Interdisciplinary Stem Cell Institute

University of Miami/ Miller School of Medicine

Clinical Research Protocol

Study Title:	***A*** Phase I/II, Randomized, Double Blind, Pilot trial to evaluate the Safety and Efficacy of Allogeneic Mesenchymal Human Stem ***C***ells infusion therapy for ***E***ndothelial Dy***S***functi***O***n in diabetic subjects. (ACESO Study)
Study Product:	Allogeneic Human Mesenchymal Stem Cells (hMSCs)
Indication:	Endothelial Dysfunction in Diabetes
FDA IND No.:	BB-IND #17054
NCT#	NCT02886884
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Protocol Version:	5.7, dated October 26, 2020

Protocol Agreement Signature:

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Joshua M. Hare, M.D (Investigator) Date

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List of Abbreviations

ADA	American Diabetes Association
ACE	Angiotensin converting enzyme
AE	Adverse event
CHF	Chronic heart failure
CRF	Case report forms
CPL	Cell Processing Laboratory
CTCAEv4	Common Terminology Criteria for Adverse Events
CVD	Cardiovascular disease
DM	Diabetes Mellitus
EPC	Endothelial progenitor cells
FDA	Food and Drug Administration
FMD%	Flow Mediated Diameter percent
GM-CSF	Granulocyte Macrophage Colony Stimulating Factor
BMMNCs	Bone marrow mononuclear cells
HIPAA	Health Insurance Portability and Accountability Act of 1996
hMSCs	Human mesenchymal stem cells
HF	Heart failure
HLA	Human Leukocyte Antigen
IL	Interleukin
IRB	Institutional review board
IV	Intravenous Infusion
LV	Left ventricular
MDRD	Modification of Diet in Renal Disease
MSC	Mesenchymal Stem Cells
NIH	National Institutes of Health
NO	Nitric Oxide
PHI	Protected health information
PRA	Panel Reactive Antibody
QOL	Quality of Life Questionnaires
ROS	Reactive oxygen species
SAE	Serious adverse event
SDF-1	Stromal derived factor 1
SCF	Stem cell factor
SLE	Systemic Lupus Erythematosus
TNF	Tumor Necrosis Factor
TNFR1	TNF-α receptor
UM	University of Miami
VEGF	Vascular endothelial growth factor

Protocol Synopsis

Sponsor: ISCI / University of Miami Miller School of Medicine	
Name of Study Therapy: Allogeneic human Mesenchymal Stem Cells (hMSCs)	
Title of Study: A Phase I/II, Randomized, Double Blind, Pilot trial to evaluate the Safety and Efficacy of Allogeneic Mesenchymal Human Stem Cells infusion therapy for Endothelial DySfunctiOn in diabetic subjects. (ACESO Study)	
Study Center: ISCI / University of Miami Miller School of Medicine	**Phase of Development:** Phase I/II

Objectives:

Primary: To demonstrate the safety of allogeneic hMSCs administered via infusion therapy for diabetic subjects with endothelial dysfunction.

Secondary:

-To determine the mechanisms underlying the endothelial effects of MSCs and whether targeting endothelial function via MSC therapy ameliorates Cardiovascular Disease (CVD) outcomes in subjects with diabetes mellitus.

-To evaluate the dose and timing of the stimulatory effect of MSCs on circulating angiogenic factor levels and EPC function as well as FMD% within 28 days post-infusion.

-To evaluate the long term effect of MSCs on circulating angiogenic factor levels and EPC function as well as FMD% at 3, 6, and 12 months post-infusion.

Design and Investigational Plan:

To evaluate the role of allogeneic mesenchymal stem cells therapy for endothelial dysfunction in diabetes.

Sixteen (16) diabetic subjects with endothelial dysfunction will be scheduled to undergo a peripheral intravenous infusion after meeting all inclusion/exclusion criteria at baseline.

