in prolonged hospitalization should be documented and reported as a serious adverse event unless specifically instructed otherwise in this protocol.

12.2 *Recording of Adverse Events*

Assuring patient safety is an essential component of this protocol. Each participating investigator has primary responsibility for the safety of the individual participants under his or her care. The Principal Investigator at each study site will evaluate all clinically important adverse events. The Study Coordinator must view patient records for possible clinically important adverse events throughout the study period.

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 is 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., $Sp0_2$ ~85%), related to positioning or suctioning. This latter event would not be considered unexpected by nature, severity or frequency.

Organ failures related to ARDS or the patient's underlying condition that are systematically captured by the protocol 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 reported in the patient's case report form. 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. Serious adverse events that are still ongoing at the end of the study period must be followed to determine the final outcome. Any serious adverse event 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 12.3**).

12.3 *Reporting of Serious Adverse Events and Unanticipated Problems*

12.3.1 Investigators' Reporting Requirements to the Sponsor

Investigators will report all ***serious AND unexpected, AND study-related*** adverse events to the study sponsor (who will run the Clinical Coordinating Center for this study) 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 investigator will then submit a detailed written report to the Clinical Coordinating Center located at University of California San Francisco.

In this trial, Vanderbilt University IRB is the central IRB to support all participating sites. The Vanderbilt IRB must also be notified in a timely manner by the Clinical Coordinating Center, as per the IRB's requirement. The Principal Investigator will then submit a detailed written report to the study sponsor no later than 5 calendar days after the investigator discovers the event. The investigator will also submit a detailed written report to the local IRB (if this is required by local IRB).

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

- Study identifier
- Study Center
- Subject number
- A description of the event
- Date of onset
- Current status
- 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

12.3.2 Sponsor Reporting: Notifying the FDA

The study sponsor is required to report certain study events in an expedited fashion to the FDA. These written notifications of adverse events are referred to as IND safety reports. The following describes the safety reporting requirements by timeline for reporting and associated type of event:

- ***Within 7 calendar days***
  Any study event that is:
  - associated with the use of the study drug, and
  - unexpected, and
  - fatal or life-threatening

- ***Within 15 calendar days***
  Any study event that is:
  - associated with the use of the study drug, and
  - unexpected, and

- serious, but not fatal or life-threatening
  -or-
- a previous adverse event that was not initially deemed reportable but is later found to fit the criteria for reporting (reporting within 15 calendar days from when event was deemed reportable)

Any finding from tests in laboratory animals that:

- suggest a significant risk for human subjects including reports of mutagenicity, teratogenicity, or carcinogenicity.

Additional IND Reporting Requirements:
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.

12.3.3 Sponsor Reporting: Notifying the DSMB

All pre-specified clinically important events and unexpected serious adverse events, including death, will be reported to the DSMB on an ongoing basis. 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.

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 and submit to the central IRB at Vanderbilt University.

12.4 *Unblinding Procedures*

The DSMB will make decisions about the need for unblinding. If unblinding the study therapy is necessary to ensure a subject's safety, this will be done by the DSMB 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.

12.5 *Stopping Rules*

The DSMB will review post-infusion safety data after 60 subjects have been enrolled and received study product; however, enrollment will not be suspended pending DSMB review. The study will be stopped for a safety evaluation by the DSMB if they have any concerns or if three subjects have

pre-specified clinically important events or unexpected serious adverse events EXCEPT death since death will be common in this critically ill population due the nature of the underlying illness (e.g., ARDS).

The study will end when 120 patients have been recruited and received the study product and have all completed 60-day follow-up, after which data analysis will commence. This Phase 2b study will stop prior to completion if mandated by the Department of Defense-appointed DSMB or the FDA due to safety concerns.

12.6 *Medical Monitoring*

It is the responsibility of the Principal Site Investigator at each of the 7 hospital sites to oversee the safety of the study at his/her site. This safety monitoring will include careful assessment and appropriate reporting of adverse events as noted above, as well as the construction and implementation of a site data and safety-monitoring plan. Medical monitoring will include a regular assessment of the number and type of serious adverse events. The study will have an independent DSMB to oversee serious adverse events.

12.6.1 Independent Data and Safety Monitoring Board

This Phase 2b study will be monitored by an independent DSMB. The DSMB will contain 3 members. There will be 2 intensivists familiar with the care of critically ill patients and ARDS, at least one of whom will be familiar with the care of critically ill patients who have suffered trauma. There will also be a biostatistician. A DSMB Charter will define the DSMB's responsibilities as described below.

The DSMB will meet by teleconference at the planned interim safety analysis (60 patients) for this Phase 2b trial. At this meeting, the DSMB will review all safety data in aggregate; individual adverse event narratives will also be available to the DSMB for review. During the trial, the DSMB will review the safety data in an unblinded fashion. The DSMB will also receive reports of serious, unexpected, and study-related adverse events and unanticipated problems on an ongoing basis.

The actual analyses will be conducted by the Clinical Coordinating Center Biostatistician who will be blinded to the randomization scheme.

- 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, the Department of Defense (DoD).

13 Data Handling and Record Keeping

13.1 *Confidentiality*

Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Those regulations require a signed subject authorization informing the subject of the following:

- What protected health information (PHI) will be collected from subjects in this study

- Who will have access to that information and why
- Who will use or disclose that information
- The rights of a research subject to revoke their authorization for use of their PHI

In the event that a subject revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of subject authorization. For subjects that have revoked authorization to collect or use PHI, attempts should be made to obtain permission to collect at least vital status (i.e., that the subject is alive) at the end of their scheduled study period.

13.2 *Source Documents*

Source data is all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents. Examples of these original documents and data records include: hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical trial.

13.3 *Case Report Forms*

The study case report forms (CRFs) are the primary data collection instruments for the study. All data requested on the CRFs must be recorded. All missing data must be explained. If a space on the CRF is left blank because the procedure was not done or the question was not asked, write "N/D". If the item is not applicable to the individual case, write "N/A". All entries should be printed legibly in black ink. If any entry error has been made, to correct such an error draw a single straight line through the incorrect entry and enter the correct data above it. All such changes must be initialed and dated. DO NOT ERASE OR WHITE OUT ERRORS. For clarification of illegible or uncertain entries, print the clarification above the item, then initial and date it.

13.4 *Records Retention*

It is the investigator's responsibility to retain study essential documents for at least 2 years after the completion and publication of the clinical trial. These documents should be retained for a longer period if required by an agreement with the sponsor. In such an instance, it is the responsibility of the sponsor to inform the investigator/institution as to when these documents no longer need to be retained.

14 Study Monitoring, Auditing, and Inspecting

14.1 *Study Monitoring Plan*

The data safety and monitoring plan for this Phase 2b trial will include a formal DSMB that will be approved by the central IRB at Vanderbilt University and the Department of Defense. Our data

safety monitoring board will include two critical care physician and a biostatistician. None of the DSMB members will be affiliated with this study or have a conflict of interest. The DSMB will meet before the clinical trial begins and subsequently at least once every six months to review the design, comment, and monitoring of the trial with special reference to safety issues. The DSMB will be available to review adverse events, which will also be reviewed by the Principal Investigators. Adverse events will be promptly report to the DSMB, the Vanderbilt IRB, and the FDA.

Taylor Thompson, MD, will be appointed as the Research Monitor and the Chair of the DSMB for this Phase 2b trial. The independent Research Monitor has the authority to stop the research protocol in progress, remove individual human subjects from a research protocol, and take whatever steps necessary to protect the safety and well-being of human subjects until the IRB assesses the independent Research Monitor's report.

A separate monitoring plan will be written to specify how the participating clinical sites will be monitored by the sponsor.

14.2 *Auditing and Inspecting*

The investigator will permit study-related monitoring, audits, and inspections by the central IRB at Vanderbilt University, the sponsor, government regulatory bodies, and University compliance and quality assurance groups of 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., bone-marrow or cell therapy laboratory, pharmacy, diagnostic laboratory).

Participation as an investigator in this study implies acceptance of potential inspection by government regulatory authorities and applicable University compliance and quality assurance offices.

15 Ethical Considerations

This study is to be conducted according to US and international standards of Good Clinical Practice (FDA Title 21 part 312 and International Conference on Harmonization guidelines), applicable government regulations and Institutional research policies and procedures.

This protocol and any amendments will be submitted to the central IRB (cIRB) at Vanderbilt University for formal approval of the study. The decision of the cIRB concerning the conduct of the study will be made in writing to the investigator, and a copy of this decision will be provided to the sponsor before commencement of this study. The reliance on the cIRB have been approved by the local IRBs of all participating sites. All study approvals and related documents will be forwarded to local institional IRBs, as requested.

All subjects for this study will be provided a consent form describing this study and providing sufficient information for subjects to make an informed decision about their participation in this

study. The consent form will be submitted with the protocol for review and approval by the central IRB for the study. The formal consent of a subject, using the central IRB-approved consent form, must be obtained before that subject undergoes any study procedure. The consent form must be signed by the subject or legally acceptable surrogate, and the investigator-designated research professional obtaining the consent.

16 Study Finances

16.1 *Funding Source*

This study is financed through a grant from the Department of Defense (DoD), and California Institute for Regenerative Medicine for funding University of California Davis as an additional enrollment site. Both the DoD and NHLBI provided support for the production of MSCs at the University of Minnesota.

16.2 *Conflict of Interest*

Any investigator who has a conflict of interest with this study (patent ownership, royalties, financial gain greater than the minimum allowable by their institution, etc.) must have the conflict reviewed by a properly constituted Conflict of Interest Committee with a Committee-sanctioned conflict management plan that has been reviewed and approved by the study sponsor prior to participation in this study. Site investigators will follow their institutions conflict of interest policy.

17 Publication Plan

Neither the complete nor any part of the results of the study carried out under this protocol, nor any of the information provided by the sponsor for the purposes of performing the study, will be published or passed on to any third party without the consent of the study sponsor. Any investigator involved with this study is obligated to provide the sponsor with complete test results and all data derived from the study.

