Clinical Study Protocol

Protocol Title:	A Phase 1 / 2, Randomized, Double-blind, Placebo-controlled, Multi-center Clinical Trial of Allogeneic Bone Marrow-derived Human Mesenchymal Stem Cells for the Treatment of Acute Respiratory Distress Syndrome
Investigational Drug:	Allogeneic Bone Marrow-derived Human Mesenchymal Stem Cells (hMSCs)
Protocol Number:	UCSF-hMSC-ARDS-P1P2-06
IND Number:	15331
Sponsor-Investigator:	Michael A. Matthay, MD University of California, San Francisco

Signature: _____

Original Date: 17 February 2015

Confidentiality Statement

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PROTOCOL Approval

Protocol:	**Version/Date:**
UCSF-hMSC-ARDS-P1P2-04	February 17, 2015
IND: [number]	**Protocol Chair:**
15331	Michael A. Matthay, MD
Short Title: Mesenchymal Stem Cells For Acute Respiratory Distress Syndrome	

I have read protocol UCSF-hMSC-ARDS-P1P2-06, 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:_____

Protocol Synopsis

Name of Sponsor – Investigator	**Michael A. Matthay, MD** **University of California, San Francisco**
Investigational Product	**Allogeneic Bone Marrow-derived Human Mesenchymal Stem Cells (hMSCs)**
Protocol Number:	UCSF-hMSC-ARDS-P1P2-06
Title:	A Phase 1/ 2, Randomized, Double-blind, Placebo-controlled, Multi-center Clinical Trial of Allogeneic Bone Marrow-Derived Human Mesenchymal Stem Cells for the Treatment of Acute Respiratory Distress Syndrome
Short Title	Mesenchymal Stem Cells For Acute Respiratory Distress Syndrome
Clinical Phase	Phase 1 and Phase 2
Methodology	Dose escalation for Phase 1 Randomized, double-blind, placebo-controlled for Phase 2
Study duration	3 years
IND Sponsor-Investigator	Michael A. Matthay, MD
Funding Sponsor	National Heart, Lung, and Blood Institute
Study Product	Allogeneic Bone Marrow-derived Human Mesenchymal Stem Cells (hMSCs)
IND Number	15331
Participating Sites	Multi-center Intensive Care Unit study sites
Study Objective	To assess the safety and efficacy of hMSCs in critically ill patients with Acute Respiratory Distress Syndrome (ARDS)
Accrual Objective	Phase 1: 9 patients receiving hMSCs (3 patients per dose) Phase 2: 60 patients (40 patients receiving hMSCs and 20 patients receiving placebo)
Study Design	Phase 1: Open label, dose escalation, multi-center Phase 2: 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 DMSO (10%), human serum albumin (5%) and Plasma-Lyte A (30%). Immediately prior to administration, the product is thawed, washed, and resuspended in Plasma-Lyte A, a clinical-grade

Name of Sponsor – Investigator	**Michael A. Matthay, MD** **University of California, San Francisco**
Investigational Product	**Allogeneic Bone Marrow-derived Human Mesenchymal Stem Cells (hMSCs)**
	infusible solution for intravenous administration. Placebo: Plasma-Lyte A For both Phase 1 and Phase 2, each dose of the hMSCs or placebo will be administered intravenously over approximately 60-80 minutes. All patients will receive one dose. Phase 1: Dose Escalation 1×10^6 cells/kg (n=3), 5×10^6 cells/kg (n=3) and 10×10^6 cells/kg (n=3) Phase 2: 10×10^6 cells/kg (n=40); placebo (n=20)
Primary Phase 1 and 2 Endpoint:	**Safety**: Tolerability of the hMSCs, defined based on the incidence of pre-specified infusion associated events and of unexpected severe adverse events in ARDS patients treated with hMSCs.
Secondary Phase 2 Endpoints:	**Efficacy:** Respiratory endpoints: Oxygenation index, PaO_2/FiO_2 ratio and acute lung injury score Systemic endpoints: Brussels organ failure-free days, mean daily Sequential Organ Failure Assessment (SOFA) score, vasopressor-free, ventilator-free, intensive care unit (ICU) free days, as well as 60 day all-cause mortality Biomarkers: Change in levels of plasma biomarkers of lung epithelial injury (Receptor for Advanced Glycation Endproducts, RAGE), pro- and anti-inflammatory markers (interleukin-6, interleukin-8, interleukin-10 and interleukin-1Ra), endothelial injury (plasma von Willebrand factor [vWF], angiopoietin-2 [Ang-2]), markers of change in other organ function (creatinine) and markers that reflect the paracrine activity of the administered hMSCs (angiopoietin-1 [Ang-1] and keratinocyte growth factor [KGF]) as well as lung protein permeability (bronchoalveolar lavage [BAL] protein) at day 2.
Inclusion Criteria	Patients will be eligible for inclusion if they meet all of the below criteria. Criteria 1-3 must all be present within a 24-hour time period and at the time of enrollment: Acute onset (defined below) of:

Name of Sponsor – Investigator	**Michael A. Matthay, MD** **University of California, San Francisco**
Investigational Product	**Allogeneic Bone Marrow-derived Human Mesenchymal Stem Cells (hMSCs)**
	1. A need for positive pressure ventilation by an endotracheal or tracheal tube with a PaO_2/FiO_2 ratio < 200 with at least 8 cm H_2O positive end-expiratory airway pressure (PEEP). A patient may be included if the PaO2/FiO2 ratio < 200 with < 8 cm H_2O PEEP if there is a contraindication to increased PEEP: evidence of barotrauma. 2. Bilateral infiltrates consistent with pulmonary edema on frontal chest radiograph 3. No clinical evidence of left atrial hypertension for bilateral pulmonary infiltrates. • Acute onset is defined as follows: The duration of the hypoxemia criterion (#1) and the chest radiograph criterion (#2) must be < 28 days at the time of randomization • Infiltrates considered "consistent with pulmonary edema" include any infiltrates not fully explained by mass, atelectasis, or effusion or opacities known to be chronic (greater than 28 days). 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 the first two inclusion criteria but has a PAOP (Pulmonary Arterial Occlusion Pressure, also known as the Pulmonary Arterial Wedge Pressure) greater than 18 mm Hg, then the first two criteria must persist for more than 12 hours after the PAOP has declined to ≤ 18 mm Hg, and still be within the 96-hour enrollment window. In addition to meeting inclusion criteria, enrollment must occur within 96-hours of first meeting ARDS criteria per the Berlin definition of ARDS.
Exclusion Criteria	1. Age less than 18 years 2. Greater than 96 hours since first meeting ARDS criteria per the Berlin definition of ARDS

Name of Sponsor – Investigator	**Michael A. Matthay, MD** **University of California, San Francisco**
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	3. Pregnant or breast-feeding 4. Prisoner 5. Presence of any active malignancy (other than non-melanoma skin cancer) that required treatment within the last 2 years 6. Any other irreversible disease or condition for which 6-month mortality is estimated to be greater than 50% 7. Moderate to severe liver failure (Childs-Pugh Score > 12) 8. Severe chronic respiratory disease with a $PaCO_2$ > 50 mm Hg or the use of home oxygen 9. Patient, surrogate, or physician not committed to full support (exception: a patient will not be excluded if he/she would receive all supportive care except for attempts at resuscitation from cardiac arrest) 10. Major trauma in the prior 5 days 11. Lung transplant patient 12. No consent/inability to obtain consent 13. Moribund patient not expected to survive 24 hours 14. WHO Class III or IV pulmonary hypertension 15. Documented deep venous thrombosis or pulmonary embolism within past 3 months 16. No arterial line/no intent to place an arterial line 17. No intent/unwillingness to follow lung protective ventilation strategy or fluid management protocol 18. Currently receiving extracorporeal life support (ECLS) or high-frequency oscillatory ventilation (HFOV)
Statistical Consideration	The primary safety analysis will be descriptive and will focus, as described above, on the incidence of pre-specified infusion associated events and of unexpected severe adverse events in ARDS patients treated with hMSCs.

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Glossary of Abbreviations

Abbreviation	Full Term
AE	Adverse event
ALI	Acute Lung Injury
ARDS	Acute Respiratory Distress Syndrome
BAL	Bronchalveolar Lavage
CFR	Code of Federal Regulations
CRF	Case report form
DSMB	Data and Safety Monitoring Board
ECLS	Extracorporeal Life Support
FDA	Food and Drug Administration
GCP	Good Clinical Practice
HFOV	High-Frequency Oscillatory Ventilation
hMSCs	Human Mesenchymal Stem Cells
IB	Investigator Brochure
ICH	International Conference on Harmonisation
IEC	Institutional Ethics Committee
IND	Investigational New Drug
IRB	Institutional Review Board
IV	Intravenous(ly)
LOCF	Last Observation Carried Forward
MSCs	Mesenchymal Stem Cells
PAOP	Pulmonary artery occlusion pressure
PEEP	Positive end-expiratory pressure
SAE	Serious adverse event
SOE	Schedule of events
SRC	Scientific Review Committee

1 BACKGROUND

1.1 Summary

We hypothesize that Allogeneic Bone Marrow-derived Human Mesenchymal Stem Cells (referred to hereafter as hMSCs) administered to patients with the Acute Respiratory Distress Syndrome (ARDS) will be safe and effective. A combined Phase 1 and Phase 2 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, including altered lung endothelial and epithelial permeability and dysregulated inflammation and infection.

1.2 Scientific Rationale

1.2.1 Significance of this work to Acute Lung Injury/Acute Respiratory Distress Syndrome

Morbidity and mortality have declined only modestly in patients with clinical acute lung injury (ALI) and its more severe form, 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 stem cells for the treatment of ALI/ARDS is attractive as a potential new therapy. Mesenchymal stem cells (MSCs) are multi-potent and have the ability to secrete multiple 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.

MSCs, also called marrow stromal stem cells, were first discovered in 1968 by Friedenstein (8) who found bone marrow stromal cells that were adherent, clonogenic, and fibroblastic in appearance. Adult mesenchymal stem 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 (9).

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.

1.3 Background Relevant to Mechanism of Action of Mesenchymal Stem Cells in ALI/ARDS

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

1.3.1 Engraftment and Trans-differentiation

Much of the initial interest in MSCs therapy stemmed from the multi-potent properties of the cells. Krause et al (10) 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 (11) 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 (10-14). However, these results were questioned by multiple groups, who observed only engraftment of leukocyte lineages (15), or low engraftment rates in lung injury models with observed rates of < 1% (16-19). 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 (20-27). There may also be important cell-contact dependent effects that may include the transfer of microvesicles and mitochondria to injured recipient host cells resulting in restoration of more normal function (28).