PILOT PHASE (Unblinded):

The dose for subjects in the pilot phase will vary from 20 million cells to 100 million cells and will be Unblinded, with subjects and study team members aware of the doses administered to the subject. The pilot phase will consist of:

Three (3) subjects will be treated with 20 million (20×10^6) allogeneic hMSC's Three (3) subjects will be treated with 100 million (100×10^6) allogeneic hMSC'

The three (3) subjects in the low dose group will not be treated less than 5 days apart and will each undergo full evaluation during those five days to show that there are no signs of treatment emergent SAE's (TE-SAE) before continuing with the treatment of further subjects.

The three (3) subjects in the high dose will not be treated until the three (3) subjects in the low dose have completed their one-month follow-up assessments and safety measures have been reviewed to confirm that there have been no treatment emergent SAE's. In addition, the three (3) subjects in the high dose group will not be treated less than 5 days apart.

Randomized Phase (Double-Blind):

After the pilot phase, subjects will be randomized to one of two treatments in a 1:1 fashion. This phase of the study will be blinded to both subjects and study team members. The randomized phase will consist of ten (10) diabetic subjects with endothelial dysfunction will be subsequently randomized to undergo a peripheral intravenous infusion after meeting all inclusion/exclusion criteria at baseline:

Five (5) subjects will be treated with 20 million (20×10^6) allogeneic hMSC's and Five (5) subjects will be treated with 100 million (100×10^6) allogeneic hMSC's

The Allo-hMSCs will be supplied from an allogeneic human mesenchymal stem cell source or commercial clinical grade bone marrow source manufactured by the University of Miami.

Follow up: Subjects will be followed at 3, 7, 14 and 28 days to evaluate the dose and timing of the stimulatory effect of MSCs on circulating angiogenic factor levels, EPC function, and FMD%, and at 3, 6, and 12 months post-infusion to complete all long-term safety and efficacy assessments.

Route of Administration: Peripheral Intravenous Infusion

Duration of Study participation: Approximately 12 months

Definition of Endpoints:

Safety (Primary): Incidence (at one-month post infusion) of any treatment-emergent serious adverse events (TE-SAEs), defined as the composite of: death, non-fatal pulmonary embolism, stroke, hospitalization for worsening dyspnea and clinically significant laboratory test abnormalities, determined per the Investigator's judgment.

Efficacy (Secondary): (Assess at Baseline, Day 3, 7, 14, 28, and Month 3, 6, and 12 following IV allogeneic MSC infusion)

1. Assess EPC-colony forming units (CFUs)
2. Assess circulating inflammatory markers (IL-1, IL-6, TNFα, and CRP)
3. Assess circulating angiogenic factors known to mobilize and recruit EPCs (VEGF, SDF-1α, and SCF)
4. Assess FMD%

Major Inclusion Criteria:

- Be ≥ 21 and ≤90 years of age.
- Provide written informed consent.

• Have endothelial dysfunction defined by impaired flow-mediated vasodilation (FMD <7%).

• Have DM type 2 documented by hemoglobin A1C ≥ 7% OR on medical therapy for diabetes.

• Females of childbearing potential must use two forms of birth control for the duration of the study. Female subjects must undergo a serum pregnancy test at screening and within 36 hours prior to infusion.

Major Exclusion Criteria:

• Be younger than 21 years or older than 90 years of age.

• Have a baseline glomerular filtration rate <35 ml/min 1.73m^2 estimated using the MDRD formula.

• Have an ejection fraction <45% by gated blood pool scan, two-dimensional echocardiogram, cardiac MRI, cardiac CT or left ventriculogram within the past year, as documented by medical history.

• Have poorly controlled blood glucose levels with hemoglobin A1C ≥ 8.5%.

• Have a history of proliferative retinopathy or severe neuropathy requiring medical treatment.

• Have a hematologic abnormality as evidenced by hematocrit < 25%, white blood cell < 2,500/ul or platelet values < 100,000/ul without another explanation.

• Have liver dysfunction, as evidenced by enzymes (AST and ALT) greater than three times the ULN.