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19 Appendices

A. Time-Events Schedule
B. Lung Injury Score
C. De-identified Data Elements for Screened, Non-enrolled Subjects
D. Ventilator Management
E. Study Product Preparation Protocol
F. Guidelines for Symptom Management During Investigational Product Infusion
G. Mini-Bronchoalveolar Lavage Procedure
H. Phase 2b Investigational Product Infusion Protocol
I. Barotrauma
J. Childs-Pugh Score for Liver Dysfunction
K. WHO Ordinal Scale for Clinical Improvement (OSCI)
L. Radiographic Assessment of Lung Edema (RALE) Score

Appendix A: Time-Events Schedule

Measurement/Event	Day 0			1	2	3	4	5	6	7	8	9	10	11	12	13	14	28	60	6m
	Prior to randomization	Prior to MSC infusion&	During and after MSC infusion																	
Demographics, History & Physical, Height, Weight	X																			
Etiology of ARDS	X																			
HCG (in females of childbearing age < 50 years old)	X																			
Baseline stability assessment (Vital signs and ventilator parameters)$[B]		X																		
Mechanism of injury and trauma registry index (if applicable)	X																			
APACHE III Score[C]	X																			
ECHO[D]	A																			
COVID-19 test	A																			
COVID-19 vaccination status	A																			
Co-enrollment with other clinical trials and COVID-19 related therapies[M]	X			A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	
Vital Signs (HR, SBP, DBP, MAP, Temp ºC, SpO2) ~$	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X			
Central Venous Pressure ~$	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A			
Record vasopressors or inotropes * (Y/N)$	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X			
Fluids in/out, crystalloids, PRBC, FFP, platelets, cryoprecipitate, urine output	X	X	X	X	X	X	X	X	X	X	X									
Modified Brussels Score and Brussels Organ Dysfunction Failure~[E]	X			X	X	X	X	X	X	X	X	X	X	X	X	X	X			
Sequential Organ Failure Score	X					X				X							X			
Ventilator Parameters (including FiO_2) #	X	X	X	X	X	X	X	X	X	X										
Arterial Blood Gases (PaO_2, $PaCO_2$, pH, SpO2, base deficit)&	X	X	X	X	X	X	A	A	A	X										
Chest X-ray (# quadrants for lung injury score, RALE score)	A			A	A	A	A	A	A	A	A	A	A							
Pulmonary dead space[F]	X			X	X	X				X										
Creatinine%[G,H]	X			A	A	X	A	A	A	X	A	A	A	A	A	A	A	A		
Total bilirubin, ALT, platelets%[H]	X			A	A	X	A	A	A	X	A	A	A	A	A	A	A			
D-dimer, lymphocyte count, neutrophil counts, fibrinogen, ferritin and serum C-reactive protein (if applicable)	A	A		A	A	A	A	A	A	A										
Glasgow coma score[J]	X	X		X	X	X				X							X			
Outpatient medication and on-study medication and study procedure[K]	X			X	X	X	X	X	X	X										
DVT Prophylaxis medication¶	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X			
Positive blood culture¶	X			X	X	X	X	X	X	X	X	X	X	X	X	X	X			
Thromboembolic complications¶	X			X	X	X	X	X	X	X	X	X	X	X	X	X	X			
Wound site infection and VAP assessment€	X			X	X	X	X	X	X	X	X	X	X	X	X	X	X			
Blood for cytokines, mediators and markers of inflammation[N]		X	X	X	X	X														
Blood for DNA/RNA		X			X															
Urine for biomarker measurements[O]		X		X	X															
Mini-BAL					X															
Ventilator history and ICU history@																		X		
Need for, timing and duration of dialysis																		X	X	
Vital Status §																		X	X	X
Ordinal Scale for Clinical Improvement (OSCI)	X									X							X	X		
Incidence of barotrauma	X			X		X				X										

X=Required; **A**=When available; ~=Data gathered on days 0-14 or until d/c from study hospital; **$**=Data recorded every 15 minutes for at least two hours during "stable baseline" period and until the initiation of study product infusion, every 15 minutes for the duration of the infusion and every hour until 6 hours, then 12, 24 and 48 hour from the initiation of study product infusion; **#**=Data recorded every 15 minutes for at least two hours during "stable baseline" period and until the initiation of study product infusion, every 15 minutes for the duration of the infusion and every hour until 6 hours, then 12, 18, 24 and 36 hour from the initiation of study drug infusion, then day 2, 3, 4, 5, 6, and 7; **&**=ABG mandatory at the following timepoints: within 6 hours of randomization, prior to the start of the infusion (within 90 minutes of starting the baseline stability period); within 15 minutes of the end of MSC infusion (~1 hour); after the initiation of the study product infusion, ABGs will also be REQUIRED at 6, 12, 18, 24, 30, 36 hours (+/- 1 hour for all timepoints), day 2 (48 hours +/- 4 hours), 3 and 7 (anytime) if patient still has arterial line after study product infusion; **€**=Record only one episode during the 14 day period after study product infusion, no need for further assessment once first diagnosis is confirmed; **%**=Labs not available in the 24 hours before randomization must be obtained; **¶**=Data gathered for first 14 days during current hospital admission; @=Measure at day 28. The 28-day follow up will be a telephone interview with structured survey if patient discharged prior to day 28; **§**=Measure at day 28, day 60, hospital discharge alive, and 6 months; the 6 month follow up will be a telephone interview with structured survey. **B**=Patient must achieve a stable baseline for ≥ 2 hours and the administration of study product must be initiated within 14 days of initial ICU admission; **C**=Record available clinical data within 24 hours prior to study randomization; **D**=Record ECHO if available, If more than one ECHO data available, select the data closest to randomization; **E**=Record clinically available worst SBP, PaO_2/FiO_2, SpO_2/FiO_2, creatinine, platelets, bilirubin, and vasopressor use; **F**=Measured on day 0, 1, 2, 3, 7 when patient is on assistant breathing; **G**=Record clinically available creatinine in the 96 hours prior to randomization, the worst creatinine of the day from day 1-14, the date and value of the highest creatinine between days 15-28 and the lowest value during the entire hospitalization obtained off dialysis; **H**=Data required prior to randomization, day 3 (+/-1 day) and day 7 (+/-1 day); **J**=Mandatory at the following timepoints: pre-randomization, pre-infusion, day 1, 2, 3, 7, 14 and the date of hospital discharge; **K**=Record outpatient medication (anticoagulants/antiplatelets/antifibrinolytic) within 7 days prior to current hospital admission; record the following medication and procedures (glucocorticoids, NMBD, inhaled vasodilators, prone positioning, recruitment maneuvers, ECMO) prior to randomization and on day 1-7; and inpatient trauma specific medications for first 14 days during current hospital admission; **M**= Record any co-enrollment of clinical trials and COVID-19 related therapies within 7 days prior to current hospitalization until hospital discharge; **N**=Blood specimens are required before the study product infusion, at 6, 12, 24 hours (+/-1 hour), and 2 (48 hours +/- 4 hours), 3 days (anytime) after the initiation of the study product infusion. **O**=Urine specimens are required before the study product infusion, at 24 hours and 2 days (48 hours +/- 4 hours) after the initiation of the study product infusion.

Appendix B: Lung Injury Score

The Lung Injury Score (116) is a validated 4-point score based on chest radiograph findings, PaO_2/FiO_2 ratio, PEEP and compliance. Scoring is performed for each individual component, and the score is the average of the 4 components.

Lung Injury Scoring	Value	Score
CXR (# of quadrants with infiltrates)		
PaO2/FiO2		
PEEP		
Compliance		
Total score		
Actual score (total points/4)		

Scoring:

	1 points	2 points	3 points	4 points
CXR (# of quadrants with infiltrates)		2 quadrants	3 quadrants	4 quadrants
PaO2/FiO2	225-299	175-224	100- 174	< 100
PEEP	6-8	9-11	12-14	≥ 15
Compliance	60-79	40-59	20-39	≤ 19

Static compliance can be calculated as: Tidal volume/(Plateau pressure – PEEP)

Appendix C: De-identified Data Elements for Screened, Non-enrolled Subjects

1. Was onset of ARDS acute?
2. Did frontal CXR show bilateral infiltrates consistent with pulmonary edema?
3. Number of quadrants with opacities?
4. Is patient intubated?
5. PaO_2
6. FiO_2
7. PEEP
8. COVID-19 test result
9. Was there evidence of left atrial hypertension or left ventricular dysfunction?
10. Month of the year that patient met screening criteria
11. Gender
12. Ethnicity
13. Age (if age >89, 89 will be entered for age)
14. Reason(s) patient excluded from study
15. If not excluded, not enrolled, why?
16. Lung injury category (e.g. sepsis, pneumonia)
17. Severe traumatic brain injury?

Appendix D: Ventilator Management

1. Ventilator Management

A modified, simplified version of the ARDS Network lung protective lower tidal volume strategy will be used in this trial. This strategy, which was associated with low mortality rates in three previous ARDS Network trials (ARMA, ALVEOLI, and FACTT), will ensure that study subjects receive the beneficial effects of lung protection while participating in this trial (The Acute Respiratory Distress Syndrome Network and Brower et al., 2004). ARDS Network personnel have substantial experience in the application of this protocol from the three completed trials noted above.

1. Any mode of ventilation capable of delivering the prescribed tidal volume (V_T, 6ml/kg predicted body weight, +/- 2ml/kg) may be used, provided the V_T target is monitored and adjusted appropriately. However, assist control ventilation is the preferred mode of ventilation.
2. V_T Goal: 6 ml/kg predicted body weight.
3. Predicted body weight (PBW) is calculated from age, gender, and height (heel to crown) according to the following equations:
    a. Males: PBW (kg) = 50 + 2.3 [height (inches) – 60]
    b. Females: PBW (kg) = 45.5 + 2.3 [height (inches) – 60]
4. Measure and record inspiratory plateau pressure (Pplat) according to ICU routine (at least every four hours and after changes in V_T and PEEP recommended).
5. If Pplat > 30 cm H_2O, reduce V_T to 5 ml/kg and then to 4 ml/kg PBW if necessary to decrease Pplat to ≤ 30 cm H_2O.
6. If V_T < 6 ml/kg PBW and Pplat < 25 cm H_2O, raise V_T by 1 ml/kg PBW to a maximum of 6 ml/kg.
7. If “severe dyspnea" (more than 3 double breaths per minute or airway pressure remaining at or below PEEP level during inspiration), then raise V_T to 7 or 8 ml/kg PBW if Pplat remains below 30 cm H_2O. If Pplat exceeds 30 cm H_2O with V_T of 7 or 8 ml/kg PBW, then revert to lower V_T and consider more sedation.
8. If pH < 7.15, V_T may be raised and Pplat limit suspended (not required).
9. Oxygenation target: 55 mm Hg < PaO_2 < 80 mm Hg or 88% < SpO_2 < 95%. When both PaO_2 and SpO_2 are available simultaneously, the PaO_2 criterion will take precedence.
10. Minimum PEEP = 5 cm H_2O.
11. Adjust F_IO_2 or PEEP upward within 5 minutes if there are consistent measurements below the oxygenation target range.

12. Adjust F_IO_2 or PEEP downward within 30 minutes if there are consistent measurements above the oxygenation target range.
13. There are no requirements for maintaining a specific PEEP to F_IO_2 ratio. The lower PEEP/higher F_IO_2 table represents a consensus approach developed by ARDS Network investigators in 1995. The higher PEEP/lower F_IO_2 table (ALVEOLI) yielded equivalent results in a randomized trial (Brower et al., 2004) and would be acceptable and perhaps preferable in patients who appear to respond with a substantial increase in arterial oxygenation in the transition from lower to higher PEEP.

Lower PEEP/Higher F_IO_2 Treatment Group

F_IO_2	.30	.40	.40	.50	.50	.60	.70	.70	.70	.80	.90	.90	.90	1.0
PEEP	5	5	8	8	10	10	10	12	14	14	14	16	18	18-24

Higher PEEP/Lower F_IO_2 Study Group

F_IO_2	.30	.30	.30	.30	.30	.40	.40	.50	.50	.50 – .80	.80	.90	1.0	1.0
PEEP	5	8	10	12	14	14	16	16	18	20	22	22	22	24

Note: Levels of PEEP in these F_IO_2/ PEEP tables represent levels set on the ventilator, not levels of total-PEEP, auto-PEEP, or intrinsic-PEEP.