1.3.2 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 (22, 23, 29, 30). 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. (20) 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 (31). 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 MSCs conditioned medium could not induce IL-10 production (20). In a model of ALI following intratracheal E. *coli* endotoxin in mice, we (32) 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. In bleomycin-induced lung injury and fibrosis in mice, Ortiz et al (33) 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 (34). These results confirmed the anti-inflammatory effects of MSCs in multiple lung injury experiments in mice (16, 17, 35-37).

Despite the well documented immunosuppressive effects of MSCs, recent literature described a dual role for MSCs as an immunostimulatory cell as well (38). 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 (39, 40). Recent evidence has also shown that MSCs can secrete IL-6 and induce production of IgG by B lymphocytes in an *in vitro* setting (41). In addition, MSCs can prevent neutrophil apoptosis and degranulation in culture without inhibiting their phagocytic or chemotactic capabilities (42). 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.

1.3.3 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 (20) as well as 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 (43). Also, we have found that the release of LL-37 by human MSCs reduces the number of bacteria in E. *coli* pneumonia in mice (44). 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 (45). Another study reported better survival following treatment with MSCs in a cecal-ligation model of peritonitis in mice (46). 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.

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

Impaired alveolar fluid clearance (AFC, i.e., delayed resolution of pulmonary edema) is common in patients with ALI/ARDS. The level of AFC impairment has significant prognostic value in determining morbidity and mortality (47, 48). Several experimental studies have investigated the mechanisms that reduce AFC in ALI, and several pathways have been implicated (49, 50). In the alveolar environment, basal AFC is determined predominately by amiloride-sensitive and insensitive sodium channels and the activity of the Na-K ATPase (49, 51-54). Several stimuli can upregulate AFC including beta-adrenergic agonists via cAMP-dependent mechanisms (49, 50). 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 (55-57).

We and other investigators have reported that pulmonary edema fluid contained high levels of several pro-inflammatory cytokines, including IL-1β, IL-8, TNF-α and TGF-β1 (58-60). 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 (61). 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 (62). 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 (63). 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 (64).

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 (65, 66), bleomycin (67, 68), hyperoxia (69, 70) and Pseudomonas *aeruginosa* (71). In rat lungs, KGF improved alveolar fluid transport in part by up-regulating αENaC gene expression (72) and Na-KATPase activity (73).

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 (74). 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 (75), anti-apoptotic effects (76) and increased transcription and/or translation of the major sodium and chloride transport proteins (72, 73). 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 (77, 78).

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 (Lee, JW et al, Manuscript submitted).

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.

1.3.5 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 (79) and vascular stabilization factor that reduces endothelial permeability and inhibits leukocyte-endothelium interactions by modifying endothelial cell adhesion molecules and cell junctions (80-83). 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 (35, 37, 84). In a study by Mei et al. (35), 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 (85).

KGF is one of several epithelial-specific growth factors produced by MSCs. In models of acute permeability edema such as α-naphthylthiourea (86), P. *aeruginosa* (71) or ventilator-induced lung injury (87), 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 (88). 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 the prevention of actin stress fiber formation and paracellular gaps among pulmonary endothelial cells injured by thrombin (89, 90). 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 (91).

1.4 Nonclinical Safety and Efficacy Studies

In anticipation of a clinical trial, we established a collaboration with 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, 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 whether given intra-venous or intra-tracheal.

We also 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 also have completed a study with hMSCs in sheep with ALI over 24 hours (Sheep Study 1). 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 they were treated 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 x 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 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). Also, 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 these 2012 sheep, the results of the first six sheep experiments (each one paired with a control) indicated that the severity of sepsis was greater in these sheep than in the prior group 1 sheep (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 above observations regarding mortality, there was no evidence of improvement in the oxygenation (P/F ratio) in the first 12 hours after the first dose of MSCs in the first six treated sheep. This raised some concern that perhaps administering the MSCs with DMSO decreased the efficacy of the MSC 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 MSCs, the possible contribution of DMSO to the reduced efficacy, and trying to reduce the bacterial injury so that it resembled the first group 1 (2011) sheep more closely.

Thus, in the next two sheep (MSC-treated and Plasmalyte control-treated), the dose of bacteria was reduced from 2.5 to 1.8 x 10^{11} *Pseudomonas aeruginosa* 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 still carried out with the same protocol of DMSO treatment and two doses of MSCs at one and eighteen hours after onset of acute lung injury. Even with the lower dose of bacteria, the MSC-treated sheep died at ≥ 20 h, suggesting again that the second dose of MSCs 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 MSCs, 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 MSCs 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 (P/F ratio) as in the prior group 1 experiments from 2011. However, there was still evidence that the second dose of MSCs given at eighteen hours was not tolerated, because one of the MSC-treated sheep died prior to 24 hours.

We 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 after an initial dose of hMSCs at 1 hour following the injury.

1.5 Description of Investigational Cellular Product and Dose Selection

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

The hMSCs are formulated in phosphate buffer saline (50%), DMSO (10%), human serum albumin (10%) and Plasma-Lyte A (30%), and cryopreserved and stored in bags suspended in liquid nitrogen.

Immediately prior to administration, the product is thawed, washed, and re-suspended in Plasma-Lyte A, a clinical-grade infusible solution for intravenous administration. The product is not further cultured or propagated prior to administration.

The placebo group will be treated with an equivalent volume of Plasma-Lyte A.

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 x 10^6 cells/kg or 10 x 10^6 cells/kg.

The initial proposed Phase 1 clinical study will evaluate single dose treatments of 1 x 10^6 cells/kg, 5 x 10^6 cells/kg and 10 x 10^6 cells/kg. The placebo-controlled Phase 2 study will evaluate the single-dose treatment of 10 x 10^6 cells/kg, or the maximum tolerated cell dose from the Phase 1 study.

1.6 Clinical Experience

There are currently 252 clinical trials listed in clinicaltrials.gov using other preparations of human mesenchymal stem 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. Among the most significant trials to date that are relevant for this application are:

Studies Focused on Lung Disease

1) A Phase 2 trial evaluating Prochymal 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 include 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 will involve 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 will include the number and severity of adverse reactions and/or the relationship between the cytokine concentrations in the BAL fluid and pulmonary artery pressure.

3) A Phase 1 trial evaluating the treatment of idiopathic pulmonary fibrosis (IPF) with human placental derived mesenchymal stem cells (NCT01385644). This is 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 will be enrolled in the study. The first 4 patients will receive a dose of 1 x 10^6 placenta-derived mesenchymal stem cells/kg. The Data Safety Management Board (DSMB) will carry out an interim safety analysis when these first 4 patients have all undergone their 3-month study visit. Should no serious adverse events be documented due to the infusion of mesenchymal stem cells, a subsequent 4 patients will receive IV infusions of 2 x 10^6 placenta-derived mesenchymal stem cells/kg.

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 is an open label, non-randomized, non-blinded, prospective clinical trial. Subjects will 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 is preceded by two intravenous infusions of hMSCs one week apart, 4 and 3 weeks prior to the second lung surgery. The dose is 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 wk interval after the first LVRS and prior to a second LVRS. Toxicity criteria will be evaluated by grade according to WHO.

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

5) 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 (92), which showed an overall response rate of 94% and complete remission rate of 77% among 32 patients who received hMSCs for acute GvHD.

6) 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.

7) 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, as did the forced expiratory volume (FEV1) 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) (93).

8) 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.

9) 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 is a single injection of 2×10^6 hMSCs/kg. The primary endpoint is time to kidney recovery, defined as a post-operative serum creatinine return to pre-operative baseline values within 30 days of dosing.

10) There are >10 clinical trials underway for the treatment of Diabetes 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.

Currently, there are no clinical trials underway studying the safety or efficacy of hMSCs in ALI/ARDS. 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 (94). 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 (93). Up to 5×10^6 cells/kg were administered in this study.

1.7 Potential Benefits and Risks to Human Participants

1.7.1 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 30-40%. Administration of hMSCs may have a benefit on mortality in this patient population. Study subjects may have a direct benefit from administration of hMSCs. 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.

1.7.2 Potential Risks

1.7.2.1 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.

1.7.2.2 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 or met or there is concern for worsening hemodynamics or oxygenation on the part of the study investigators.

1.7.2.3 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 hMSCs 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.

1.7.2.4 Risks of blood draws

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

1.7.2.5 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.

1.7.2.6 Mini-bronchoalveolar lavage (Mini-BAL)

In the Phase 2 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. We will not perform a mini-BAL in the Phase 1 dose escalation trial or prior to hMSCs

or placebo infusion in the Phase 2 trial since the focus of our Phase 1 and Phase 2 studies is safety, and since one of our pre-specified infusion associated adverse events is deterioration of oxygenation, which is also a known risk of the mini-BAL procedure. However, 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.

1.8 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 has been generated demonstrating the potential efficacy and safety of hMSCs administration for the treatment of ALI/ARDS. Based on these studies, we propose a Phase 1 dose escalation trial followed by a Phase 2 randomized, double blind, placebo controlled clinical trial of hMSCs for the treatment of moderate-to-severe ARDS, a condition that remains associated with mortality rates in the range of 30-40%.

2 Study Objectives

2.1 Primary Objective

The primary objective of this study is to assess the safety of intravenous infusion of hMSCs in patients with ARDS.

2.2 Secondary Objective

The secondary objectives of this study are to assess the potential efficacy of intravenous infusion of hMSCs in patients with ARDS and to acquire mechanistic data regarding the activity of hMSCs in patients with ARDS.

3 Study Design

This clinical study design starts with an open label dose escalation Phase 1 study that rolls into a randomized, double-blinded placebo-controlled Phase 2 study.

3.1 Phase 1 Study Design

Phase 1 is an open label dose escalation pilot study in which cohorts of subjects with ARDS will receive increasing doses of a single infusion of hMSCs. There are 3 cohorts with 3 subjects/cohort who will receive doses of 1 x 10^6 cells/kg, 5 x 10^6 cells/kg, and 10 x 10^6 cells/kg. The first subject in each cohort will receive an infusion and will be observed for 7 days prior to enrollment of the remaining subjects in that cohort. The scientific review committee (SRC) made-up of two investigators and the study medical monitor, will review a 7 day report of clinical data and adverse events from the first subject in the cohort by teleconference.