• Have a bleeding diathesis or coagulopathy (INR > 1.3), cannot be withdrawn from anticoagulation therapy, or will refuse blood transfusions.

• Be an organ transplant recipient or have a history of organ or cell transplant rejection.

• Have a clinical history of malignancy within the past 5 years (i.e., subjects with prior malignancy must be disease free for 5 years), except curatively treated basal cell or squamous cell carcinoma, or cervical carcinoma.

• Have a condition that limits lifespan to < 1 year.

• Have a history of drug or alcohol abuse within the past 24 months.

• Be serum positive for HIV, Syphilis – VDRL (Confirmation with FTA-ABS if needed (Syphilis)), hepatitis B surface antigen, or viremic hepatitis C.

• Be currently participating (or participated within the previous 30 days) in an investigational therapy/medication or device trial.

• Be pregnant, nursing, or of childbearing potential while not practicing effective contraceptive methods.

• Any other condition that in the judgment of the Investigator would be a contraindication to enrollment or follow-up.

1. INTRODUCTION

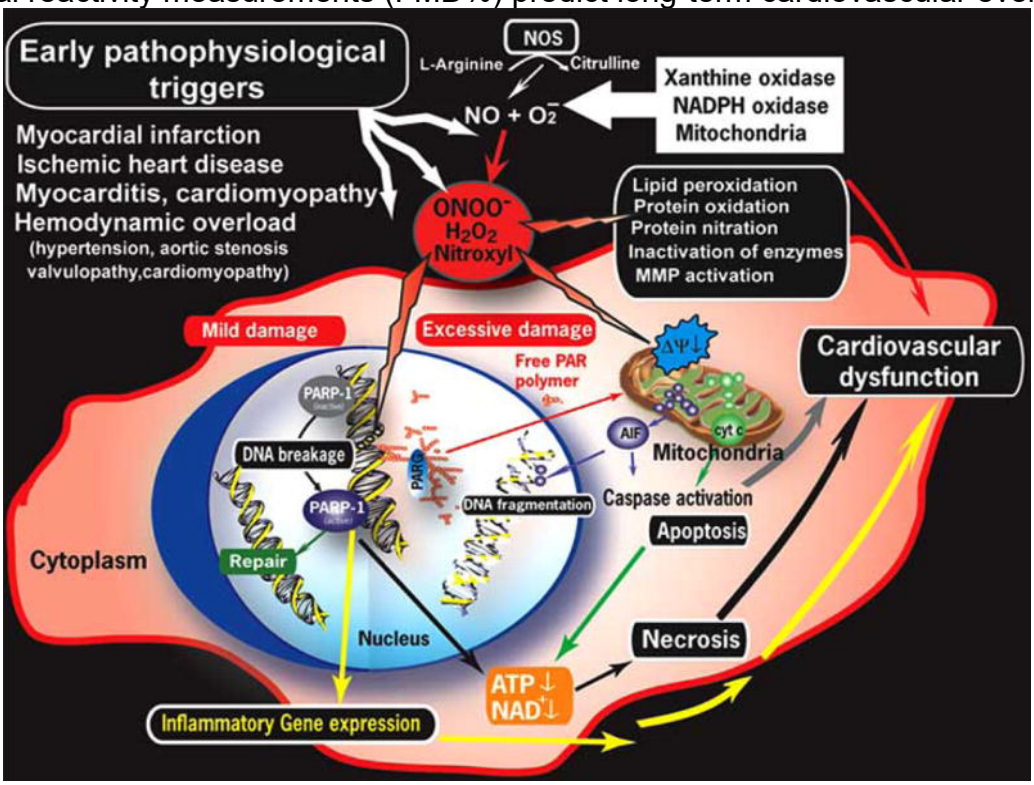
1.1 Background

Cardiovascular disease (CVD) is the leading cause of death and disability among people with type 2 diabetes mellitus (DM)[1]. Not only are people with DM more likely to have a myocardial infarction (MI), their prognosis is worse, highlighting the need for novel targeted therapies[1]. It has long been appreciated that endothelial dysfunction underlies the high rates of CVD associated with long-term DM[2-5]. The persistent hyperglycemia and other metabolic abnormalities directly affect the endothelium, contributing to the pathophysiology of disease[2;6;7]. Numerous cell-based therapy clinical trials in subjects with ischemic heart disease illustrate that mesenchymal stem cell (MSC) administration improves cardiac structure, function and quality of life[8-11]. Our recent observation that MSCs improve endothelial progenitor cell (EPC) function (measured by colony forming assay) and endothelial function (measured by brachial artery flow-mediated vasodilation, FMD%) in subjects with ischemic as well as non-ischemic cardiomyopathy[12], independent of their age or DM, suggests that we now have a means to target a primary cause of the CV manifestations of DM. Moreover, the effect on EPCs and FMD% was sustained at 3 months after MSC administration, and evident in subjects receiving allogeneic but not autologous MSCs[12]. The mechanisms underlying the therapeutic effects of MSCs are due to a combination of multilineage differentiation, secretion of anti-inflammatory and proangiogenic paracrine factors, as well as stimulation of endogenous progenitor cells growth and differentiation[12;13]. Moreover, comorbidities and aging are potential factors underlying the difference in efficacy between autologous (subject-derived) and allogeneic (healthy donor) MSCs[14-16].