14. No specific rules for respiratory rate. It is recommended that the respiratory rate be increased in increments to a maximum set rate of 35 if pH < 7.30.
15. No specific rules about I:E. It is recommended that duration of Inspiration be ≤ duration of Expiration.
16. Bicarbonate is allowed (neither encouraged nor discouraged) if pH < 7.30.
17. Changes in more than one ventilator setting driven by measurements of PaO_2, pH, and Pplat may be performed simultaneously, if necessary.

References:

The Acute Respiratory Distress Syndrome Network. Ventilation with lower tidal volumes as compared with traditional tidal volumes for acute lung injury and the acute respiratory distress syndrome. The Acute Respiratory Distress Syndrome Network. 2000; 342:1301-1308.

Brower RG, Lanken PN, MacIntyre N, et al. Higher versus lower positive end-expiratory pressures in patients with the acute respiratory distress syndrome. N Engl J Med 2004; 351:327-336.

2. Weaning

Commencement of Weaning (applicable to patients ventilated invasively or non-invasively):
Patients will be assessed for the following weaning readiness criteria each day between 0600 and 1000. If a patient procedure, test, or other extenuating circumstance prevents assessment for these criteria between 0600 and 1000, then the assessment and initiation of subsequent weaning procedures may be delayed for up to six hours.

1. At least 24 hours since enrollment in the trial
2. $F_IO_2 \leq 0.40$ and PEEP $\leq$ 8 cm H_2O or $F_IO_2 \leq 0.50$ and PEEP = 5 cm H_2O
3. Values of both PEEP and $F_IO_2 \leq$ values from previous day (comparing Reference Measurement values, section 6.3)
4. Not receiving neuromuscular blocking agents and without neuromuscular blockade
5. Patient exhibiting inspiratory efforts. If no efforts are evident at baseline, ventilator set rate will be decreased to 50% of baseline level for up to 5 minutes to detect inspiratory efforts
6. Systolic arterial pressure $\geq$ 90 mm Hg without vasopressor support ($\leq$ 5 mcg/kg/min dopamine or dobutamine will not be considered a vasopressor)

Spontaneous Breathing Trial Procedure and Assessment for Unassisted Breathing:
If criteria 1-6 above are met, then initiate a trial of up to 120 minutes of spontaneous breathing with $F_IO_2 < 0.5$ using any of the following approaches:

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

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

Monitor for tolerance using the following:

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

If any of the goals a-e are not met, revert to previous ventilator settings or to PS greater than or equal to 10 cm H_2O with Positive End-expiratory Pressure and F_IO_2 = previous settings and reassess for weaning the next morning. The patient will be reassessed for weaning (Section E2) the following day.

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

3. Definition of Unassisted Breathing

1. Spontaneously breathing with face mask, nasal prong oxygen, high flow nasal oxygen, or room air, OR
2. T-tube breathing, OR
3. Tracheostomy mask breathing, OR
4. CPAP ≤ 5 without PS or IMV assistance, OR
5. Use of CPAP or BIPAP solely for sleep apnea management.

4. Definition of Extubation

1. Removal of an oral or nasotracheal tube.
2. If a patient receives a tracheostomy, the time of extubation is defined as the time when the patient achieves unassisted breathing as defined in **Section 3**.

5. Completion of Ventilator Procedures

Patients will be considered to have completed the study ventilator procedures if any of the following conditions occur:

1. Death
2. Hospital discharge
3. Alive 28 days after enrollment

If a patient requires positive pressure ventilation after a period of unassisted breathing, the study ventilator procedures will resume unless the patient was discharged from the hospital or > 28 days elapsed since enrollment.

6. Removal from the Ventilator Management Protocol

Patients may be removed from the 6 ml/kg PBW tidal volume ventilation requirement if they develop neurologic conditions where hypercapnia would be contraindicated (e.g., intracranial bleeding, GCS < 8, cerebral edema, mass effect [midline shift on CT scan], papilledema, intracranial pressure monitoring, fixed pupils).

Appendix E: Study Product Preparation Protocol

Allogeneic Bone Marrow-derived Human Mesenchymal Stromal Cells (hMSCs)

Document Date

February 24, 2020

To be used in support of

Protocol Title:	A Phase 2b, Randomized, Double-blind, Placebo-controlled, Multi-center Clinical Trial of Allogeneic Bone Marrow-derived Human Mesenchymal Stromal Cells for the Treatment of Acute Respiratory Distress Syndrome
Protocol Number:	UCSF-hMSC-ARDS-P1P2-14
Sponsor-Investigator:	Michael A. Matthay, MD University of California, San Francisco

1. Background

Allogeneic, bone marrow-derived human mesenchymal stromal cells (hMSCs) will be received frozen from:

University of Minnesota Medical Center (UMMC)
Clinical Cell Therapy Laboratory
Molecular and Cellular Therapeutics (MCT)
1900 Fitch Avenue
Saint Paul, MN 55108

The cellular product is cryopreserved for long-term storage. The cryopreserved hMSCs are formulated in CryoStor (90%) and DMSO (10%).

Once received at the clinical site, the hMSCs will be stored under controlled conditions in liquid nitrogen tanks in the Clinical Bone Marrow Transplant Facility (or equivalent).

Just prior to use, the cellular product will be thawed and diluted with 1:1 mix of 5% human serum albumin and 10% Dextran 40 for intravenous administration in the Clinical Bone Marrow Transplant Facility (or equivalent) by trained facility technicians.

→ The hMSCs administration must be completed within 4 hours of the start of the thaw procedure. The hMSCs will be administered over 60-80 minutes.

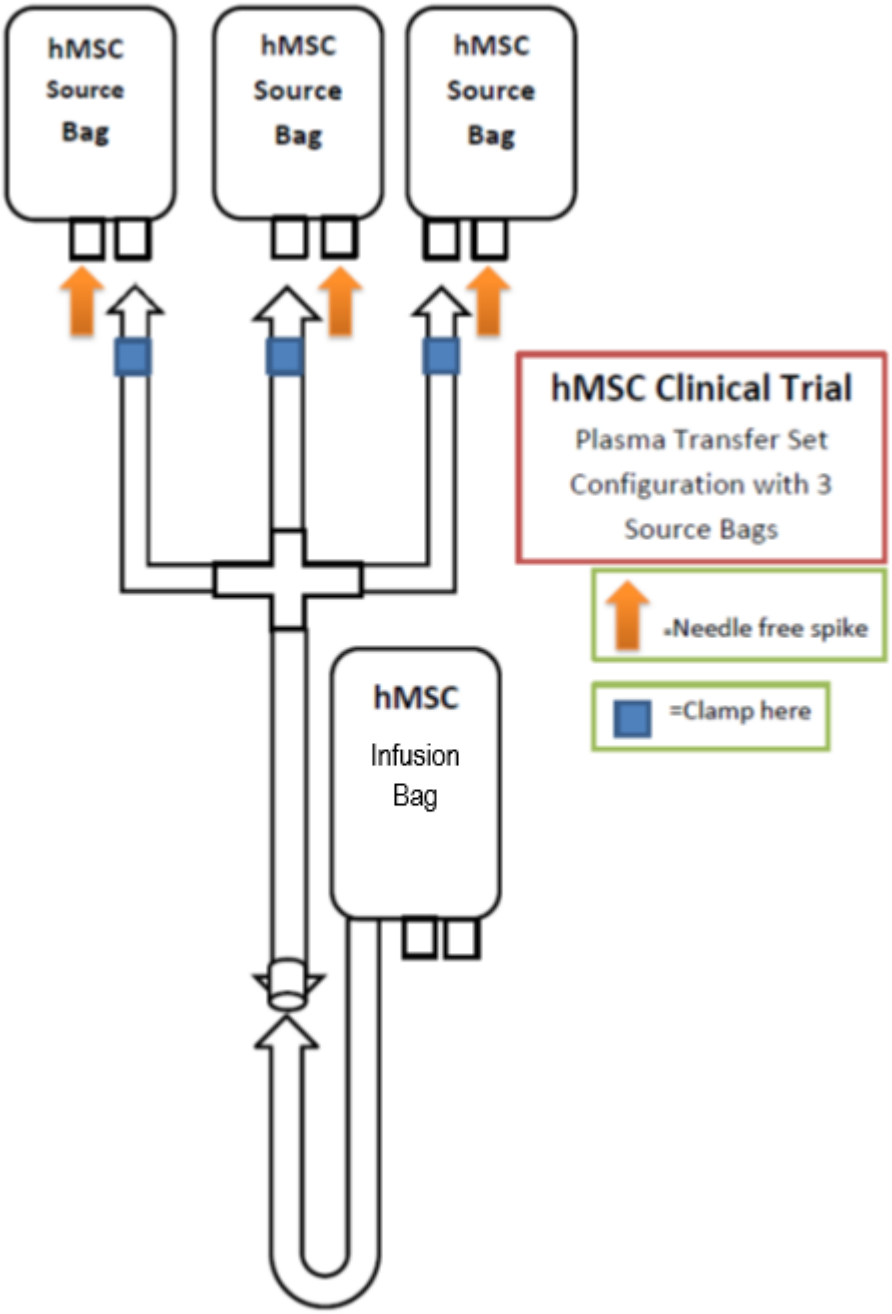
→ Each Clinical Bone Marrow Transplant Facility (or equivalent) will develop their own site-specific Standard Operating Procedure in support of this study protocol. The laboratory-specific standard operating procedure at each site will be reviewed by the Clinical Coordinating Center including the affiliated Clinical Bone Marrow Transplant Facility prior to initiating the clinical trial at that site. To assist with this process, the Clinical Coordinating Center will share the SOP developed by the UCSF Clinical Bone Marrow Transplant Facility (see below).