- If there are any concerns on the part of the SRC or if the first subject has met the stopping rule criteria (pre-specified infusion associated event or serious adverse event including death within 7 days), enrollment will be suspended pending review by the independent Data Safety and Monitoring Board (DSMB).
- If stopping rule criteria are not met, and there are no concerns by the SRC, the next two subjects in that cohort may be treated.

The second and third subjects in the cohort may be enrolled concurrently; however if the second subject experiences a pre-specified clinically important event or unexpected serious adverse event including death prior to enrollment/dosing of the third study subject, enrollment/dosing will be suspended pending review by the DSMB.

After completion of enrollment of each study cohort and 7 days of follow-up for all 3 individuals in the cohort, an aggregate 7 day report of clinical data and all adverse events will be reviewed by the SRC and DSMB by teleconference for each cohort prior to proceeding to the next dosing cohort.

After completion of the Phase 1 study (28 days of follow-up for all nine study subjects), the SRC will review the data and propose a cell product dose for the Phase 2 study. This recommendation will be submitted to the DSMB for approval prior to initiating the Phase 2 study. For planning purposes, Phase 2 has been designed using the higher dose of 10 x 10^6 cells/kg, assuming it will be the maximal tolerated dose.

Importantly, there is the possibility that patients may be enrolled in the study but not receive hMSCs due to clinical instability prior to the cell infusion (see **Section 7.4**); since this is a safety study, three subjects must be treated with hMSCs at each dose prior to any consideration of dose escalation by the SRC and DSMB. Therefore, additional subjects may be enrolled to complete the three subjects per dose.

3.2 Phase 2 Study Design

Phase 2 is a randomized, double-blinded placebo-controlled study using the 10 x 10^6 cell/kg dose of hMSCs or the maximal tolerable dose as determined by the DSMB. Subjects will be randomized in a 2:1 randomization scheme to receive hMSCs or Plasma-Lyte A placebo; the

study will enroll 60 patients who achieve a stable clinical baseline and receive study product (either hMSCs or the Plasma-Lyte A placebo) as described in **Section 7.4**.

The DSMB will review 28 day post infusion safety data after 20 and 40 subjects have been enrolled and received study product; enrollment will continue during DSMB review. During Phase 2, 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 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).

4 Endpoints

Analysis of the primary and secondary endpoints will be conducted on an as-treated basis since, as described in detail in **Section 7.4**, 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.

4.1 Primary Study Endpoint: Phase 1 and Phase 2

The primary study endpoint for both Phase 1 and Phase 2 will focus on the safety and tolerability of the hMSCs product. This analysis will be largely descriptive in nature for Phase 1, but will also examine the incidence of pre-specified infusion associated events and (2) unexpected severe adverse events in ARDS patients treated with hMSCs.

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 hMSCs infusion.

Pre-specified infusion associated events will be defined as:

Within 6 hours of the hMSCs infusion:

1. An increase in vasopressor dose greater than or equal to the following:
    - Norepinephrine: 10 mcg/min
    - Phenylephine: 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 or more to maintain Sp02 in the target range of 88-95%

5. Clinical scenario consistent with transfusion incompatibility or transfusion-related infection (e.g. urticaria, new bronchospasm)

Within 24 hours of the hMSCs 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.

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 the Phase 2 study, the primary endpoint will be the incidence of pre-specified infusion associated events occurring within 6 hours of hMSCs administration and any cardiac arrest or death occurring within 24 hours of hMSCs administration and unexpected severe adverse events in ARDS patients treated with hMSCs compared to patients treated with placebo.

4.2 Secondary Endpoints: Phase 2

We will test three categories of efficacy endpoints in the Phase 2 trial: respiratory, systemic and biologic.

1. **Respiratory**: Respiratory efficacy endpoints will include:
   - The ALI score [Lung Injury Score (LIS)] (**Appendix B**) at day 3, since improvement in the LIS has been shown to be associated with other clinical outcomes (5, 95, 96), including an increased number of ventilator free days and improved survival. The LIS is a composite scoring system including the PaO_2/FiO_2, the level of positive end-expiratory pressure, the extent of infiltrates on the chest radiograph, and static respiratory compliance.
   - The other respiratory efficacy endpoints will be the PaO_2/FiO_2 ratio and oxygenation index (OI) at day 3, which incorporates mean airway pressure and the PaO_2/FiO_2. OI is independently predictive of mortality in patients with ALI (97, 98).

2. **Systemic**: Efficacy endpoints will include:
   - Mean SOFA score (99) at day 3 as well as

- Ventilator-free, ICU-free, vasopressor-free, and organ failure free days and
- 60 day all-cause mortality, although this initial clinical trial of 60 patients will be underpowered for these endpoints.

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 randomization, 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.

To assess organ failure, we will measure serum creatinine (renal function), bilirubin (hepatic function), and platelet count (hematology) at day 0, 3, 7 and 14.

We will also calculate Organ failure days defined according to the most abnormal vital sign or clinically available lab value for each calendar day (with LOCF for missing values), according to the Brussels Organ Failure methodology using the clinically significant organ failure thresholds for cardiovascular, renal, coagulation and hepatic failure (100). Patients will be followed for development or resolution of organ failures to death, hospital discharge or study day 14, whichever comes first. Each day a patient is alive and free of an organ failure will be scored as an organ failure-free day. Any day that a patient is alive and free of all 4 organ failures will represent days alive and free of all organ failure.

3. **Biologic**: Biological endpoints will include:
   - A plasma biomarker of lung epithelial injury (RAGE), measured at baseline at day 3
   - Plasma pro- and anti-inflammatory markers (interleukin-6, interleukin-8, interleukin-10 and interleukin-1Ra), measured at baseline and day 3
   - Plasma biomarkers of endothelial injury (vWF, Ang-2), measured at baseline and day 3
   - Plasma markers of change in other organ function (creatinine), measured at baseline, day 3 and day 7
   - Plasma biomarkers that may reflect the paracrine activity of the administered hMSCs (Ang-1 and KGF), measured at baseline and day 3
   - A marker of lung epithelial permeability (total protein concentration in a mini-BAL obtained 48 hours after product infusion) as well as the total and differential cell count in the mini-BAL specimen in the Phase 2 study.

5 Study Population and Enrollment

5.1 Number/Source/Screening

The Phase 1 trial will enroll 9 patients over 6-9 months and the Phase 2 trial will enroll 60 patients over a 1-2 year interval. Patients with ARDS will be recruited from ICUs at multiple study centers.

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, etc; see **Appendix C** for a listing of the de-identified data to be collected on screened, non-enrolled subjects).

5.2 Inclusion criteria

Patients will be eligible for inclusion if they meet all of the below criteria. 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 < 200 with at least 8 cm H_2O positive end-expiratory airway pressure (PEEP). A patient may be included if the PaO_2/FiO_2 ratio < 200 with < 8 cm H_2O PEEP if there is a contraindication to increased PEEP: evidence of barotrauma (see Appendix K).
2. Bilateral infiltrates consistent with pulmonary edema on frontal chest radiograph, and
3. No clinical evidence of left atrial hypertension, or if measured, a Pulmonary Arterial Occlusion Pressure (PAOP) less than or equal to 18 mm Hg.

- "Acute onset" is defined as follows: the duration of the hypoxemia criterion (#1) and the chest radiograph criterion (#2) must be ≤28 days at the time of randomization.
- 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 (> 28 days). The findings of vascular redistribution, indistinct vessels, and indistinct cardiac borders are not considered "consistent with pulmonary edema".
- If a patient meets the first two 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, and the patient must still be within the 96-hour enrollment window.

5.3 Exclusion Criteria

1. Age younger than 18 years.
2. Greater than 96 hours since first meeting ARDS criteria per the Berlin definition of ARDS
3. Pregnant or breast-feeding
4. Prisoner
5. Presence of any active malignancy (other than non-melanoma skin cancer) that required treatment within the last 2 years
6. Any other irreversible disease or condition for which 6-month mortality is estimated to be greater than 50%
7. Moderate to severe liver failure (Childs-Pugh Score > 12)

Measure	1 point	2 points	3 points	Units
Bilirubin (total)	<34 (<2)	34-50 (2-3)	>50 (>3)	µmol/l (mg/dl)
Serum Albumin	>35	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*

8. Severe chronic respiratory disease with a $PaCO_2$ > 50 mm Hg or the use of home oxygen
9. Patient, surrogate, or physician not committed to full support (Exception: a patient will not be excluded if he/she would receive all supportive care except for attempts at resuscitation from cardiac arrest).
10. Major trauma in the prior 5 days
11. Lung transplant patient
12. No consent/inability to obtain consent
13. Moribund patient not expected to survive 24 hours
14. WHO Class III or IV pulmonary hypertension
15. Documented deep venous thrombosis or pulmonary embolism within past 3 months
16. No arterial line/no intent to place an arterial line
17. No intent/unwillingness to follow lung protective ventilation strategy or fluid management protocol
18. Currently receiving extracorporeal life support (ECLS) or high-frequency oscillatory ventilation (HFOV)

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: Greater than 96 hours would diminish the likelihood of efficacy
Criteria 3 and 4: Standard exclusion of vulnerable patient populations

Criteria 5 and 6: Those with active malignancies within the past 2 years or other irreversible disease or condition for which 6-month mortality is estimated to be greater than 50% will be excluded because these individuals are at an increased risk of death

Criteria 7, 8, 9 and 13: Intended to exclude patients unlikely to survive to the day 28 primary study endpoint or whose underlying condition or ventilation management complicates assessment of the secondary endpoint of VFDs.

Criteria 10 and 11: The pathogenesis of trauma-associated ARDS and ARDS post-lung transplant may be different than that of other forms of ARDS, and the intent is to focus on a group of severely ill patients with similar pathogenetic mechanisms to reduce heterogeneity
Criteria 14 and 15: Intended to exclude patients at increased risk if pulmonary arterial pressures increase after administration of MSCs.
Criteria 16: Given Phase 1/2 nature of study, continuous blood pressure monitoring is a mandatory component of the protocol.
Criteria 17 and 18: Intended to exclude patients in which the ECLS circuit or the use of alternative ventilation or fluid management strategies would complicate assessment of primary and secondary endpoints. Those on ECLS are also excluded because the effect of the ECLS circuit on MSC half-life is unknown.