Endothelial Dysfunction in Subjects with Cardiovascular Diseases

Endothelial dysfunction – defined by impaired flow-mediated vasodilation (FMD) and endothelial progenitor cell (EPC) dysfunction – is a crucial component of the pathophysiology of cardiovascular disease (CVD) and manifests in subjects with cardiovascular risk factors such as atherosclerosis, hypertension, chronic kidney disease, metabolic syndrome, and diabetes mellitus (DM)[17]. The endothelium plays an essential role in maintaining circulatory homeostasis by the release of factors that relax and contract vascular smooth muscle, ensuring appropriate blood flow to tissues. As a major regulator of peripheral blood flow, it controls the balance between nitric oxide (NO), reactive oxygen species, vasomotor tone, and inflammation[18;19]. Any change in the vasomotor regulatory balance may be characterized as endothelial dysfunction that leads to impaired control of vascular tone and participates in the pathogenesis of CVD. By stimulating the release of NO from the endothelium, EPCs play a pivotal part in maintaining vascular homeostasis as well as in mediating vascular repair in damaged endothelium. EPCs regulate the health of the vasculature by incorporating into the endothelium, replacing injured endothelial cells, and secreting angiogenic factors that activate mature endothelial cells[20]. Subjects with CVD have decreased circulating EPC levels and bioactivity[21]. Indeed, circulating EPC levels serve as a predictor of CV events[22;23]. Low numbers of EPC-colony forming units (EPC-CFUs) have been linked to

high Framingham risk scores for adverse cardiovascular health outcomes[23]. Similarly, brachial reactivity measurements (FMD%) predict long-term cardiovascular events[24;25].



Mesenchymal Stem Cells Transplantation for Patients with CVD and Endothelial Dysfunction

The field of cell-based therapy for CVD, particularly ischemic heart disease, has had major advances in the past few years[2;8-12]. Several studies support the safety and efficacy of MSC based therapy for this large group of subjects who are at major risk for MI, heart failure (HF), sudden cardiac death, and other major CV complications[17-22]. The DM subject population is at an especially high risk for ischemic heart disease and has a worse prognosis. According to the 2014 National Diabetes Statistics Report, 29.1 million Americans, or 9.3% of the population, had DM in 2012 (mostly type 2). In 2003-2006, CVD death rates were about 1.7 times higher among age-matched adults with DM than among those without DM. Similarly, in 2010 hospitalization rates for MI were 1.8 times higher among adults with DM than among those without DM.