<table>
<tr><td>Protocol Title: (Check Protocol)</td><td colspan="2">STAT: A Phase 2b, Randomized, Double-blind, Placebo-controlled, Multi-center Clinical Trial of Allogeneic Bone Marrow-Derived Human Mesenchymal Stromal Cells for the Treatment of Acute Respiratory Distress Syndrome</td></tr>
<tr><td>Protocol Overview:</td><td colspan="2">Protocol: UCSF-hMSC-ARDS-P1P2-14
PI: Matthay, Michael Michael.Matthay@ucsf.edu
MD: Liu, Kathleen Dori Kathleen.Liu@ucsf.edu
CRC: Kimberly Yee 415-502-1596 | Email: kimberly.yee2@ucsf.edu
Research Analyst: Hanjing Zhuo (415) 502-7434 Hanjing.Zhuo@ucsf.edu
QC Testing: Jenny Fang Xiaohui.Fang@ucsf.edu

Randomized, double-blind, placebo-controlled, multi-center.
Subjects with ARDS after trauma or not traumatic cases of ARDS will receive a single infusion of hMSCs or placebo. A Phase 2b trial with this cell-based therapy will be used to test the safety and efficacy based on potential treatment of the major abnormalities that underlie ARDS after trauma, including oxygenation, carbon dioxide excretion, degree of pulmonary edema on the chest radiograph, systemic organ dysfunction, secondary infections, and altered lung endothelial and epithelial permeability and inflammation.</td></tr>
<tr><td rowspan="2">Manufacturing Facility:
List names, addresses, & responsibilities</td><td colspan="2">Minnesota Cell Therapy Clinical Laboratory - contract manufacturer, manufacture, QC testing, Storage, release, & distribution
UCSF Medical Center- IND holder, study oversight, coordinating center, eligibility determination
BMTL- Storage, randomization, distribution, thawing/pooling, QC testing and Release for Infusion</td></tr>
<tr><td>M Health Fairview | Cell Therapy Clinical Laboratory
MCT Facility | Dock B
Contact Information:
Molecular & Cellular Therapeutics
1900 Fitch Avenue
Saint Paul, MN 55108
Contacts:
Main lab - (612) 625-8204
Darin Sumstad (sumst003@umn.edu)
Diane Kadidlo (kadid003@umn.edu)
David McKenna, MD (mcken020@umn.edu; pager 612-899-7375)</td><td>UCSF Medical Center
BMT Laboratory
505 Parnassus Avenue, M547
San Francisco, CA 94143Contact:
Lizette.caballero@ucsf.edu, ph. 415-353-1351
Melanie.Mcmillan@ucsf.edu ph. 415-353-1789</td></tr>
<tr><td>Materials Needed</td><td colspan="2">Water Bath
Sterile Docking Device
Heat Sealer
Plasma-Lyte A
Sterile Water
Aerobic culture bottle
Angel Wings Blood Transfer Set
300ml Transfer Pack
Sterile Zip-Lock Bag
Sterile syringes - 3ml, 5ml, 10ml, 60ml
Hemostat
Tip Cap Red
Frozen Gel Packs
Infusion Filter
Amber Bag and IV Tubing Cover
Hemacytometer
Pipette
Microscope
Laminar Flow Hood
Wafers
Scale
Trypan Blue
Alcohol Pads
Anaerobic culture bottle
Needle free spike
Charter Medical 6 Lead Harness Set
Pipette tips
Sterile towel
Plastic BagSyringe
Transport cooler
Thermometer
0.9% Sodium Chloride
5% Human Serum Albumin
Dextran 40
400ml Transfer Pack</td></tr>
<tr><td colspan="3">CLINICAL STUDY- PROTOCOL SPECIFIC SOP (PSS)</td></tr>
</table>

SOP/ Step #	STAT: A Phase 2b, Randomized, Double-blind, Placebo-controlled, Multi-center Clinical Trial of Allogeneic Bone Marrow-Derived Human Mesenchymal Stromal Cells for the Treatment of Acute Respiratory Distress Syndrome	Tech Initials /Date
Section A: hMSC Product Receipt		
A.1	When receiving hMSC products, follow Instruction located on **the hMSC Receipt Instructions** document located in the Tree. HPCT Adult Lab >>Immuno>>hMSC ARDS Trauma >>Current Forms >>hMSC Receipt Instrutions	
Attention Lab Staff: Keep doctors, nurses, and research personnel blind at all times.		
Section B: Preparation and Randomization on the Day of Infusion		
B.1	Assign a UCSF DIN. □ Call PIC to assign a DIN number on the Apheresis Collection Number Accountability Log □ Place the sticker on page one of this PSS.	
B.2	Fax order received: ARDS MSC STUDY ORDERS: Preparation and Randomization for cell infusion. This order will include all the information needed in the Randomization Portal (A second order must be received before thawing cells) Note: There is a time window of about 45 minutes unitl the next order is faxed.	
B.3	**Randomization Procedure:** 1. To access the electronic randomization system, go to www.studydata.net. 2. Login and select “statrand” from the dropdown box. 3. From the Main Menu dropdown select “New Randomization”. 4. To randomize, enter all the data in the green section and press the Save button. 5. Note the Randomization time below. 6. Print a copy of the randomization page. 7. After you have pressed Save, exit this page by returning to the Randomization List form the Main Menu **Randomized to: □ INTERVENTION (CELLS) □ CONTROL (PLACEBO)** **Randomization Date/Time: _____** If patient is randomized to cell dose proceed to step B.4. **If patient is randomized to Placebo proceed to B.7**	
B.4	Prepare water bath and biosafety hood for thawing and reconstitution of product. Refer to: SOP-0906 Cellular Therapy Thawing Procedure SOP-0959 Laminar Flow Hood Operation and Maintenance Procedure	**□ N/A PLACEBO**
B.5	Determine the number of cells to thaw. • Use the Dose Table located in the Study Binder. • Use the Height/Gender provided on the order (see B.1). **Minimum number of cells to thaw based on Dose Table:_____ x10^6**	**□ N/A PLACEBO**

<table>
<tr><th>SOP/ Step #</th><th>STAT: A Phase 2b, Randomized, Double-blind, Placebo-controlled, Multi-center Clinical Trial of Allogeneic Bone Marrow-Derived Human Mesenchymal Stromal Cells for the Treatment of Acute Respiratory Distress Syndrome</th><th>Tech Initials /Date</th></tr>
<tr><td>B.6</td><td>Select bags from current inventory to reach minimum number of cells to thaw in B.5.
□ Look for hMSC products by using the hMSC/ARDS Investigational Drug Accountability Log FORMS-0585
□ Waste as few cells as possible.
□ Select hMSC products from a single donor by using bags with the same UMMC DIN
TABLE 1<table><tr><th>Product Code
UMMC DIN
Include flag character</th><th>Viable Cell Dose (X10^6)</th><th>Bag Volume (ml)</th><th>EU Dose
(from CoA)[1]</th><th>Tank Location</th></tr><tr><td></td><td></td><td></td><td></td><td></td></tr><tr><td></td><td></td><td></td><td></td><td></td></tr><tr><td></td><td></td><td></td><td></td><td></td></tr><tr><td></td><td></td><td></td><td></td><td></td></tr><tr><td></td><td></td><td></td><td></td><td></td></tr><tr><td>TOTAL</td><td>(A)</td><td>(B)</td><td>(2)</td><td></td></tr></table>[1]Endotoxin Dose (EU) documented on the COA based on viable cell dose frozen
[2]Total EU_____ ÷ PBW _____(kg) = _____EU/kg
[3]Acceptable limit = <5EU/kg (If the result is ≥5.0 EU/Kg then select another combination of MSC</td><td>Calculated by/Date:
_____
Verified by/Date:
_____
□ N/A PLACEBO</td></tr>
<tr><td>B.7</td><td>Prepare a 1-10°C credo cooler for product transport. Refer to section that describes how to set up credo in SOP-0929 “Transport and Shipping of Cellular Therapy Products”. This PSS will describe the documents necessary for transport in this clinical trial.</td><td></td></tr>
<tr><td>B.8</td><td>Prepare an Infusion Bag
□ Label an empty 400mL TP with a partial label: Patient name, MRN, DIN
□ Label as “Infusion Bag”
□ Log all reagents and supplies on a Reagents and Supplies Sheet (FORMS-0080)</td><td></td></tr>
<tr><td>B.9</td><td>Prepare the Reconstitution Media Bag:
□ Label a 400mL transfer pack (TP) “Reconstitution Media”.
□ Seal and remove excess tubing.
□ Add 160 mL of Dextran 40.
□ Add 160 mL of 5% Human serum albumin (HSA).
□ Aseptically spike one port with a needle free spike.
□ Keep refrigerated.
Note: If 5% Human Serum Albumin is not available: mix 200 ml 0.9% Saline + 50 ml 25% Human Serum Albumin in a 300mL transfer pack labeled “5% Human Serum Albumin” + Date. Seal and remove excess tubing.</td><td></td></tr>
</table>

SOP/ Step #	STAT: A Phase 2b, Randomized, Double-blind, Placebo-controlled, Multi-center Clinical Trial of Allogeneic Bone Marrow-Derived Human Mesenchymal Stromal Cells for the Treatment of Acute Respiratory Distress Syndrome	Tech Initials /Date
B.10	If more than one product bags is needed prepare a Pooling 6 lead harness set: □ Close all tubing clamps from spikes in the pooling harness. □ Spike the "**Infusion Bag"** from step B.8 into the pooling harness. □ Heat seal and remove any excess tubing lines from the harness. ○ For example. If three hMSC bags will be thawed and pooled, heat seal and remove 3 tubing lines. 	□ **N/A** □ **N/A PLACEBO**

SOP/ Step #	STAT: A Phase 2b, Randomized, Double-blind, Placebo-controlled, Multi-center Clinical Trial of Allogeneic Bone Marrow-Derived Human Mesenchymal Stromal Cells for the Treatment of Acute Respiratory Distress Syndrome	Tech Initials /Date
Section C: Confirmation of Subject Stability on the Day of Infusion		
C.1	Patient has met the clinical stability criteria, confirmation to thaw cells received VIA fax: ARDS MSC STUDY ORDERS: Confirmation of subject stability (Start thaw of cells within 15 minutes of receipt of this order). **Time notification received:_____**	
Section D: Thaw and Reconstitution of Product		
D.1	**If PLACEBO proceed to Step D.7** **THAW PROCEDURE:** Remove the hMSCs from inventory and thaw up to 2 bags at a time if needed. Refer to SOP-0906 "Cellular Therapy Thawing Procedure" ☐ Inspect the bags using FORMS-0120 "Frozen Bag Inspection Prior to Release" ☐ Remove bag(s) from cassette and place into zip lock bag. ☐ If the product bag has leaks or cracks, do not use it and replace with new bag. ☐ Immerse the sealed zip bag containing the frozen hMSCs in the water bath, gently rocking the bag to mix the suspension during the thawing.	☐ **N/A PLACEBO**
D.2	**DOCUMENT:** Water Bath Temp:_____ (37^0C +/- 3^0C) Thaw Start Time (first bag):_____ (Start timer for 30min. Perform viability between 30 min to 1 hours post start of thaw) Thaw Finish Time (last bag):_____ Thaw Product Expiration Date/Time:_____ (4 hours post thaw start time first bag)	**Documented by/Date:** _____ **Checked by/Date:** _____ ☐ **N/A PLACEBO**