5.4 Study Initiation Time Window

In addition to meeting inclusion criteria, patients must be enrolled within 96 hours of first onset of ARDS, as per the Berlin Definition of ARDS:

- Bilateral opacities not full explained by effusions, lobar/lung collapse, or nodules; PaO2/FIO2 < 300 mm Hg with PEEP or CPAP ≥ 5 cm H2O not fully explained by cardiac failure or fluid overload; within 1 week of known clinical insult or new/worsening respiratory symptoms.

All ARDS criteria must occur within the same 24-hour period. The onset of ARDS is when the last criterion is met. Subjects who meet inclusion criteria must be enrolled within 96 hours of first meeting criteria for ARDS, and the MSC infusion must be initiated within 120 hours of first meeting criteria for ARDS.

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 and the fluid management strategy must be initiated within one and four hours respectively (if not already being utilized). The fluid management strategy will be held for the four hours prior and the six hours after the MSC infusion.

5.5 Subject Recruitment and Informed Consent

Study subjects will be recruited in the ICU by a study investigator. Because these study subjects may be unable to consent for themselves, written informed consent will be obtained from a surrogate where appropriate. Written informed consent will be obtained by one of the study investigators before enrollment in the trial. No study procedures will be conducted before obtaining informed consent. 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.

5.6 Randomization

For the Phase 2 trial, after informed consent is given, an assignment will be made by computer-generated randomization to administer either hMSCs therapy or placebo with a 2:1 allocation to the hMSCs:placebo arms. Randomization will occur at the start of the 2 hour planned baseline stability period and must occur in time to allow the study team to initiate the study infusion within 120 hours. The study participant must have an arterial blood gas with a PaO_2/FiO_2 ratio <

200 with PEEP ≥ 8 cm H_20 within 6 hours of randomization. (The PEEP may be < 8 cm H_2O if there is a contraindication to higher PEEP as described in Appendix K.)

5.7 Early Withdrawal of Subjects

5.7.1 When and How to Withdraw Subjects

Since the study treatment is a single infusion of hMSCs, 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 or after hMSCs administration. As described below, if a subject or surrogate withdraws consent for study participation after hMSCs administration, we will work with them to allow collection of follow-up data to ensure safety.

5.7.2 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.

6 Study Drug

6.1 Description

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

The hMSCs are formulated in DMSO (10%), human serum albumin (5%) and Plasma-Lyte A (30%), and cryopreserved and stored in bags suspended in liquid nitrogen.

Immediately prior to administration, the product is thawed, washed, and re-suspended in Plasma-Lyte A, a clinical-grade infusible solution for intravenous administration. The product is not further cultured or propagated prior to administration.

The placebo group will be treated with an equivalent volume of Plasma-Lyte A.

6.2 Treatment Regimen

For this trial, a single dose of the hMSCs product or placebo Plasma-Lyte A will be administered intravenously over approximately 60-80 minutes. All patients will receive one dose.

The infusion will be administered using a standard clinical infusion pump using a standardized procedure (see **Appendix G**).

6.3 Preparation and Administration of Cellular Product

Allogeneic, unrelated donor hMSCs will be manufactured by:

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

Cells will be shipped frozen to the clinical site.

6.3.1 Cellular Product Preparation

Just prior to use, the cellular product will be thawed, washed and prepared for intravenous administration in the clinical site's clinical bone marrow transplant (BMT) facility (or equivalent) by facility technicians. See **Appendix F** for cellular product preparation instructions.

6.3.2 Placebo Product Preparation

The placebo for the Phase 2 trial will be the same volume of Plasma-Lyte A (100 mL). See **Appendix F** for cellular product preparation instructions.

6.4 Subject Compliance Monitoring

The hMSCs will be administered as a single intravenous dose over 60-80 minutes on the date of the study enrollment. Study personnel will be on site for safety and compliance monitoring for the baseline stability period, and for 6 full hours starting from the time the hMSC infusion is initiated.

6.5 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. Patients will not be permitted to be enrolled in other experimental therapy trials while participating in this study.

6.6 Blinding of Cellular Product

As in prior studies of hMSCs (93), 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.

6.7 Receiving, Storage, Dispensing and Return

6.7.1 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 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.

6.7.2 Storage

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

6.7.3 Dispensing of Cellular Product

The hMSCs 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 F**).

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.

6.7.4 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.

7 Study Procedures

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

7.1 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 approached and asked if we have permission to approach the patient and their surrogate for potential study participation.

7.2 Informed Consent and Randomization

Informed consent will be obtained from each patient or surrogate before enrollment in the trial. No study procedures will be conducted before obtaining informed consent. For the Phase 2 study, randomization will occur after informed consent as described in **Sections 5.5 and 5.6**. An arterial blood gas must be obtained to confirm a $PaO_2/FiO_2 < 200$ on PEEP $\geq$ 8 cm H_20 within 6 hours of randomization. (The PEEP may be < 8 cm H_2O if there is a contraindication to higher PEEP as described in Appendix K.)

If a pregnancy test is not available before informed consent, a blood or urine pregnancy tests will be obtained in women of childbearing age 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.

7.3 Baseline Study Procedures

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

If the patient does not have an arterial line, an arterial line will be placed prior to administration of the hMSCs.

7.4 hMSC Administration

The hMSCs will be administered intravenously according to the following dose schedule. The hMSCs will be administered intravenously over approximately 60-80 minutes.

In the P**hase 1 trial**, we will enroll 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 will use a standard dose escalation model, with a plan for safety assessments as detailed in **Section 3.1**.

B. The initial dose will be 1 x 10^6 cells/kg given intravenously to 3 patients. After approval from the DSMB, we will progress to the next dose. The second dose delivered will be 5.0 x 10^6 cells/kg given intravenously to 3 patients. The third dose level will be 10.0 x 10^6 cells/kg given intravenously to 3 patients.

In the **Phase 2 trial,** we will enroll 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 will be conducted by the independent DSMB, and the sponsor and investigators will remain blinded. The safety reviews will be conducted after treatment of 20 and 40 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 able to 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. If the patient is on vasopressin, investigators will be instructed not to titrate the vasopressin dose during this 2-hour period.

B. The following patients will be considered clinically unstable and will **NOT** receive hMSCs:

   a. Patients requiring an FiO_2 of > 0.8 or a PEEP of > 20 cm H_2O, in order to maintain transcutaneous oxygen saturation 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 epinephrine for blood pressure support
   OR
   c. Patients who do not meet stability criteria in the supine position, as baseline stability period and infusion must occur in the supine position

C. Patients who are clinically unstable will be monitored closely; if they achieve a stable baseline within 120 hours of meeting the entry criteria for the study and at the time of the stable baseline still meet criteria for ARDS ($PaO_2/FiO_2 \leq 300$ with bilateral infiltrates and no evidence of left atrial hypertension) with a PEEP of ≥ 8 (or lower PEEP if there is evidence of barotrauma), they can receive the cell product. Thus, patients may be enrolled within 96 hours of first meeting criteria for ARDS and then there is a 24-hour window during which hMSC therapy can be administered after the enrollment window if the patient achieves a stable baseline.

D. An arterial blood gas will be required prior to the hMSC infusion, during the last 30 minutes of the baseline stability period. Patients will not receive the MSC infusion if the PaO_2/FiO_2 ratio is greater than 300 mmHg on this pre-infusion arterial blood gas. Arterial blood gases will also be obtained at the end of MSC infusion (~1 hour) and 4 hours thereafter.

E. During infusion of hMSCs, patients will have continuous monitoring of arterial blood pressure, heart rate, rhythm as well as oxygen saturation. Study personnel will be available for the duration of the infusion to monitor the patient. Body temperature will be monitored at a minimum at the start, midway through, and at the end of the infusion. 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 hMSCs 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.

7.5 Ventilator Management

Ventilator management, including weaning, will follow the modified ARDS Network lower tidal volume (6 ml/kg PBW) protocol (**Appendix E**). Using this ventilator management protocol will

standardize the application of PEEP, which is a component of one of the respiratory efficacy endpointss, the LIS, thus reducing the potential for bias. If not already being used, this low tidal volume protocol for mechanical ventilation must be initiated within one hour of informed consent for the Phase 1 clinical trial and within one hour of randomization for the Phase 2 clinical trial. 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 E**).

7.6 Concomitant Therapy

Patients will be managed with a conservative fluid management protocol. The fluid management strategy will be held for the four hours prior and the six hours after the MSC infusion to reduce the likelihood that the fluid management strategy impacts hemodynamic stability around the time of the MSC infusion. We will record the use of other pharmacologic agents that are sometimes used by clinicians in the treatment of ARDS, including glucocorticoids and inhaled vasodilators. We will also record if patients are turned into a prone position or receive alternative non-pharmacologic strategies such as high frequency ventilation or extra-corporeal membrane oxygenation.

7.7 Biospecimens for Biological Endpoint Measurements

Blood and urine samples will be obtained before the infusion of study product, then 6 hours and 1, 2, 3, and 7 days (+/- 2 days for last sample only) after the initiation of the study product infusion for biomarker measurements, which include measurements of epithelial injury, inflammation and of hMSC administration. Plasma obtained from two 10 ml EDTA anti-coagulated blood samples will be divided immediately after centrifugation into 0.5 mL and 1 mL aliquots and frozen at –70°C. Urine obtained from the patients 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 –70°C. Finally, the blood cell pellet from the baseline blood specimen will be collected for DNA banking.

A mini-BAL will be performed 2 days (48 hours) after the initiation of the study product infusion in patients in the Phase 2 clinical trial (See **Appendix A**, Time-Events Schedule) for total protein measurement, a marker of lung epithelial permeability.

7.8 Day 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). In addition, patients will have an arterial blood gas and chest x-ray on day 3 (after administration of the study cellular product) since these are secondary endpoints of the Phase 2 trial. 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.

7.9 Day 7 Measurements

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 for safety monitoring if they are still hospitalized.

7.10 Day 14 Measurements

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 14 (+/- 1 day) after administration of the study product for safety monitoring if they are still hospitalized.

7.11 Day 28 Measurements

Patients will be followed daily for adverse events through day 28, death or hospital discharge, whichever occurs first. If a subject is discharged before day 28, investigators will follow-up by phone with the study subject on day 28 to ensure that no adverse events have occurred. Decisions about hospital discharge will not be made by study personnel; rather such decisions will be made by clinicians caring for the study subject and for the underlying condition that led to the hospitalization.