MSCs, under evaluation as a regenerative therapeutic approach for ischemic and non-ischemic cardiomyopathy[8;9;11;12;26], have significant potential for clinical benefit in CVD by virtue of their antifibrotic[27], anti-inflammatory, and pro-angiogenic properties[26, 27], as well as their ability to stimulate endogenous progenitor cells and capacity to differentiate into endothelial cells[20, 28, 29]. Moreover, there is evidence that NO deficient environments stimulate MSC involvement in angiogenesis[12;28]. We demonstrated in porcine models of ischemic cardiomyopathy that MSCs reduce infarct size by 35% and significantly improve global and regional left ventricular function[29;30]. These effects are due to cell engraftment, differentiation into myocytes and blood vessels, and stimulation of endogenous cardiac

stem cell proliferation and differentiation[13]. Given this capacity of MSCs and the role of impaired EPCs in CVD[31;32], we tested the hypothesis that MSCs stimulate EPC function and augment vascular relaxation in subjects with heart failure due to idiopathic dilated or ischemic cardiomyopathy[12]. We found that allogeneic, but not autologous, MSCs improve EPC bioactivity and endothelial function (FMD%) in heart failure subjects, regardless of etiology. These findings demonstrated a novel clinical beneficial effect of allogeneic MSCs administration in patients with heart failure and have implications for all disorders associated with endothelial dysfunction, such as DM. Notably, DM subjects have impaired EPC mobilization and trafficking from bone marrow as well as functionally impaired MSCs[33;34].

Numerous cell-based therapy clinical trials in subjects with ischemic heart disease illustrate that MSC administration improves cardiac structure and function and quality of life[8-11]. Our recent observation that MSCs improve EPC function (measured by colony forming assay) and endothelial function (measured by brachial artery flow-mediated dilation, FMD%) in subjects with ischemic as well as non-ischemic cardiomyopathy[12], independent of age or DM, suggests that we now have a means to target a primary cause of the cardiovascular manifestations of DM. Moreover, the effect on EPCs and FMD% was sustained at 3 months after MSC administration, and evident in subjects receiving allogeneic but not autologous MSCs[12]. The mechanisms underlying the therapeutic effects of MSCs are due to a combination of multilineage differentiation, secretion of anti-inflammatory and proangiogenic paracrine factors, and stimulation of endogenous progenitor cells growth and differentiation[12;13]. Moreover, comorbidities and aging are potential factors underlying the difference in efficacy between autologous (subject-derived) and allogeneic (healthy donor) MSCs[14-16].

2. STUDY OBJECTIVES AND ENDPOINTS

2.1 Study Objectives

2.1.1 Primary Objective

- To demonstrate the safety of allogeneic hMSCs administered via infusion therapy for diabetic subjects with endothelial dysfunction.

2.1.2 Secondary Objectives

- To determine the mechanisms underlying the endothelial effects of MSCs and whether targeting endothelial function via MSC therapy ameliorates CVD outcomes in subjects with diabetic mellitus.

- To evaluate the dose and timing of the stimulatory effect of MSCs on circulating angiogenic and inflammatory factor levels and EPC function, as well as FMD%.

2.2 Study Endpoints

2.2.1 Primary Endpoints (Safety)

Safety (Primary): Incidence (at one-month post infusion) of any treatment-emergent serious adverse events (TE-SAEs), defined as the composite of: death, non-fatal pulmonary embolism, stroke, hospitalization for worsening dyspnea and clinically significant laboratory test abnormalities, determined per the Investigator's judgment.

2.2.2. Secondary Endpoints (Efficacy)

(Assess at Baseline, Day 3, 7, 14, and 28, and Month 3, 6 and 12 following IV allogeneic MSC infusion)

1. Assess EPC-colony forming units (CFUs)
2. Assess circulating inflammatory markers (IL-1, IL-6, TNFα, and CRP)
3. Assess circulating angiogenic factors known to mobilize and recruit EPCs (VEGF, SDF-1α, and SCF)
4. Assess FMD%

3. STUDY DESIGN

3.1 Description of the Study

Sixteen (16) diabetic subjects with endothelial dysfunction will be scheduled to undergo a peripheral intravenous infusion after meeting all inclusion/exclusion criteria at baseline. The study will consist of a Pilot Phase and Randomized Phase.