SOP/ Step #	STAT: A Phase 2b, Randomized, Double-blind, Placebo-controlled, Multi-center Clinical Trial of Allogeneic Bone Marrow-Derived Human Mesenchymal Stromal Cells for the Treatment of Acute Respiratory Distress Syndrome	Tech Initials /Date
D.3	*If multiple hMSC bags were thawed, mark this section as N/A and proceed to Step D.4 **RECONSTITUTION PROCEDURE FOR 1 BAG OF hMSC:** 1) Aseptically transfer the thawed bag into the hood. □ Remove the bag from the zip bag. □ Spray bag with 70% Isopropanol 2) Aseptically spike the thawed hMSC bag with a needle free spike. 3) Aseptically spike the empty "Infusion Bag" bag with a needle free spike. 4) Using a sterile syringe, draw the hMSC product out of the thawed bag, note the volume, and add the product to the "Infusion Bag". 5) Using a sterile syringe add reconstitution media **equal to the amount** of hMSC in the "Infusion Bag". **Refer to volume (B) from step B.6.** **_____mL + _____mL = _____mL** **Product Volume(B) Reconstitution Media to add Final Volume (C)**	□ **N/A PLACEBO**
D.4	*If one hMSC bag was thawed, mark this section as N/A and proceed to Step D.3 **RECONSTITUTION PROCEDURE FOR >1 BAGS OF hMSC:** 1) Aseptically transfer the thawed bags into the hood. □ Remove the bags from the zip bag. □ Spray bags with 70% Isopropanol 2) Connect thawed hMSC bags to the pooling harness. □ Ensure the clamps on the pooling harness are closed. □ Spike the hMSC bags with the spikes on the harness. 3) Insert a needle free spike into the other port on all of the thawed hMSC bags. 4) Open the clamps and transfer the hMSC product into the empty 400mL "Infusion Bag". Close the all clamps. 5) Using a sterile syringe add reconstitution media **equal to the amount** of hMSC in the empty hMSC bags. Each bag may have different volumes. **Refer to volume (B) from step B.6.** 6) Gently swirl the hMSC bags with the reconstitution media. Open the clamps and allow the reconstitution media to transfer into the "Infusion Bag" with the hMSC product. Use air to facilitate the transfer if necessary. Close the clamps. 7) Heat seal and disconnect pooling harness from bag. Leave about 4inches of tubing on the bag. **_____mL + _____mL = _____mL** **Product Volume (B) Reconstitution Media added Final Volume (C)**	□ **N/A PLACEBO**

SOP/ Step #	STAT: A Phase 2b, Randomized, Double-blind, Placebo-controlled, Multi-center Clinical Trial of Allogeneic Bone Marrow-Derived Human Mesenchymal Stromal Cells for the Treatment of Acute Respiratory Distress Syndrome	Tech Initials /Date
D.5	Mix gently and allow the cells to equilibrate for 5 minutes. **Time End of Equilibration: _____**	**☐ N/A PLACEBO**
D.6	While cells are in equilibration **weigh** the product bag to determine the final product volume**.** _____g - _____g = _____mL **Weight of product bag (g)** **Weight of transfer pack (g)** **Final Product volume* (Q)** ***Note: g = mL** Calculate the volume of reconstitution media needed to achieve a final volume of 300mL. **300mL - _____ mL = _____mL** **Final Product volume (Q)** **Reconstitution Media to add (D)**	**☐ N/A PLACEBO**
D.7	Cell product - After equilibration, bring volume up to 300mL. ☐ Add reconstitution media volume (D) to the "Infusion Bag" via needle free spike. Mix gently. ☐ Mix gently. ☐ This is the Final Invesgtigational Product Bag. Placebo: ☐ 300mL of reconstitution media is the Final Investigational Product Bag ☐ Remove 20mL of reconstitution media for QC testing ○ Use a sterile syringe and syringe tip cap ○ Label with Study ID and "QC sample" ☐ Re-label transfer pack as "Infusion Bag" ☐ Go to step E.1	

<table>
<tr><th>SOP/ Step #</th><th>STAT: A Phase 2b, Randomized, Double-blind, Placebo-controlled, Multi-center Clinical Trial of Allogeneic Bone Marrow-Derived Human Mesenchymal Stromal Cells for the Treatment of Acute Respiratory Distress Syndrome</th><th>Tech Initials /Date</th></tr>
<tr><td>D.8</td><td>
<table>
<tr><th colspan="2">TABLE 2</th></tr>
<tr><th colspan="2">Calculate the Volume of cells to infuse and for QC</th></tr>
<tr><td>% Cells to infuse =

_____ ÷ _____ * 100
Assigned dose to infuse x10^6 (Z) Total cell dose thawed x10^6 (A)</td><td>(F)</td></tr>
<tr><td>Volume of cells to infuse =

300 mL * _____
% to infuse (F)</td><td>(G)</td></tr>
<tr><td>Volume for QC =

300 mL – _____
Volume (mL) to infuse (G) Note: Minimum of 8mL needed</td><td>(N)</td></tr>
<tr><td>Reconstitution media volume needed to re-suspend final product =

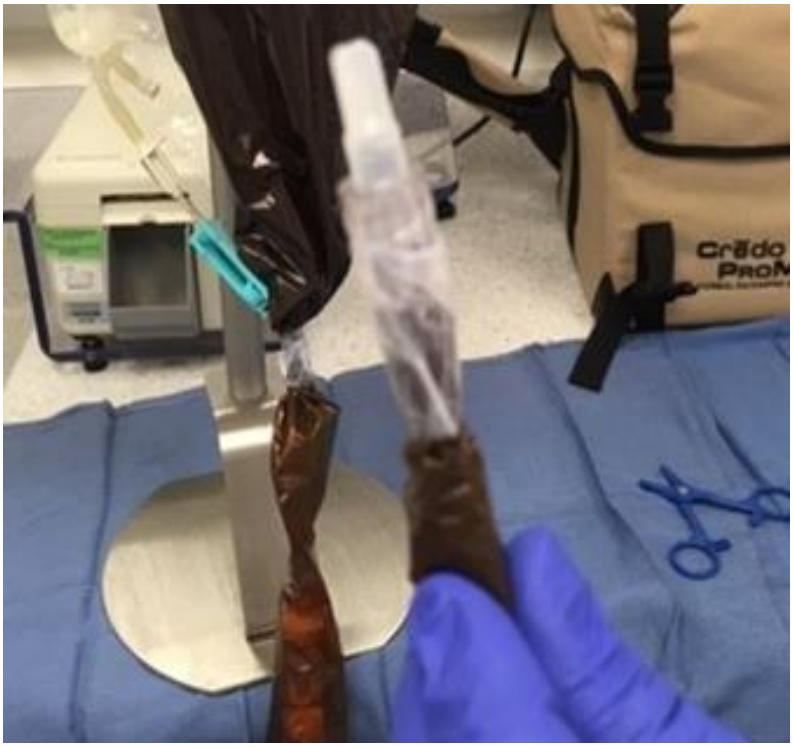
300 mL - _____
Volume (mL) to infuse (G)</td><td>(O)</td></tr>
</table>

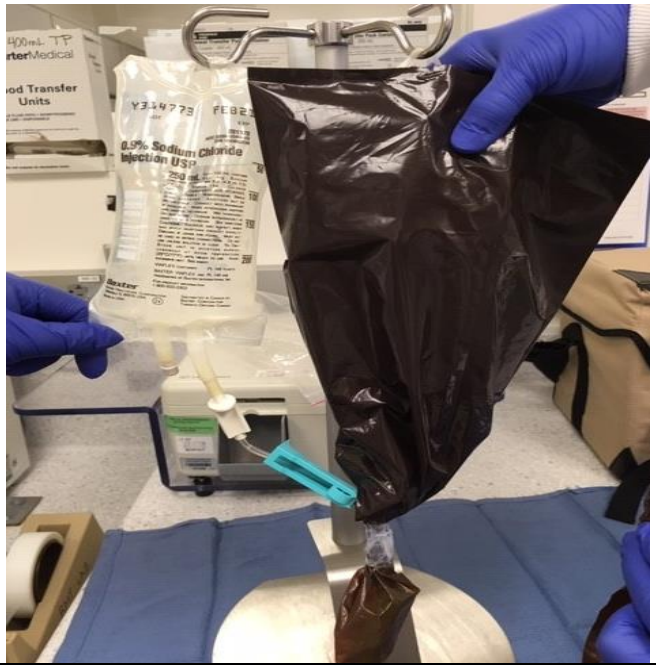
Remove QC sample and bring final infusion volume to 300mL:
1. Mix bag well with gentle swirls.
2. Using an appropriate size syringe, remove the QC sample volume (N).
3. Cover syringe with a syringe tip cap.
4. Re-suspend the Final Investigational Product to 300mL by adding reconstitution media. Refer to volume (O).
</td><td>Calculated by/Date:
_____

Verified by/Date:
_____

☐ N/A PLACEBO</td></tr>
<tr><td colspan="3">Section E: Labeling Product</td></tr>
<tr><td>E.1</td><td>Prepare Final Label. Refer to SOP-0887 “Labeling Procedure used in the BMT Laboratory.” Print two labels with the same information. One to be affixed directly to the cell product/placebo bag and the second to be affix outside the amber bag cover:

Use the Product Code S2511. Standard Label.
☐ Scan final product DIN (assigned by UCSF)
☐ Blood Type: Unknown
☐ Donation Type: For use by intended recipient(s) only
☐ Donor Type: Leave blank
☐ Donor info: Leave blank
☐ Recipient info: Name. MRN, DOB
☐ Collection center: Leave blank
☐ Processing Facility: BMT Lab
☐ Collection date/time: handwrite N/A
☐ Expiration date/time: 4 hours post start of thaw or For Placebo: see E.2
☐ Volume: 300 ml</td><td></td></tr>
</table>

SOP/ Step #	STAT: A Phase 2b, Randomized, Double-blind, Placebo-controlled, Multi-center Clinical Trial of Allogeneic Bone Marrow-Derived Human Mesenchymal Stromal Cells for the Treatment of Acute Respiratory Distress Syndrome	Tech Initials /Date
E.2	Placebo: Generate a "fake" expiration Date/Time for Placebo to keep the study blinded. □ Assign an expiration using the receipt time of the "Confirmation of Subject Stability Order". □ Add 10-30 minutes (choose at random) to the receipt time and then add 4 hours to calculate the expiration time. • Calculation: _____ + _____+ 4 hours = _____ Receipt time (10-30min) Expiration time	□ N/A CELLS
E.3	□ Obtain a check of the **two labels** by a second trained staff. □ Apply the Product Label to the Final Investigational Product Bag.	**Labeled by/Date:** _____ **Verified by/Date:**
Section F: Preparing Infusion Filter and Amber Plastic Covering		
F.1	Cover Y-type Infusion Set with amber plastic tubing. □ Leave access to the 2 spikes. □ Tape the end that will be inserted to the infusion line to the amber cover. This will prevent the amber cover from falling off. Ensure the infusion line cap can be easily removed. □ See Figure 1 below. **FIGURE 1** 	