7.12 Post-hospitalization Follow-up

Vital status as well as the need for dialysis will be collected at 6 and 12 months after study enrollment. This data will be collected at 6 months via a structured telephone interview and at 12 months via an in-person follow up visit with a limited history and physical examination.

8 Data Collection

8.1 Medical History

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

1. Demographic and admission data
2. Pertinent medical history and physical examination
3. Height; gender, measured body weight; calculated predicted body weight
4. Time on ventilator prior to enrollment
5. Type of Admission
   a. Medical
   b. Surgical scheduled
   c. Surgical unscheduled
   d. Trauma
6. Acute or chronic renal failure and use of dialysis
7. Alcohol Use Disorders Identification Test (AUDIT) tool (**Appendix D,** to be answered by patient/surrogate)
8. Survey of smoking history including (if need be, will ask patient/surrogate for answers):
   • Ever smoker (> 100 cigarettes in lifetime)?
   • If yes, current smoker?
   • Estimate of pack years (# packs per day) x (# years smoked)
   • If former smoker, when did the subject quit smoking?

8.2 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.

8.2.1 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 (including non-invasive ventilation), tidal volume, FiO_2 and PEEP, inspiratory plateau pressure, and mean airway pressures. If on a pressure-cycling mode, peak pressure during inspiration will be assumed to be the plateau pressure.
4. Arterial PaO_2, $PaCO_2$, pH and transcutaneous oxygen saturation
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 7.3** if not available from testing obtained as part of clinical care.

8.3 Assessment after Enrollment: Determination of Stable Baseline for hMSCs Administration, 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 hMSC infusion:

1. Respiratory: FiO_2, PEEP, transcutaneous oxygen saturation; arterial blood gas pH, PaO_2 and $PaCO_2$ and 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 in the last 30 minutes of the baseline stability period, at the end of the hMSC infusion (~1 hour), and 4 hours after the initiation of MSC infusion; additional blood gases will be recorded if obtained as part of clinical care as above.

8.4 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.

8.4.1 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 with the patient supine.

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 days 0-14
5. Vasopressor use (Y/N), worst systolic BP, creatinine, bilirubin, and platelet count for the day. The date and value of the highest creatinine between days 15-28

6. Safety laboratory studies; all creatinine and bilirubin measurements will be recorded as above. Creatinine, bilirubin and ALT will be measured and recorded as a safety labs on day 7 if not obtained as part of clinical care
7. Frontal chest radiograph – Lung Injury Score
8. Suspected or known infection

8.4.2 Specimen Collection

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

8.5 Endpoint Determinations

3. Vital status at 28 days or until discharged home on unassisted breathing.
4. Brussels Organ dysfunction failures at days 0-14
5. SOFA scores at 3, 7 and 14 days
6. Time of initiation of unassisted breathing (assuming a patient achieves 48 consecutive hours of unassisted breathing)
7. Need for re-instituting assisted or mechanical ventilation after achieving 48 consecutive hours of unassisted breathing
8. Need for, timing, and duration of dialysis; change in renal function as measured by creatinine
9. Status 48 hours after initiation of unassisted breathing
10. ICU length of stay in calendar days including ICU days after readmission to ICU.
11. Hospital length of stay in calendar days and discharge disposition (home, other facility, with or without assisted ventilation)

8.6 Assessments After Hospitalization

Vital status as well as the need for dialysis will be collected at 6 and 12 months after study enrollment. We will collect this data through telephone interviews with patients at 6 months and an in-person follow up visit with a limited history and physical examination at 12 months.

9 Statistical Plan

9.1 Sample Size Determination

The sample size for the Phase 1 study will be 9 patients, 3 cohorts of 3 patients with escalating doses of hMSCs. The sample size for this portion of the study was determined based on the number of escalating doses of hMSCs that we planned to study and a reasonable number of patients in each cohort to detect a safety signal. The sample size for the Phase 2 portion of the trial will be 40 patients in the hMSCs arm and 20 patients in the placebo arm, for a total of 60 patients. This sample size was determined on the basis of feasibility and in order to generate enough clinical and biological data to aid in sample size projections for subsequent Phase 2b/3 clinical trials.

9.2 Statistical Methods

The primary focus of both the Phase 1 and Phase 2 trials is safety. Therefore, the primary statistical analyses will be largely descriptive. In Phase 1, we will describe the baseline characteristics of treated subjects using standard statistical tests, including the Pearson's chi-square test for categorical variables, analysis of variance and Student's t-test for continuous variables, and Kruskal-Wallis or other non-parametric tests for non-normally distributed variables. We will describe the incidence of serious adverse events, including death, as well as the incidence of pre-specified infusion associated events and non-serious adverse events felt to be related to the study infusion. Since up to 3 cohorts of subjects will receive escalating doses of hMSCs, as detailed in the full Statistical Analysis Plan, we will compare the incidence of adverse events by treatment cohort using the Pearson's chi-square test or the Student's t-test to compare the mean number of adverse events by treatment cohort, depending on the number of AEs observed.

For the Phase 2 study, the group treated with hMSCs will be compared to the placebo control group using a variety of baseline patient characteristics, including socio-demographic characteristics (age, gender, race), smoking history, acute illness severity (APACHE III scores, non-pulmonary organ failures), and severity of ALI (oxygenation, respiratory compliance, airway pressures, the 4-point lung injury score). Here, we will describe the incidence of serious adverse events, including death, as well as the incidence of pre-specified infusion associated events and non-serious adverse events felt to be related to the study infusion in the hMSC-treated versus placebo arms. Analysis of the primary safety endpoint will be focused on characterizing the AE proportion in each treatment arm, whereas the secondary efficacy endpoints will be used for the design of larger efficacy studies. We will again compare the incidence of adverse events by treatment cohort using the Pearson's chi-square test or the Student's t-test. The per-treatment arm sample sizes were generated based on an assumption of a 28-day AE proportion of 30%. In this setting, the 95% confidence interval (CI) length for a binomial proportion is 24% ranging from 19% to 43%.

The Phase 2 part of this trial is not adequately powered for clinical efficacy endpoints such as mortality and ventilator free days and has limited power for physiological endpoints since the primary focus of the Phase 2 study is safety. If hMSCs are safe, a larger Phase 2b study will be

needed to better define the potential treatment effect size of administration of hMSCs on clinical efficacy endpoints to inform a Phase 3 clinical trial.

Therefore, the focus of the statistical analysis of secondary endpoints for the Phase 2 trial will be to test the effect of hMSCs on respiratory, systemic and biological endpoints that will reflect the effect of hMSCs on the severity of lung injury. This analysis will be based on an approach using standardized effect sizes (the difference in mean values between treatment and control divided by the standard deviation). This approach allows us to evaluate the efficacy of hMSCs in a small sample size by comparing the effect size observed in our small Phase 2 trial to effects observed in larger trials of therapies known to be efficacious. In the case of ARDS, the comparison trials would be those of lower tidal volume ventilation (4) and fluid conservative therapy (5). We will use the incidence of adverse events along with an overall assessment of the potential efficacy of hMSCs using the pre-specified respiratory, clinical and biological endpoints to make a determination as to whether or not a larger Phase 2b study is safe and warranted.

9.3 Subject Population(s) for Analysis

The subject population for all study analyses will be the all-treated population, that is, any subject randomized into the study who at least starts to receive hMSCs or placebo, regardless of whether or not the infusion is completed. This population has been selected instead of an all-randomized population because study subjects must achieve a clinically stable baseline prior to study drug infusion. It is possible that some enrolled subjects may never meet these criteria for clinical stability and thus would never be treated. Therefore, the all-treated population analysis will most accurately reflect the effects of the study infusion and allow us to analyze sufficient subjects who receive the study drug to detect a safety concern, if one exists.

10 Safety and Adverse Events

10.1 Definitions

Investigators will determine daily if any clinical adverse experiences occur during the period from enrollment through study day 28. The investigator will evaluate any changes in laboratory values and 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 the study product, regardless of the "expectedness" of the event for a patient with ALI.
   or:
2. The following pre-specified infusion associated events that occur 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
     - Phenylephine: 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 or more to maintain Sp02 in the target range of 88-95%
   - Clinical scenario consistent with transfusion incompatibility or transfusion-related infection (e.g., urticaria, new bronchospasm)
3. Any cardiac arrest or death occurs within 24 hours from the start of study product will be reported as a pre-specified infusion-associated significant event. Any cardiac arrest or death occurs during the study period will be a reportable event that will be reviewed in detail.

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 (101):

- 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; and 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 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.

10.2 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 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 forms. 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 up 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 10.3**).

10.3 Reporting of Serious Adverse Events and Unanticipated Problems

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

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

10.3.2 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.

10.3.3 Sponsor Reporting: Notifying the DSMB

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

It is the responsibility of the study sponsor to notify all participating investigators, in a written IND safety report, of any adverse event associated with the use of the drug that is both serious and unexpected. Additionally, sponsors are also required to identify in IND safety reports all previous reports concerning similar adverse events and to analyze the significance of the current event in light of the previous reports. IND safety reports will be sent to the participating investigators immediately after submission to the IND.

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

10.4 Unblinding Procedures for Phase 2

The study will have an external Medical Monitor separate from the Investigator/Sponsor and who will not be affiliated with any of the study sites or participate in any study procedures (e.g.,

screening of subjects, consenting of subjects or surrogates, administration of hMSCs or study follow-up). If unblinding the study therapy is necessary to ensure a subject's safety, this will be done by the external medical monitor after review of the clinical events. If this is done in conjunction with the site Principal Investigator, the study sponsor must be informed within 24 hours by phone, fax or email of the unblinding event, followed by a detailed written narrative within 48 hours of the event by the Principal Investigator at the study site.

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

10.5 Stopping Rules

Phase 1 is an open label dose escalation pilot study in which cohorts of subjects with ARDS will receive increasing doses of a single infusion of hMSCs. We plan 3 cohorts with 3 subjects/cohort who will receive doses of 1 x 10^6 cells/kg, 5 x 10^6 cells/kg, and 10 x 10^6 cells/kg. The first subject in each cohort will receive an infusion and will be observed for 7 days prior to enrollment of the remaining subjects in that cohort. A Scientific Review Committee (SRC) made up of two investigators and the study medical monitor, will review a report of clinical data and adverse events from the first subject in the cohort by teleconference.