PILOT PHASE

To test the safety of allogeneic mesenchymal stem cells therapy for endothelial dysfunction in diabetes subjects administered via peripheral intravenous infusion, a non-randomized, unblinded pilot phase of six subjects will be performed.

Three (3) subjects will be treated with 20 million (20×10^6) allogeneic hMSCs
Three (3) subjects will be treated with 100 million (100×10^6) allogeneic hMSCs.

The three (3) subjects in the low dose group will not be treated less than 5 days apart and will each undergo full evaluation during those five days to show that there are no signs of treatment emergent SAE's before continuing with the treatment of further subjects.

The three (3) subjects in the high dose will not be treated until the three (3) subjects in the low dose have completed their one-month follow-up assessments and safety measures have been reviewed to confirm that there have been no treatment emergent SAE's. In addition, the first three (3) subjects in the high dose group will not be treated less than 5 days apart.

RANDOMIZED PHASE

After the Pilot Phase, the Randomized Phase will begin. Ten (10) diabetic subjects with endothelial dysfunction will be randomized to undergo a peripheral intravenous infusion in a 1:1 ratio after meeting all inclusion/exclusion criteria at baseline.

Five (5) subjects will be treated with 20 million (20×10^6) allogeneic hMSCs Five (5) subjects will be treated with 100 million (100×10^6) allogeneic hMSCs

The Allo-hMSCs will be supplied from an allogeneic human mesenchymal stem cell source manufactured by the University of Miami.

Electronic randomization will be performed by the University of Miami Biostatistics Collaboration and Consulting Core (BCCC) and communicated to cellular laboratory personnel who have no contact with the investigators or subjects. After each infusion, subjects will be monitored for immediate complications.

Continued safety and tolerability with review of adverse events (AEs) will be monitored at each visit. Efficacy parameters (EPC's, FMD%, and inflammatory markers) will be assessed at baseline, Day 3, 7, 14, and 28, Month 3, Month 6 and Month 12 following the IV infusion of the allocated investigational product. Clinical laboratory tests to assess safety will be performed at every clinic visit, excluding the screening visit.

4. SUBJECT SELECTION

4.1 Inclusion Criteria

In order to participate in this study, a subject MUST:

- Be ≥ 21 and ≤90 years of age.
- Provide written informed consent.
- Have endothelial dysfunction defined by impaired flow-mediated vasodilation (FMD <7%).
- Have DM type 2 documented by hemoglobin A1C ≥ 7% or on medical therapy for diabetes.
- Females of childbearing potential must use two forms of birth control for the duration of the study. Female subjects must undergo a serum pregnancy test at screening and within 36 hours prior to infusion.

4.2 Exclusion Criteria

In order to participate in this study, a subject MUST NOT:

- Be younger than 21 years or older than 90 years of age.
- Have a baseline glomerular filtration rate <35 ml/min $1.73m^2$ estimated using the MDRD formula.

- Have an ejection fraction < 45% by gated blood pool scan, two-dimensional echocardiogram, cardiac MRI, cardiac CT or left ventriculogram within the past year, as documented by medical history if history of heart failure.
- Have poorly controlled blood glucose levels with hemoglobin A1C $\geq$ 8.5%.
- Have a history of proliferative retinopathy or severe neuropathy requiring medical treatment.
- Have a hematologic abnormality as evidenced by hematocrit < 25%, white blood cell < 2,500/ul or platelet values < 100,000/ul without another explanation.
- Have liver dysfunction, as evidenced by enzymes (AST and ALT) greater than three times the ULN.
- Have a bleeding diathesis or coagulopathy (INR > 1.3), cannot be withdrawn from anticoagulation therapy, or will refuse blood transfusions.
- Be an organ transplant recipient or have a history of organ or cell transplant rejection.
- Have a clinical history of malignancy within the past 5 years (i.e., subjects with prior malignancy must be disease free for 5 years), except curatively-treated basal cell or squamous cell carcinoma, or cervical carcinoma.
- Have a condition that limits lifespan to < 1 year.
- Have a history of drug or alcohol abuse within the past 24 months.
- Be on chronic therapy with immunosuppressant medication, such as corticosteroids or TNFα antagonists.
- Be serum positive for HIV, Syphilis – VDRL (Confirmation with FTA-ABS if needed (Syphilis)), hepatitis BsAg, or viremic hepatitis C.
- Be currently participating (or participated within the previous 30 days) in an investigational therapeutic or device trial.
- Be pregnant, nursing, or of childbearing potential while not practicing effective contraceptive methods.
- Any other condition that in the judgment of the Investigator would be a contraindication to enrollment or follow-up.