SOP/ Step #	STAT: A Phase 2b, Randomized, Double-blind, Placebo-controlled, Multi-center Clinical Trial of Allogeneic Bone Marrow-Derived Human Mesenchymal Stromal Cells for the Treatment of Acute Respiratory Distress Syndrome	Tech Initials /Date
F.2	Attach Y-type Infusion Set to the saline bag using aseptic technique. □ **Close all of the clamps** □ Spike the saline bag with one of the infusion filter spikes □ Prime the Y-type Infusion Set: □ Close all clamps. □ Insert one spike of the Y-type infusion set into the 250 mL saline bag. Open the clamp. □ Squeeze and release to allow solution to fill filter chamber half way. Leave room so nurse can count drips. □ Open the regulating clamp to prime remainder of the tubing. Purge remaining air in the tubing. There should not be any air bubbles left in the tubing. □ Close the clamp close to the saline bag. Attach the Y-type Infusion Set to the Final Investigational Product Bag □ Close all clamps. □ Insert the other spike of the Y-type infusion set into the Final Investigational Product Bag. □ Ensure all clamps are closed.	
F.3	Cover the product bag using the amber plastic cover. □ Tape the bottom of the amber plastic cover to the amber tubing cover. □ Make sure the bag content is not visible. Refer to FIGURE 2. □ Ensure access to the roller clamp on the saline line is available. □ Clamp the loop at the top of the transfer pack with a hemostat to give the nurse easy access □ Affix duplicate of the cell dose product/placebo label outside of the amber plastic cover. **FIGURE 2**  	

<table>
<tr><th>SOP/ Step #</th><th>STAT: A Phase 2b, Randomized, Double-blind, Placebo-controlled, Multi-center Clinical Trial of Allogeneic Bone Marrow-Derived Human Mesenchymal Stromal Cells for the Treatment of Acute Respiratory Distress Syndrome</th><th>Tech Initials</th></tr>
<tr><td colspan="3">Section G: Packing final product for transport at 1-10°C and Release for Infusion</td></tr>
<tr><td>G.1</td><td>Prepare final product for transport:
☐ Place the final product/placebo bag into a biohazard bag. Then place into the prepared Credo cooler.Refer to SOP-0929 Transport and Shipping of Cellular Therapy Products.
☐ Place a thermometer in the cooler. Ensure the thermometer probe is in the pocket with the product.
☐ Place a Circular of Information for the use of 3rd party mesenchymal stromal cells inside a plastic bag and place inside cooler. Copies are in the study binder.</td><td></td></tr>
<tr><td>G.2</td><td>Fill out Allogeneic Bone Marrow-Derived Human Mesenchymal Stromal Cells Infusion Form with the following information:

☐ Recipient Name, MR#, DOB and Study ID
☐ Patient’s Weight = Predicted Body Weight
☐ Product Identification = DIN
☐ Randomization Date/Time
☐ Product Expiration Date/Time
☐ Signatures: Reviewed by and Verified by
☐ Signatures: Removed from lab by and Verified by
☐ Insert this form and circular of information inside a plastic bag and place inside cooler.</td><td></td></tr>
<tr><td>G.3</td><td>FINAL RELEASE

<table><tr><th colspan="4">TABLE 3</th></tr><tr><th>Cell Dose Product assessment</th><th>Specification</th><th>Results</th><th>Pass/Fail</th></tr><tr><td>Endotoxin</td><td>< 5.0 EU/kg</td><td>EU/kg[3]</td><td>❑ Pass ❑ Fail</td></tr><tr><td>Single Donor Used</td><td>Single Donor Used</td><td>❑ Yes ❑ No</td><td>❑ Pass ❑ Fail</td></tr></table>Manufacturer Final Certificate of Analysis for Bone Marrow-Derived Mesenchymal Stromal Cells filed?
❑ Yes ❑ No
Product receipt and storage without incident?
❑ Yes ❑ No

Released by:_____
Date:_____ Time Released: _____
NOTE: Release product if the answer to all of the questions is YES and PASS. If product does not meet release criteria, approval as urgent medical need from Medical Director is required.</td><td>☐ N/A PLACEBO</td></tr>
<tr><td>G.4</td><td>SF General (ZSFGH) Patients ONLY:

Call AM-TRAN 1-877-243-8733 and schedule STAT pick-up going to San Francisco General.</td><td>☐ N/A</td></tr>
</table>

<table>
<tr><th>SOP/ Step #</th><th>STAT: A Phase 2b, Randomized, Double-blind, Placebo-controlled, Multi-center Clinical Trial of Allogeneic Bone Marrow-Derived Human Mesenchymal Stromal Cells for the Treatment of Acute Respiratory Distress Syndrome</th><th>Tech Initials</th></tr>
<tr><td>G.5</td><td>UCSF Parnassus Heights Patients ONLY:

Deliver cells to bedside nurse or doctor.</td><td>☐ N/A</td></tr>
<tr><td>G.6</td><td>Document product issue using FORMS-0124 “Issue Log”.

Time Released to Courier:_____ ☐N/A if transported by BMT Lab</td><td></td></tr>
<tr><td colspan="3">DO NOT TAKE THIS PSS TO ICU</td></tr>
<tr><td colspan="3">Section H: Quality Control (QC) Testing</td></tr>
<tr><td>H.1</td><td>Use QC sample (from step D.7 or D.8) to inoculate sterility cultures.
☐ Use technique described in SOP-0912 “Sterility Testing for Cellular Therapy Products and management of Positive Cultures”.
☐ Label bottles with: DIN, Date, hMSC Clinical Trial and Tech’s initials.

Aerobic & Anaerobic sterility testing Sent on:_____ by:_____</td><td></td></tr>
<tr><td>H.2</td><td>Sterility Results:

☐ Positive Notified to:_____ Date/Time:_____

☐ Negative</td><td>Sterility Result review by/date:</td></tr>
<tr><td>H.3</td><td>Using QC sample (from step D.8) perform:
☐ WBC count via hemocytometer
☐ Trypan Blue Viability Testing: Refer to SOP-0935 “Trypan Blue Viability Procedure”
☐ Time Viability was Done: _____
☐ Calculations using memacytomer:

<table>
<tr><th></th><th>Viable Cell Count</th><th>Total Cell Count (Viable + Non-Viable)</th></tr>
<tr><td>Side 1</td><td></td><td></td></tr>
<tr><td>Side 2</td><td></td><td></td></tr>
<tr><td>TOTAL COUNT</td><td>(H)</td><td>(I)</td></tr>
</table>
_____ (H) ÷ _____ (I) X 100 = _____% % Cell Viability (J)

If % Cell Viability is <70 notify bmtlab@ucsf.edu and Kathleen.Liu@ucsf.edu within 24 hours.</td><td>Calculated by/Date:

_____

Verified by/Date:

_____

☐ N/A PLACEBO</td></tr>
</table>

<table>
<tr><th>SOP/ Step #</th><th>STAT: A Phase 2b, Randomized, Double-blind, Placebo-controlled, Multi-center Clinical Trial of Allogeneic Bone Marrow-Derived Human Mesenchymal Stromal Cells for the Treatment of Acute Respiratory Distress Syndrome</th><th>Tech Initials</th></tr>
<tr><td>H.4</td><td>Calculate TNC count and recovery %:

<table>
<tr><th colspan="4">TABLE 4</th></tr>
<tr><th colspan="4">QC SAMPLE: Total Cell Count Thawed/Recovered</th></tr>
<tr><td>Patient Name:</td><td>Study ID:</td><td>DIN:</td><td>Product Code(s)</td></tr>
<tr><td colspan="3">Total Cell Count (Viable + Non-Viable)</td><td>(I)</td></tr>
<tr><td colspan="3">Total Cells/mL= (10^6)=(I) ÷ (# of large squares counted) * 2 (TB Dilution factor) * (any other dilution) * 1 X10^4</td><td>(K)</td></tr>
<tr><td colspan="3">Total Cells thawed/recovered=(x10^8)= (K) * 300mL</td><td>(L)</td></tr>
<tr><td colspan="3">Total Viable Cells = (10^8)=(L) * (J)</td><td>(M)</td></tr>
<tr><td colspan="3">Viable Cells Recovery (%) = (M) / (A) x 100</td><td></td></tr>
</table>
If % Viable Cells Recovery is <70 notify bmtlab@ucsf.edu and Kathleen.Liu@ucsf.edu within 24 hours.</td><td>Calculated by/Date:

Calculated by/Date:

Verified by/Date:

Verified by/Da

–

□ N/A PLACEBO
□ N/A PLACE</td></tr>
<tr><td colspan="3">Section I: Clerical Documentation</td></tr>
<tr><td>I.1</td><td>The remaining volume from the QC sample will be given to the clinical research team for testing. Contact Jenny Fang by text (415) 316-9895 and email Xiaohui.Fang@ucsf.edu to schedule a pickup.

□ Place a syringe tip cap on the syringe and store at 2-8°C.
□ Make a copy of TABLE 4 and send with QC sample.</td><td></td></tr>
<tr><td>I.2</td><td>Order IECTW to the ZZ account using Sunquest:

Accession # _____</td><td></td></tr>
<tr><td>I.3</td><td>Infuse cells in the BMT Inventory Database
□ Using the Tank Summary screen, select a bag that was thawed by its location in the database.
□ Then select Discard/Transfuse Collection from the drop down
□ Select “discard”
□ In the Disposition box, enter the patients Name and MRN
□ Select the check boxes that correlate with the bags that were infused to the patient
□ Submit Edits
□ Print the screen and file in the patients records</td><td>□ N/A PLACEBO</td></tr>
</table>

SOP/ Step #	STAT: A Phase 2b, Randomized, Double-blind, Placebo-controlled, Multi-center Clinical Trial of Allogeneic Bone Marrow-Derived Human Mesenchymal Stromal Cells for the Treatment of Acute Respiratory Distress Syndrome	Tech Initials
I.4	Update the hMSC/ARDS Investigation Drug Accoutabilty Log FORMS-0585 □ Complete the Patient Information section that correlates to the bags that were infused.	□ N/A PLACEBO

Approval/Review of Protocol Summary

All processes required by the protocol that differ from BMT Lab SOPs are listed above. The descriptions of the protocol and processes have been reviewed and approved by the BMT Lab Senior Supervisor and Director. They are considered acceptable protocol specific processes and do NOT require documentation or handling as a Deviation.

PSS Summary Reviewed/ Approved By:	_____ BMT Lab Supervisor/Designee Signature	_____ Date
	_____ Andrew Leavitt, MD BMT Lab Medical Director	_____ Date

Appendix F: Guidelines for Symptom Management During Study Product Infusion

Adverse Reactions	Signs and Symptoms	Management
Hypersensitivity Hypersensitivity to allogeneic plasma proteins.	• Dyspnea • Hypotension • Fever • Urticaria • Tachycardia • Hypoxemia	• Pause MSC infusion • Check vital signs and O2 saturation • Hydrocortisone 1mg/kg IV • If still symptomatic after hydrocortisone, then diphenhydramine 25-50 mg IV • Increase supplemental O2 if O2 saturation is <93% and/or dyspnea • Resume MSC infusion after reasonable resolution of signs and symptoms.
Leukoagglutination Because some leukocytes may be present in the MSC preparation (although MSC purity will typically be >95%), leukoagglutination symptoms may occur.	• Cough • "Tickle in throat" • Dyspnea • Hypoxemia • Chest pressure	• Pause MSC infusion. • Check vital signs and O2 saturation • Increase supplemental O2 if O2 saturation is <93% and/or dyspnea. • Resume MSC infusion after reasonable resolution of signs and symptoms.
Volume Overload The volume of the MSC will be set at 300 ml total. Pulmonary edema may occur in this population. This can occur with or without concurrent cardiac dysfunction.	• Dyspnea • Hypoxemia • Frothy, clear or pink sputum • Tachycardia • Lack of fever • Distended neck veins	• Pause MSC infusion. • Check vital signs and O2 saturation. • Increase supplemental O2 if O2 saturation is <93% and/or patient is dyspneic. • Furosemide (≥ 10 mg IV) • Resume MSC infusion after reasonable reduction of signs and symptoms.
Transfusion Incompatibility The infusion of allogeneic MSC can be incompatible based on the presence of allo-antibodies in the donor and/or recipient serum. Transfusion incompatibility	• Fever • Hypotension • Dyspnea • Low back pain • Dark urine • Drop in blood hemoglobin	• Stop MSC infusion. • Check vital signs and O2 saturation. • IV fluid resuscitation immediately if hypotensive. • Forced diuresis and urinary alkalinization with IV D5W + 2 amps/l sodium bicarbonate.