- If no pre-specified infusion associated event or unexpected serious adverse event including death is observed and if review of the case report forms does not show any concerning events or trends, the SRC may recommend that the next two subjects in that cohort may be treated.
- The SRC will document in writing the rationale for continuing the dosing within that cohort. The second and third subjects in the cohort may be enrolled concurrently.
- If there are any concerns on the part of the SRC or if the first subject has a protocol-specified infusion-related adverse event or any serious adverse event including death, enrollment will be suspended pending review by the DSMB.
- If the second subject experiences a pre-specified infusion associated event or unexpected serious adverse event including death prior to enrollment of the third study subject, enrollment will be suspended pending review by the DSMB.
- If a subject experiences a pre-specified infusion associated event or unexpected serious adverse event including death at a given dose of hMSC, the DSMB will have the discretion to add another subject to the cohort at the same dose or to determine that the maximum tolerable dose has been achieved.
- If another subject is added to the cohort, that subject must have 7 days of follow-up without a pre-specified infusion associated event or unexpected serious adverse event prior to enrollment of the next subject in the cohort.
- Finally, dose escalation cannot proceed unless 3 subjects are successfully enrolled at a given dose without a pre-specified infusion associated event or unexpected serious adverse event including death prior to day 7.

After completion of enrollment of each study cohort, an aggregate report of clinical data and all adverse events will be reviewed by the SRC and DSMB by teleconference prior to dose escalation to the next cohort

After completion of the Phase 1 study, the SRC will review all of the data and along with the principal investigator propose a cell product dose for the Phase 2 study. The maximum tolerable dose will be defined as the dose that was tolerated by three subjects as defined above. This recommendation will be reported to the DSMB for approval prior to the Phase 2 study.

For the Phase 2 study, the DSMB will review 28 day post infusion safety data after 20 and 40 subjects have been enrolled and received study product; however, enrollment will not be suspended pending DSMB review. During Phase 2, 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 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 Phase 2 study will end when 60 patients have been recruited and received the study product and when all 60 subjects have completed 28-day follow-up, after which data analysis will commence. The Phase 2 study will stop prior to completion if mandated by the NHBLI-appointed DSMB or the FDA due to safety concerns.

10.6 Medical Monitoring

It is the responsibility of the Principal Investigator 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 external medical monitor who will be available to investigators to independently evaluate adverse events and to assist with unblinding, if this becomes necessary.

10.6.1 Independent Data and Safety Monitoring Board

Both the Phase 1 and Phase 2 studies will be monitored by an independent DSMB. The DSMB will contain at least 3 members, 2 intensivists familiar with the care of patients with ARDS and a biostatistician. A DSMB Charter will define the DSMB's responsibilities as described below.

The DSMB will meet by teleconference prior to the start of the Phase 1 trial, after each cohort of 3 patients is completed (and earlier if needed) during Phase 1, and at the planned interim safety analyses (20 and 40 patients) for the Phase 2 trial. At these meetings, the DSMB will review all safety data in aggregate; individual adverse event narratives will also be available to the DSMB for review. At the end of the Phase 1 trial, the DSMB will also review in data the hemodynamic and oxygenation data from Phase 1 subjects around the time of the hMSCs infusion. During the Phase 2 trial, the DSMB will review the safety data in an unblinded fashion.

As described in detail in the prior section, the dosing in a given cohort be stopped for DSMB review if a subject has a pre-specified infusion associated event or unexpected serious adverse event (including death) during the Phase 1 component of the study; the DSMB will also receive

reports of serious, unexpected, and study-related adverse events and unanticipated problems on an ongoing basis.

The external medical monitor will serve as the liaison between the DSMB and the study sponsor and Principal Investigator. The actual analyses will be conducted by the Clinical Coordinating Center Biostatistician; who will ensure that the unblinded analyses are not available to study investigators.

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

11 Data Handling and Record Keeping

11.1 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.

11.2 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.

11.3 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.

11.4 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.

12 Study Monitoring, Auditing, and Inspecting

12.1 *Study Monitoring Plan*

The data safety and monitoring plan for this Phase 1/2 trial will include a formal DSMB that will be approved by the IRB at the University of California, San Francisco. Our data safety monitoring board will include a critical care physician, a cell products specialist with expertise in Phase 1/2 clinical trials, and an ethicist. None of the DSMB members will be affiliated with this study or have a conflict of interest. Members will be from both the UCSF faculty and at least one outside institution. 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 UCSF IRB, the NHLBI and the FDA. Since this is a Phase 1 clinical trial focused on safety and since the patient population is critically ill and at high risk for complications related to their underlying severity of illness, we will also have a designated external medical monitor. The person will assist the PI with adjudication of severe adverse events on a case-by-case basis without potential conflict of interest.

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

12.2 Auditing and Inspecting

The investigator will permit study-related monitoring, audits, and inspections by the IRB, 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., pharmacy, diagnostic laboratory, etc.).

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

13 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 a properly constituted independent IRB, in agreement with local legal prescriptions, for formal approval of the study conduct. The decision of the IRB 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 investigator should provide a list of IRB members and their affiliate to the sponsor.

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 IRB for the study. The formal consent of a subject, using the 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.

14 Study Finances

14.1 Funding Source

This study is financed through a grant from the National Institutes of Health National Heart Lung, and Blood Institute.

14.2 Conflict of Interest

Any investigator who has a conflict of interest with this study (patent ownership, royalties, or 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. All University of California investigators will follow the University conflict of interest policy.

15 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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17 Appendices

A. Time-Events Schedule
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Appendix A: Time-Events Schedule

Measurement/Event	Day 0			1	2	3	4	5	6	7	8	9	10	11	12	13	14	28	6m	12m
	Prior to randomization	Prior to MSC infusion[&]	During and after MSC infusion																	
Demographics, History & Physical, Height, Weight	X																			
Etiology of ARDS, site of sepsis if septic etiology	X																			
HCG (in females of childbearing age)	X																			
Alcohol Use Disorders Identification Test (AUDIT)	X																			
APACHE III Score [C]	X																			
Vital Signs (HR, SBP, DBP, MAP, Temp °C) *$	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			
Fluids (In and Out) *	X	X	X	X	X	X	X	X	X	X										
Modified Brussels Score and Brussels Organ Dysfunction Failure[B] ~	X			X	X	X	X	X	X	X	X	X	X	X	X	X	X			
Sequential Organ Failure Score	X			X	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) and SpO_2[D]	X	X	X	A	A	X	A	A	A	A										
Creatinine[E]	X			A	A	X	A	A	A	X	A	A	A	A	A	A	X	A		
Chest X-ray (# quadrants for lung injury score)	X			A	A	X	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			
Suspected or known site of infection	X									X							X			
Total bilirubin, ALT, platelets[F]	X					X				X							X			
Blood and urine for cytokines, mediators and markers of inflammation[G]	X		X	X	X	X				X										
Blood cell pellet for DNA banking (if consent obtained)	X																			
Mini-BAL (Phase 2 only)					X															
Vital Status §																		X	X	X
Need for, timing and duration of dialysis																		X	X	X

X=Required; A=When available; [C]=Labs not available in the 24 hours before randomization must be obtained; *= Data gathered at times indicated or until 48 hours UAB, whichever occurs first
[B]=Record clinically available creatinine, platelets, bilirubin, SBP and vasopressor use; ~=Data gathered on days 0-28 or until d/c from study hospital; #=Measure during reference period (0600-1000); other values may be obtained closest to 0800 on the specified calendar date; §=Measure at 28 days, 6 months and 12 months; the 12 month follow up visit will be an inperson visit with a focused history and physical examination
[D]= ABG mandatory at the following timepoints: within 6 hours of randomization, prior to the start of the infusion (during the final 30 minutes of the baseline stability period); within 15 minutes of the end of MSC infusion (~1 hour) and 4 hours after the initiation of the infusion; [E]=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; [F]=Data required prior to randomization, day 3 (+/-1 day), day 7 (+/-1 day) and day 14 (+/-1 day); [$]=Data recorded every 15 minutes for at least two hours during "stable baseline" period and until the initiation of study drug infusion, every 15 minutes for the duration of the infusion and every hour until 6 hours from the initiation of study drug infusion; [G]= Blood and urine specimens are required before the hMSC infusion, at 6 hours and 1, 2, 3, 7 days (+/- 2 day) after the initiation of the study product infusion.

Appendix B: Lung Injury Score

The Lung Injury Score (Murray JF *et al*., 1988) is a validated 4-point score based on chest radiograph findings, PaO2/FiO2 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)

Reference: Murray JF, Matthay MA, Luce JM, *et al*. An expanded definition of the adult respiratory distress syndrome. Am Rev Respir Dis 1988;138:720–3; erratum 1989;139:1065.

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. Was there evidence of left atrial hypertension?
8. Month of the year that patient met screening criteria
9. Gender
10. Ethnicity
11. Age (if age >89, 89 will be entered for age)
12. Patient location (e.g. MICU, SICU, etc.) and if regularly screened
13. Reason(s) patient excluded from study
14. If not excluded, not enrolled, why?
15. Lung injury category (e.g. sepsis, pneumonia)

Appendix D: Alcohol Use Disorder Identification Test (AUDIT)

The Alcohol Consumption Questionnaire is important to administer because there is a common association between alcohol abuse and ARDS (Moss M *et al*., 1996). It will be important to have this information for a subgroup analysis.