Adverse Reactions	Signs and Symptoms	Management
reactions are usually symptomatic and occasionally life-threatening.	• Disseminated-intravascular coagulation	• Send recipient blood for a "transfusion reaction analysis" to the blood bank. • Consider mannitol 25 gm IV if suspicion of a transfusion ABO incompatibility reaction is high. • Consider blood cultures (and culture of the MSC product) followed by broad spectrum anti-bacterial antibiotics. • The attending BMT physician will be informed of the episode and will decide whether to restart the MSC infusion or abort the MSC infusion. • If the MSC infusion is aborted, the balance of the MSC product will be quarantined in the BMT laboratory refrigerator for possible additional analysis.
Reactions to Cryopreservatives (e.g. DMSO) DMSO is added to all MSC products being cryopreserved to protect the MSC from hypothermal damage.	• Metallic/garlic taste • Nausea/emesis • Flushing of face • Intestinal cramps • Acute hypertension • Bradycardia (first, second, or third degree atrioventricular block on electrocardiogram)	• Pause MSC infusion. • Check vital signs and O2 saturation. • Administer additional anti-emetics if appropriate. • If bradycardia, perform an electrocardiogram. If patient is asymptomatic with first or second degree atrio-ventricular block on EKG, resume MSC infusion. If symptomatic, stop infusion and administer atropine 0.5 -1mg. • If bradycardia is present and electrocardiogram shows third degree atrio-ventricular block, consider placement of a temporary pacemaker. Consult Cardiology. • Infuse remainder of previously non-thawed MSC after reasonable resolution of the signs and symptoms of an acute DMSO reaction and acquisition of hemodynamic stability.
Infection/Endotoxemia	• Hypotension	• Stop the MSC infusion

Adverse Reactions	Signs and Symptoms	Management
The infusion of infected MSC products is usually fatal. A rapid response to the acute adverse signs/symptoms of the infusion of the infected MSC may be life-saving.	• Fever • Oliguria/anuria • Tachycardia • Dyspnea/ hypoxemia • Disseminated intravascular coagulation • Signs/symptoms within "minutes" of MSC infusion	• Culture the recipient's blood and the MSC product. • Immediately start broad spectrum antibiotics. The usual default should be vancomycin, meropenem or imipenim, and tobramycin. • Use hemodynamic support as appropriate (IV fluids +/- vasopressors). • Notify the BMT laboratory clinical scientists of the suspicion of an infected MSC product.

Appendix G. Mini-Bronchoalveolar Lavage Procedure

The mini-BAL procedure involves blind specimen sampling from distal airspaces of patients with endotracheal or tracheal intubation. Specimens are obtained with the Combicath™ (Plastimed) catheter that is commonly used for the diagnosis of ventilator-associated pneumonia. The catheter is introduced from its protective sheath into the endotracheal or tracheal tube through a standard bronchoscopy adapter, and then gently advanced into the lungs until it becomes wedged in a distal airway. The catheter then is withdrawn approximately 3 cm to allow room for the inner catheter to be advanced into the distal airway. This is accomplished by removing the white protective spacer and gently advancing the inner catheter to its full length and securing it to the outside catheter by slightly twisting it into the outside catheter. Then, two 30-mL syringes containing 20 mL normal saline and a 5mL air bolus are injected rapidly into the lungs. Once the air bolus clears the second syringe, gentle aspiration is applied to the syringe for approximately 10 seconds to retrieve as much of the instilled fluid as possible (usually 5-10 ml). When completed, the catheter is removed from the endotracheal tube. The recovered aspirated samples are then emptied into a standard screw-top specimen container used for BAL samples. If the clinical condition warrants it, 1 ml can be deposited into a sterile container to be sent to the microbiology laboratory for culture.

Note: The mini- BAL procedure can be done with either one or two clinicians but it is easier to do (and typically more effective) when two clinicians are involved: one to advance, wedge, and manipulate the Combicath and the other to instill and aspirate the lavage. The patient should be pre-oxygenated on an FiO_2 of 1.0 for 10 minutes prior to the procedure and routine endotracheal suctioning should be done first to remove sputum from the airways.

After the mini- BAL, routine suction should be repeated to remove any excess BAL fluid that may be present in the airways. Assess patient's oxygenation and ventilatory status and return patient to their baseline FiO_2 when clinically indicated, usually within 5-10 minutes. The ventilator circuit should be returned to its original configuration by replacing the bronchoscopy swivel adapter with a clean adapter that was originally present in the circuit.

The mini-BAL should NOT be performed under the following circumstances:

1. FiO_2 greater than 0.70 AND PEEP greater than 14 cm H_2O
2. Hemodynamic instability (despite fluid resuscitation/pressor support)
3. Open external ventricular device or intracranial pressure greater than 15mmHg or unstable
4. Most recent INR greater than 2.0 or PTT > 40
5. Most recent Platelets less than $50x10^3/mm^3$ (within 36 hours of BAL)

Since this is a study procedure, any associated adverse event should be reported as a study related event. Transient hypoxemia is considered an expected event for ARDS, but prolonged or severe hypoxemia following mini-BAL should be reported as an adverse event.

Appendix H. Phase 2b Investigational Product Infusion Protocol

BACKGROUND: Human mesenchymal stromal cells (hMSCs) are being tested in a placebo-controlled trial as a novel therapy for the acute respiratory distress syndrome. Several factors are essential to the safe administration of hMSCs. They include accurate identification of the donor, the recipient; the product; and the cell dose. Therapies that can damage hMSCs include hemodialysis. In addition, recognition and management of the potential side effects associated with hMSC infusion are also important.

Each study site will develop its own site-specific Standard Operating Procedure in support of this protocol. The site-specific standard operating procedure will be reviewed by the Coordinating Center prior to initiating the clinical trial at that site.

1. OBJECTIVES

1.1 To ensure the safe and effective infusion of either clinical grade allogeneic bone marrow-derived human mesenchymal stromal cells (hMSCs) or the placebo.
1.2 To ensure the administration of the full hMSC dose (10 million cells/kg predicted body weight based on cell viability at the time of initial cell preparation at UMMC).
1.3 To blind investigators to the product being infused (hMSCs or placebo).

2. MATERIALS/EQUIPMENT

2.1 250mL Normal Saline bag (delivered by BMT lab)
2.2 Standard blood filter tubing Y set (170-260 micron filter): At UCSF Medical Center, this is ICU Medical B9211 (delivered by BMT lab)
2.3 Opaque bag to blind investigational product bag (delivered by BMT lab)
2.4 Semi-opaque tubing sleeve to cover tubing (delivered by BMT lab)
2.5 Gloves (in ICU)
2.6 One 3-way luer stopcock (in ICU)
2.7 10mL luer-lock syringe (in ICU)
2.8 Pressure bag (in ICU) – can be used for COVID-19 patients who have their IV poles outside the room

3. POLICIES

3.1 A study physician from the study must initiate and be present for the entire duration of the investigational product infusion.
3.2 A study physician must write an order to administer the investigational product.
3.3 For subjects randomized to receive hMSCs, the investigational product infusion must be completed within 4 hours of the start of the cell thaw protocol. To keep study investigators blinded, the investigational product expiration time will be documented on the investigational product bag by the bone marrow transplant (BMT) laboratory staff. This time will be 4 hours to the minute after the start of the cell thaw protocol. Placebo products will be assigned a product expiration time by the BMT lab to maintain blinding. Infusion supplies

will be picked up from the laboratory by a study personnel or transported to the ICU by BMT laboratory staff. Product expiration time will be documented by the BMT lab on the STAT Infusion Record and will be reviewed with study personnel. The start and end of the infusion will be documented on the STAT Infusion Record.

3.4 **Never discard investigational product**. In the event of an adverse reaction terminating the infusion, return any un-transfused product to the BMT laboratory immediately.

- Note: For COVID-19 suspected or confirmed positive patients, investigational product bags and tubing do not need to be returned to the BMT Lab

3.5 No prophylactic pre-medications (e.g. acetaminophen or diphenhydramine) will be administered prior to the investigational product infusion. If such medications are ordered by the clinical team and given prior to or during the infusion, however, their use does not preclude proceeding with the infusion.

3.6 The following medications should be immediately available at the patient's bedside during the infusion and the post-infusion monitoring period: epinephrine 1 mg for intravenous administration, hydrocortisone 100 mg for intravenous administration, and diphenhydramine 50 mg for intravenous administration.

3.7 The investigational product can be infused via peripheral or central venous access. If peripheral, the IV should preferably be 18 gauge, but no smaller than 20 gauge.

3.8 Ideally a separate port or line should be used for the investigational product infusion. The infusion should not be "piggy backed" into a medication line. If absolutely necessary, a stopcock can be used at the lumen connection to create a separate, dedicated line for the investigational product infusion. Avoid administration with dextrose containing solutions; Normal saline, Plasmalyte, and Lactated Ringers are acceptable.

3.9 Dialysis Patients: Patients receiving intermittent dialysis may be dialyzed prior to the investigational product infusion, but should not be dialyzed until 24 hours or more after receiving the infusion if possible. Continuous renal replacement therapy will be allowed to continue during the hMSC or placebo infusion.

3.10 Positioning: Patients must remain in the supine or prone position for the duration of the infusion and the post-infusion observation period.

3.11 Apply standard precautions and aseptic technique at all times.

3.12 Verify patient's identity per National Patient Safety Goals (two identifiers). (At UCSF Medical Center, the Department of Nursing Blood and Blood Components Administration Procedure should be followed).

3.13 Monitor the patient for reaction to the investigational product infusion during administration (see **Appendix F** for potential side effects and symptom management guidelines).

4. PROCEDURE

4.1 Study Physician to write order for investigational product infusion. This order also confirms the date, and time of the investigational product infusion. No investigational product will be thawed, processed, or released from the BMT laboratory without this order. (At UCSF Medical Center: Fax infusion order to BMT Lab, fax #353-1227. Confirm infusion time with BMT laboratory at 353-1789).