1. How often do you have a drink containing alcohol? (0) Never [Skip to Qs 9-10] (1) Monthly or less (2) 2 to 4 times a month (3) 2 to 3 times a week (4) 4 or more times a week ☐	6. How often during the last year have you needed a first drink in the morning to get yourself going after a heavy drinking session? (0) Never (1) Less than monthly (2) Monthly (3) Weekly (4) Daily or almost daily ☐
2. How many drinks containing alcohol do you have on a typical day when you are drinking? (0) 1 or 2 (1) 3 or 4 (2) 5 or 6 (3) 7, 8, or 9 (4) 10 or more ☐	7. How often during the last year have you had a feeling of guilt or remorse after drinking? (0) Never (1) Less than monthly (2) Monthly (3) Weekly (4) Daily or almost daily ☐
3. How often do you have six or more drinks on one occasion? (0) Never (1) Less than monthly (2) Monthly (3) Weekly (4) Daily or almost daily *Skip to Questions 9 and 10 if Total Score for Questions 2 and 3 = 0* ☐	8. How often during the last year have you been unable to remember what happened the night before because you had been drinking? (0) Never (1) Less than monthly (2) Monthly (3) Weekly (4) Daily or almost daily ☐
4. How often during the last year have you found that you were not able to stop drinking once you had started? (0) Never (1) Less than monthly (2) Monthly (3) Weekly (4) Daily or almost daily ☐	9. Have you or someone else been injured as a result of your drinking? (0) No (2) Yes, but not in the last year (4) Yes, during the last year ☐
5. How often during the last year have you failed to do what was normally expected from you because of drinking? (0) Never (1) Less than monthly (2) Monthly (3) Weekly (4) Daily or almost daily ☐	10. Has a relative or friend or a doctor or another health worker been concerned about your drinking or suggested you cut down? (0) No (2) Yes, but not in the last year (4) Yes, during the last year ☐
If total is greater than recommended cut-off, consult User's Manual.	Record total of specific items here ☐

Reference: Moss M, Bucher B, Moore FA, *et al.* The role of chronic alcohol abuse in the development of acute respiratory distress syndrome in adults. JAMA 1996; 275:50-54

Appendix E: 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. If airway pressure release ventilation (APRV) is used, tidal volume is defined as the sum of the volume that results from the ventilator pressure-release and an estimation of the average spontaneous V_T.
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 remains 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 12 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, or room air, OR
2. T-tube breathing, OR
3. Tracheostomy mask breathing, OR
4. CPAP ≤ 5 without PS or IMV assistance
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 F: Clinical Cell Wash Protocol

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

Clinical Cell Wash Protocol

Document Date:

21 January 2014

To be used in support of

Protocol Title:	A Phase 1/2, Randomized, Double-blind, Placebo-controlled, Multi-center Clinical Trial of Allogeneic Bone Marrow-derived Human Mesenchymal Stem Cells for the Treatment of Acute Respiratory Distress Syndrome
Protocol Number:	UCSF-hMSC-ARDS-P1P2-06
Sponsor-Investigator:	Michael A. Matthay, MD University of California, San Francisco

1. Background

Allogeneic, bone marrow-derived human mesenchymal stem 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 DMSO (10%), human serum albumin (5%) and Plasma-Lyte A (30%).

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, washed and prepared 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 protocol. The laboratory-specific standard operating procedure at each site will be reviewed by the 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 Coordinating Center will share the SOP developed by the UCSF Clinical Bone Marrow Transplant Facility.

2 Reagents, Supplies and Equipment

Each Clinical Bone Marrow Transplant Laboratory supporting this clinical trial will develop their own check list of reagent, supply and equipment checklist as part of the laboratory-specific standard operating procedure. These will include but are not limited to:

Reagents:
- Plasma-Lyte A (Baxter) for cell wash and resuspension
- Trypan blue for cell counts

Supplies:
- Sterile cell transfer pack, 300 mL
- Blood culture bottles for sterility testing

Equipment:
- Balance
- Centrifuge
- Heat sealer
- Hemostat
- Hematocytometer
- Laminar Flow Hood
- Microscope
- Plasma Expressor
- Water Bath

3 Procedure

3.1 Equipment Preparation

3.1.1 Confirm that the water bath is ready and fill with sterile water according to local practices. The water bath should be maintained according to local practices to avoid microbiological contamination. This protocol will be reviewed by the Sponsor prior to enrollment of the first patient.

3.1.2 Bring water bath to 37°C.

3.1.3 Turn on and clean biocontainment hood before use.

3.1.4 Turn on centrifuge. Set to refrigerated temperature of 2-5°C and spin for 15 minutes at 400g. Verify temperature and rpms during initial use.

3.1.5 All calculations will be done on a hMSC Calculation Worksheet, which will be retained by the Clinical Bone Marrow Transplant Facility as a source document.

3.2 Thawing of hMSCs

3.2.1 Locate appropriate bags of hMSCs (stored in liquid nitrogen in the Clinical Bone Marrow Transplant Facility).

> **NOTE: Prior to thawing, laboratory staff must calculate the number of cells needed for the study subject based on predicted body weight, and they must determine the number of cell bags they will need to thaw to have this dose of cells available. No more than 1 x 10^9 cells (100 kg x 10 x 10^6 cells/kg) will be administered to any subject.**
>
> At the same time, the Clinical Bone Marrow Transplant Facility will calculate the amount of endotoxin present in the original hMSC product based on the lot release information supplied by the University of Minnesota. The acceptance limit is <5.0 EU/Kg.

3.2.2 Cells should not be thawed until the laboratory has staff available to start the wash procedure immediately after the thaw procedure is complete. Thaw time, time wash was completed, and infusion start/stop times will be recorded.

3.2.3 Remove bag from cassette and place into sealable plastic bag.

3.2.4 If either the product bag or sealable outer bag has leaks or cracks, do not use it and replace with new bag.

3.2.5 Immerse the sealed zip bag containing the frozen hMSCs in the water bath, gently rocking the bag to mix the suspension during the thawing.

3.2.6 Aseptically transfer the thawed bag into the hood.

3.3 General Washing Instructions for hMSCs

3.3.1 The cells should be washed according to the standard protocol of the local Clinical Bone Marrow Transplant Facility (or equivalent). This protocol will be reviewed by the Sponsor prior to enrollment of the first patient.

3.3.2 The general principles of the wash procedure are as follows:

(1) Cells should be transferred from the original bag to a 300 mL transfer pack for centrifugation under sterile conditions, along with the appropriate volume of Plasma-

Lyte A for the wash. If multiple bags of cells are thawed, pool cells prior to the wash procedure.

(2) Cells should be placed in the centrifuge and centrifuged 400g for 15 minutes at 2-5˚C.

(3) Supernatant should be removed aseptically using a plasma expressor. The laboratory should determine ahead of time what the maximum volume of supernatant that will be removed is and this should be part of the standard operating procedure developed by the laboratory.

(4) Cells should be resuspended in Plasma-Lyte A to a product of 100 mL

(5) Remove a 5.0 mL sample of **POST-Wash/Reconstituted Cells** to use for the following tests:

- 0.5 mL for total cell count; cells should be counted undiluted, 1:5 and 1:10
- 0.5 mL for viability testing by Trypan Blue
- 2 mLs for sterility cultures

3.3.3 The final product will have a volume of 100 mL. Plasma-Lyte A will be added to the volume of cells required from the **POST-Wash/Reconstituted Cells** to a final volume of 100 mL.

3.3.4 Clinical Bone Marrow Transplant Facility staff should complete the "BMT Lab" section of the Infusion Form. This will include product thaw date/time, expiration date/time, and product identification number.

4 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.

Appendix G: Human Mesenchymal Stem Cell Infusion Procedure

BACKGROUND: Human mesenchymal stem cells (hMSCs) are being administered here 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. Toxicity from DMSO used to protect hMSCs during cryo-preservation is not a major concern since the hMSCs will be washed after the thaw and prior to infusion; renal toxicity from red blood cell disruption during the freeze/thaw procedure is also not a concern since these cells are cultured prior to cryopreservation.

Each study site will develop their 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. OBJECTIVE:

1.1 To ensure the safe and effective infusion of clinical grade allogeneic bone marrow-derived human mesenchymal stem cells (hMSCs).
1.2 To ensure the administration of the total prescribed hMSC dose

2. MATERIALS/EQUIPMENT

2.1 Standard blood filter tubing Y set (170-260 micron filter): At UCSF Medical Center, this is Baxter 4C6772
2.2 Gloves
2.3 Normal saline bag
2.4 One 3-way luer stopcock
2.5 Large luer lock syringe (10 mL or greater)

3. POLICIES

3.1 A study physician from the study must initiate and be present for the entire duration of the hMSC infusion (or placebo in Phase 2).
3.2 A study physician must write an order to administer hMSCs.
3.3 The hMSC infusion must be completed within 4 hours of the start of the cell thaw protocol, which will be documented on the cellular product by the BMT laboratory staff. Cells will be picked up from the laboratory by a study investigator or transported to the ICU by BMT laboratory staff. Time of completion of the thaw and wash procedure and the start and end of the infusion will be documented on the hMSC Infusion Record.
3.4 **Never discard hMSCs**. In the event of an adverse reaction terminating the infusion, return any un-transfused hMSCs to the BMT laboratory immediately.
3.5 No prophylactic pre-medications (e.g. acetaminophen or diphenhydramine) will be administered prior to the hMSCs. If such medications are ordered by the clinical team and given prior to or during the hMSC 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 hMSCs 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 hMSC infusion. The hMSC 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 hMSC 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 hMSC infusion, but should not be dialyzed until 24 hours or more after the hMSC infusion if possible. Continuous renal replacement therapy will be allowed to continue during the hMSC or placebo infusion.

3.10 Prone positioning: Patients must be in the supine position for the pre-infusion baseline stability period, and must remain in the supine position for the duration of the infusion. The patient should also remain supine for at least 1 hour after the completion of the infusion and preferably for the full 5 hours of additional bedside observation that follows the infusion. If after 1 hour post-infusion there is evidence of respiratory deterioration, however, the study MD and primary clinical team at bedside may elect to return the patient to the prone position.

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 hMSC infusion during administration (see attached potential side effects).

4. PROCEDURE

4.1 Study Physician to write order for cell infusion. This order also confirms the date, and time of the hMSC infusion. No hMSCs are to 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 Set up IV tubing as follows: normal saline bag, "Y-set" standard blood filter tubing (170 - 260 micron filter tubing, for example Baxter 4C6772) with one 3-way luer-lock stopcock at the end. Saline prime the blood filter tubing setup. Do NOT use bifuse tubing. DO NOT INFUSE THROUGH A NEEDLELESS CAP.

4.3 hMSCs will be administered as 100 mL in a 300 mL transfer bag; in Phase 2 this bag will be covered with aluminum foil to blind investigators to the content. In addition, in phase 2, the BMT laboratory will label both the transfer bag and the aluminum foil cover with the patient identifiers.

4.4 The Study Physician will:

- Confirm that the patient has met criteria to receive the hMSC infusion: the 2 hour stable baseline period has been achieved, and the PaO2/FiO2 ratio on a blood gas

obtained during the last 30 minutes of the stable baseline prior to infusion does not exceed 300. If the PaO2/FiO2 ratio is greater than 300, the patient is no longer eligible to receive hMSCs.

- 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.
- Double-check the information on the hMSC bag with the bedside RN for the following:
    - Recipient name/ID#
    - Recipient date of birth
    - Product Unique Identifier (DIN)
    - Product Proper Name

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 and will directly oversee the entire infusion procedure:

- Spike the hMSC bag and attach to the saline primed filter tubing
- The cells can be spiked onto the filter tubing and the infusion started immediately after the cells arrive at the bedside from the BMT laboratory and all patient checks have been completed. There is no need to wait for the cells to come to room temperature.
- Attach the primed filter tubing with the three-way stopcock to the intravenous line through which the hMSCs are to be infused
- Confirm that all IV connections are secure and that the infusion lines from the MSC to the patient are visible.
- Document the start date and time of the hMSC infusion on the hMSC Infusion Record.

4.6 Start the infusion:

- At each center, the priming volume of the filter set must be measured prior to enrollment of the first study patient:
    - For example, for Baxter 4C6772, the priming volume ranges from 80-100 mL, depending on the drip chamber prime volume
    - 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
- Run HALF of the estimated priming volume in over 10-15 minutes, checking the drop rate at the start of the infusion and approximately 5 minutes later. The drop

rate should be checked by counting the number of drops over 30 seconds and calculating the number of drops/minute.

- Run the remain HALF of the estimated priming volume and the cells in over 50-60 minutes, checking the drop rate at the start of the infusion, approximately 5 minutes after the start, and periodically throughout the infusion
- For the Baxter 4C6772 filter set:
    - Infuse 40 mL over 10-15 minutes, calculating the drop rate/minute
        - The # drops/mL for this priming set per the manufacturer is approximately 15
        - Therefore 40 mL = 40 mL * 15 drop/mL = 600 drops
        - 600 drops/10 minutes = 60 drops/minute = 30 drops/30 seconds
        - 600 drops/15 minutes = 40 drops/minute = 20 drops/30 seconds
        - **Therefore the target infusion rate is 25-30 drops/30 seconds for the first 10 minutes of the infusion**
    - For the second half of the prime volume and 100 mL of cells,
        - (40 mL prime + 100 mL cell volume) * 15 drops/mL = 2100 drops
        - 2100 drops/50 minutes = 42 drops/minute = 21 drops/30 seconds
        - 2100 drops/60 minutes = 35 drops/minute = 17.5 drops/30 seconds
        - **Therefore the target infusion rate is 18-20 drops/30 seconds for the next 50-60 minutes**

4.7 **Do not** run normal saline concurrently with the cells.

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

4.9 If the cells do not infuse easily by gravity, attach the luer-lock syringe to the three-way stop-cock and push hMSCs through line slowly (2-5 mL/min) via a syringe.

4.10 Observe the infusion line for clumping and change the filter set if clumping is observed. Save original filter set for the bone marrow transplant laboratory. Document if clumping is observed on the case report forms.

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 the 30 minutes prior to the start of the infusion (DURING 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 300, the patient should NOT receive hMSCs.
- See **Appendix H** for potential side effects of transfusion. 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 cell bag and flush the line**, ensuring that the cells have been rinsed from the bag and infused through the priming setup. **The target infusion rate during the rinse and flush procedure is increased, as the patient has already received a large portion of the infusion, and the rinse is expected to be more dilute.**

- At the end of the infusion, rinse the hMSC bag with normal saline using this protocol. To do this: (1) Clamp the hMSC line to the patient (2) Unclamp the saline

line and lower the hMSC bag to allow for saline to enter the hMSC bag; (3) After a small amount of saline (approximately 20 mL) has entered the hMSC bag, reclamp the saline line; (4) Swirl the saline in the hMSC bag until the sides of the bag are clear of cells (in Phase 2 this should be done by the bedside RN and not the study investigator, who must remain blinded to treatment); (5) Re-hang the hMSC bag, then open the line and infuse the saline rinse from the hMSC bag into the patient until the bag is empty; (6) Finally, allow 80 mL (priming volume) of saline to run through the filter to flush the remaining rinse volume into the patient. **The rinse and flush volume should be infused over approximately 20 minutes:**

- (20 mL rinse + 80 mL flush) * 15 drops/mL = 1500 drops
- 1500 drops/20 minutes = 75 drops/minute = 32.5 drops/30 seconds
- **Therefore the target infusion rate for the rinse and flush is approximately 30 drops/30 seconds for 20 minutes**

- If cell clumps are still seen in the filter, clamp the hMSC line and unclamp the saline line. Attach a large syringe to the stopcock nearest the patient. Flip the stopcock to allow for saline to be drawn through the filter and tubing into the syringe. Aspirate saline into the syringe to flush the filter (the aspiration of saline through the filter serves as a filter flush). When the syringe is full, turn the stopcock (open to the patient's IV) to allow for the contents of the syringe to be injected into the patient. Empty the syringe slowly (2-5mL/min). Repeat this process until the filter is clear of cells.

4.13 hMSC bags and tubing should be returned to the BMT laboratory

4.14 Obtain and document vital signs at completion of infusion.

5. Documentation:

5.1 Complete the hMSC 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 and to the University of Minnesota.

5.2 The study investigator will inform the bedside RN of the total volume (hMSC 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 any confirmation of the appropriate product, MSC dose infused, and any complications or adverse reactions.

Appendix H: Guidelines for Symptom Management During hMSCs 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 O_2 saturation • Hydrocortisone 1mg/kg IV • If still symptomatic after hydrocortisone, then diphenhydramine 25-50 mg IV • Increase supplemental O_2 if O_2 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 O_2 saturation • Increase supplemental O_2 if O_2 saturation is <93% and/or dyspnea. • Resume MSC infusion after reasonable resolution of signs and symptoms.
Volume Overload Although the volume of the MSC will be set at 100 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 O_2 saturation. • Increase supplemental O_2 if O_2 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 reactions are usually symptomatic and occasionally life-threatening.	• fever • hypotension • dyspnea • low back pain • dark urine • drop in blood hemoglobin • disseminated-intravascular coagulation	• Stop MSC infusion. • Check vital signs and O_2 saturation. • IV fluid resuscitation immediately if hypotensive. • Forced diuresis and urinary alkalinization with IV D5W + 2 amps/l sodium bicarbonate. • 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

Adverse Reactions	Signs and Symptoms	Management
		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. The cell product will be washed to minimize the infusion of DMSO, but the infusion of DMSO has side effects and consequences that study personnel need to be aware of	• 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 O_2 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 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.	• hypotension • fever • oliguria/anuria • tachycardia • dyspnea/ hypoxemia • disseminated intravascular coagulation • signs/symptoms within “minutes” of MSC infusion	• Stop the 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 I. Mini-Bronchoalveolar Lavage Procedure

The mini-BAL procedure involves blind specimen sampling from distal airspaces. 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 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 J. START Phase 2 Investigational Product Infusion Protocol

BACKGROUND: Human mesenchymal stem 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. Toxicity from DMSO used to protect hMSCs during cryopreservation is not a major concern since the hMSCs will be washed after the thaw and prior to infusion; renal toxicity from red blood cell disruption during the freeze/thaw procedure is also not a concern since these cells are cultured prior to cryopreservation.

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 stem cells (hMSCs) or Plasmalyte A placebo.
1.2 To ensure the administration of the full hMSC dose (10 million cells/kg predicted body weight).
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 Baxter 4C6772 (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)

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

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 Prone positioning: Patients must remain in the supine 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 H 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 Baxter 4C6772). 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 100 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 PaO2/FiO2 ratio on a blood gas obtained during the last 30 minutes of the stable baseline prior to infusion cannot exceed 300. If the PaO2/FiO2 ratio is greater than 300, 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 will review the information on the investigational product bag with the bedside RN or study physician for the following:
    - Recipient name/ID#
    - Recipient date of birth
    - Product expiration date/time

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.
- 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 START 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 prior to enrollment of the first study patient:
  - For example, for Baxter 4C6772, the priming volume ranges from 80-100 mL, depending on the drip chamber prime volume
  - 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 HALF of the estimated priming volume in over 10-15 minutes, checking the drop rate at the start of the infusion and approximately 5 minutes later. 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.
- Run the remain HALF of the estimated priming volume and the investigational product in over 50-60 minutes, checking the drop rate at the start of the infusion, approximately 5 minutes after the start, and periodically throughout the infusion
- For the Baxter 4C6772 filter set:
  - Infuse 40 mL over 10-15 minutes, calculating the drop rate/minute
    - The # drops/mL for this priming set per the manufacturer is approximately 15
    - Therefore 40 mL = 40 mL * 15 drop/mL = 600 drops
    - 600 drops/10 minutes = 60 drops/minute = 30 drops/30 seconds
    - 600 drops/15 minutes = 40 drops/minute = 20 drops/30 seconds
    - **Therefore the target infusion rate is 25-30 drops/30 seconds for the first 10 minutes of the infusion**
  - For the second half of the prime volume and 100 mL of product,
    - (40 mL prime + 100 mL product) * 15 drops/mL = 2100 drops
    - 2100 drops/50 minutes = 42 drops/minute = 21 drops/30 seconds
    - 2100 drops/60 minutes = 35 drops/minute = 17.5 drops/30 seconds
    - Therefore the target infusion rate is 18-20 drops/30 seconds for the next 60 minutes

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, 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 since it was not an issue for Phase 1. 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.

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 the 30 minutes prior to the start of the infusion (DURING 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 300, the patient should NOT receive the investigational product.
- See **Appendix H** 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. **The target infusion rate during the rinse and flush procedure is increased, as the patient has already received a large portion of the infusion, and the rinse is expected to be more dilute.**

- 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 (priming volume) of saline to run through the filter to flush the remaining rinse volume into the patient. **The rinse and flush volume should be infused over 20 minutes:**
  - (20 mL rinse + 80 mL flush) * 15 drops/mL = 1500 drops
  - 1500 drops/20 minutes = 75 drops/minute = 32.5 drops/30 seconds
  - **Therefore the target infusion rate for the rinse and flush is approximately 30 drops/30 seconds for 20 minutes**

4.13 Investigational product bags and tubing should be returned to the BMT laboratory

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 and to the University of Minnesota.

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 K. Barotrauma

Evidence of barotrauma includes the following:

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