4.2 Personnel from the BMT lab will set up IV tubing as follows: 250mL normal saline bag spiked on to one limb of "Y-set" standard blood filter tubing (170 - 260 micron filter tubing,

for example ICU Medical B9211). The BMT lab will saline prime the blood filter tubing setup. The IV tubing will be covered with the blinding sleeve.

4.3 The investigational product will be administered as 300 mL in a 300 mL transfer bag; this bag will be covered with an opaque bag to blind investigators to the content. The BMT laboratory will label both the transfer bag and the blinding bag with the patient identifiers, then spike the investigational product bag onto the saline-primed IV tubing.

4.4 The Study Physician will:

- Confirm that the patient has met criteria to receive the investigational product infusion: in addition to meeting inclusion criteria and having no exclusion criteria, the 2 hour stable baseline period must have been achieved, and the PaO_2/FiO_2 ratio on a blood gas obtained within 90 minutes of starting the baseline stability period cannot equal or exceed 250. If the PaO_2/FiO_2 ratio is equal or greater than 250, the patient is no longer eligible to receive the investigational product.
- Follow National Patient Safety Goals to identify the patient (two identifiers). (At UCSF Medical Center: Verify patient identification per Department of Nursing Blood and Blood Components Administration Procedure).
- Verify that the signed consent is in the patient's medical record.
- Review the infusion order.
- BMT lab personnel or a study physician will review the information on the investigational product bag with the bedside RN or study physician for the following:
    - Recipient name/medical record number (MRN)
    - Recipient date of birth
    - Product expiration date/time
    - Product identification number
    - Product volume

One person should read the information on the product and a second person should compare it with the information on the paperwork and patient identification band. One of these two individuals should be a study investigator.

- Prior to infusion, the study physician should ensure that the following medications are available at the patient's bedside:
    - Epinephrine: 1 mg
    - Hydrocortisone: 100 mg
    - Benadryl: 50 mg

4.5 The study physician will be present for the entire infusion procedure:

- The investigational product bag will arrive from the BMT lab already spiked and attached to the saline-primed tubing, and covered with the semi-opaque sleeve.
    - The investigational product can be infused immediately after the product arrives at the bedside from the BMT laboratory and all patient checks have been completed. There is no need to wait for the bag to come to room temperature.
- For COVID-19 patients with the IV pole outside of the patient's room, the study personnel can wrap the investigational product with a pressure bag.
- The study physician will attach the primed filter tubing to a three-way stopcock and the intravenous line through which the investigational product is to be infused.

- Do NOT use bifuse tubing. DO NOT INFUSE THROUGH A NEEDLELESS CAP.

- The study physician will confirm that all IV connections are secure and that the infusion lines from the investigational product bag to the patient are visible but covered with the semi-opaque sleeve.
- Document the start date and time of the investigational product infusion on the STAT Infusion Record.
- If there is accidental unblinding, contact CCC and the investigator on-call to discuss how to proceed.

4.6 Start the infusion:

- At each center, the priming volume of the filter set must be measured and infusion drip rates should be calculated prior to enrollment of the first study patient:
  - For example, for ICU Medical B9211, the priming volume is 16.9 mL. The # drops/mL for this filter set per the manufacturer is approximately 10.
  - The drip chamber should not be primed to "full" since the infusion rate will be set based on the number of drops/minute that are used
  - The clamps CAN be opened and closed through the blinding tubing. If you are having difficulty opening and closing the clamps, however, you can cut a small vertical hole in the sleeve to allow direct access to the clamp.
- Run the entire volume of investigational product (300 mL) at the same rate over 60 minutes, checking the drop rate at the start of the infusion, approximately 5 minutes later, and periodically throughout the infusion. These can be visualized through the semi-opaque tubing sleeve. The drop rate should be checked by counting the number of drops over 30 seconds and calculating the number of drops/minute.
  - If using a pressure bag, pump the bag until the target drop rate is reached.
- For the ICU Medical B9211filter set:
  - The target infusion drop rate is 40 drops/30 seconds for the entire infusion
  - Continue checking the drop rate periodically throughout the infusion. If the infusion drop rate falls to 20-25 drops/30 seconds, increase the drop rate back to 40 drops/30 seconds.

4.7 **Do not** run normal saline concurrently with the investigational product.

4.8 **When the** investigational product **bag is empty, proceed to the RINSE AND FLUSH PROCEDURES** (4.12) to rinse the bag and flush the line.

4.9 If the investigational product does not infuse easily by gravity or using a pressure bag, you may attempt to attach the luer-lock syringe to the three-way stopcock and push the investigational product through line slowly (2-5 mL/min) via a syringe.

4.10 We do not anticipate that clumping should be an issue. However, save original filter set for the bone marrow transplant laboratory; they should visually examine the filter set for clumping prior to disposal. If clumping is significant and leads to unblinding of the bedside investigator or is noted by the BMT laboratory, contact the CCC and the investigator on call immediately.

Note: For COVID-19 suspected or confirmed positive patients, investigational product bags and tubing do not need to be returned to the BMT Lab

4.11 Monitoring:

- Blood pressure, heart rate and oxygen saturation will be continuously monitored for the duration of the infusion and documented every 15 minutes during the infusion.
- An arterial blood gas will be obtained within 90 minutes prior to beginning the baseline stability period and within 15 minutes of the end of the infusion. If the PaO2/FiO2 ratio on the blood gas obtained during the baseline stability period is greater than 250, the patient should NOT receive the investigational product.
- See **Appendix F** for potential side effects of the infusion. Monitor periodically during the infusion for these side effects. If the patient experiences significant side effects, consider pausing or stopping infusion.

4.12 RINSE AND FLUSH PROCEDURE: Approximately **100 mL of saline total should be used to rinse the investigational product bag and flush the line**, ensuring that the investigational product has been rinsed from the bag and infused through the priming setup.

- At the end of the infusion, rinse the investigational product bag with normal saline using this protocol. To do this: (1) Clamp the investigational product line to the patient (2) Unclamp the saline line and lower the investigational product bag to allow for saline to enter the investigational product bag; (3) After a small amount of saline (approximately 20 mL) has entered the investigational product bag, reclamp the saline line; (4) Swirl the saline in the investigational product bag; (5) Re-hang the investigational product bag, then open the line and infuse the saline rinse from the investigational product bag into the patient until the bag is empty; (6) Finally, allow 80 mL of saline to run through the filter to flush the remaining rinse volume into the patient. **The rinse will include 100 mL used to flush the bag and will be infused over 12-15 minutes:**
  - 100 mL * 10 drops/mL = 1000 drops/(80 drops/minute) = 12.5 minutes
  - **The target infusion rate for the rinse and flush is 40 drops/30 seconds (same as the start of the infusion) and the infusion should be complete in 12-15 minutes**
    - **Note: If using a pressure bag, after the initial 20 mL of saline has been infused, the next 80 mL of saline needs to be manually pushed with a syringe.**

4.13 Investigational product bags and tubing should be returned to the BMT laboratory
- Note: For COVID-19 suspected or confirmed positive patients, investigational product bags and tubing do not need to be returned to the BMT Lab.

4.14 Obtain and document vital signs at completion of infusion.

5. Documentation:

5.1 Complete the Infusion Record. The Study Physician must perform patient assessment for any adverse events. A copy of this infusion record should be placed in the patient's paper medical record or scanned into the electronic medical record; a hard copy must also be sent to the local BMT lab.

5.2 The study investigator will confirm with the bedside RN the total volume (investigational product and saline wash) used during the infusion procedure so this can be documented in the patient's medical record.

5.3 Study MD writes an event note documenting the procedure in the electronic medical record, including confirmation of the appropriate product and any complications or adverse reactions.

Appendix I. Barotrauma

Evidence of barotrauma includes the following:

- Pneumothorax
- Bronchopleural fistula
- Tracheobronchial fistula
- Pneumomediastinum
- Subcutaneous emphysema
- Pneumoatocoele

Appendix J. Childs-Pugh Score for Liver Dysfunction

Measure	1 point	2 points	3 points	Units
Bilirubin (total)	<34 (<2)	34-50 (2-3)	>50 (>3)	µmol/l (mg/dl)
Serum Albumin	>3.5	28-35	<28	g/l
INR	<1.7	1.71-2.20	> 2.20	*no unit*
Ascites	None	Suppressed with medication	Refractory	*no unit*
Hepatic Encephalopathy	None	Grade I-II (or suppressed with medication)	Grade III-IV (or refractory)	*no unit*

Appendix K. Ordinal Scale for Clinical Improvement (OSCI)

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

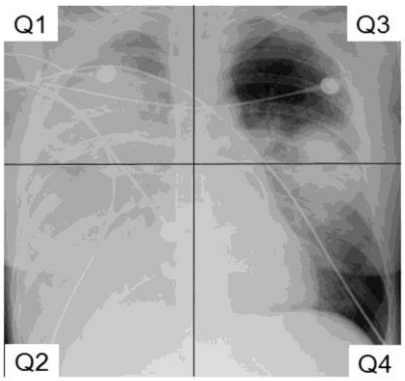
Appendix L. Radiographic Assessment of Lung Edema (RALE) Score

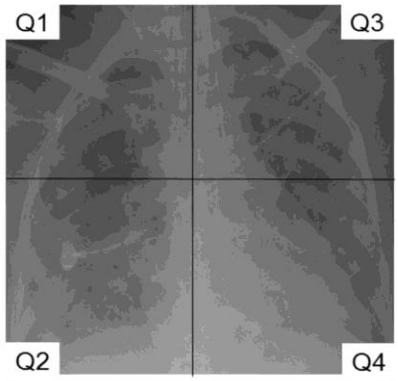
A

Consolidation[a]	
Consolidation Score	Extent of alveolar opacities
0	None
1	<25%
2	25-50%
3	50-75%
4	>75%
Density[b]	
Density Score	Density of alveolar opacities
1	Hazy
2	Moderate
3	Dense
Final RALE Score[c]	
Right Lung	**Left Lung**
Upper Quadrant **Cons x Den = Q1 score**	Upper Quadrant Cons x Den = Q3 score
Lower Quadrant **Cons x Den = Q2 score**	Lower Quadrant Cons x Den = Q4 score
Total RALE = Q1+ Q2 + Q3 + Q4	

[a]Consolidation is scored for each quadrant
[b]Density is scored for each quadrant that has a consolidation score ≥ 1
[c]If Quadrant consolidation score is – then Quadrant score is 0

B





Calculation of the RALE Score for Left Radiograph					
Score	**Q1**	**Q2**	**Q3**	**Q4**	**Total**
Consolidation	4	4	1	2	
Density	3	3	3	3	
Quadrant Score	4 x 3 = 12	4 x 3 = 12	1 x 3 = 3	2 x 3 = 6	**33**

Calculation of the RALE Score for Right Radiograph					
Score	**Q1**	**Q2**	**Q3**	**Q4**	**Total**
Consolidation	4	4	4	4	
Density	1	2	1	2	
Quadrant Score	4 x 1 = 4	4 x 2 = 8	4 x 1 = 4	4 x 2 = 8	**24**

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