4.3 Concomitant Treatments, Procedures, and Nondrug Therapies

All concomitant medications (prescription or over-the counter) as well as procedures or nondrug therapies (e.g. continuous positive airway pressure, pulmonary rehabilitation) will be recorded at the initial screening visit and updated at each subsequent visit. Except for other experimental treatments or medications with putative disease modifying effects in endothelial dysfunction, subjects will continue all prior concomitant medications for comorbid diseases to ensure optimal general medical care.

4.4 Withdrawal Criteria

Subjects will be informed that they have the right to withdraw from the study at any time and for any reason without prejudice to future or continued medical care. Subjects must be withdrawn for the following reasons:

- Subject's request.
- Subject is unable or unwilling to comply with the protocol.
- Medical reasons, at the discretion of the investigator.

Reason for withdrawal will be recorded in the subject's case report form. In order to adequately monitor for safety and potential efficacy outcomes, subjects who are withdrawn for any reason after receiving the first infusion should be encouraged to return for all assessments through the end of the study period. All efforts should be made to continue to record safety data and lung function parameters for all withdrawn subjects. Subjects who withdraw for reasons unrelated to the study or study drug (e.g. withdrawal of consent or loss to follow-up) may be replaced if deemed necessary to meet study objectives. Replacement subjects will be assigned unique identification numbers.

5. DONORS

5.1 Normal Donor Eligibility

Donors (male or female) between the ages of ≥ 18 to ≤ 45 (inclusive) will be screened as potential BM donors. Donors will be evaluated by history and physical examination. The history will include:

- History of malignancy
- Bleeding abnormalities
- Prior deep venous thrombosis
- Known cardiac or pulmonary conditions
- Prior blood transfusions
- Vaccinations
- Questions to identify persons at risks of infectious disease transmission, including Zika virus
- Questions to identify persons at risk of transmitting hematological or immunological disease
- A physician will administer the National Marrow Donor Program (NMDP) Questionnaire (a donor health history screening questionnaire).

The physical examination will include review of vital signs and evaluation for potential risks associated with the BM aspiration procedure. Prospective donors will have infectious disease testing including:

- Hepatitis B surface antigen (HBsAg)
- Anti-Hepatitis B core antibody (HBcAb)
- Anti-Hepatitis C virus antibody (HCV Ab)
- Anti-Human Immunodeficiency Virus (HIV) antibody (HIV 1/2)
- Cytomegalovirus antibody (CMV)
- HCV/HIV Nucleic Acid test
- West Nile Virus Nucleic Acid test
- Human T-lymphotropic Virus I/II (HTLV I/II)
- *T. cruzi* ELISA test (Chagas disease)

- *Zika Virus (RNA qualitative Real Time RT-PCR and/or Virus Antibody (IgM), MAC-ELISA)*
- Rapid Plasma Reagin (RPR) or *VDRL (confirmation with FTA-ABS if needed (Syphilis))*

Prospective donors will also have the following blood and urine tests:

- Complete blood count with differential
- Comprehensive metabolic panel, magnesium, calcium, and uric acid
- Urinalysis
- Serum pregnancy test (Female only)

Eligibility Criteria for Normal Donors will include:

- Male and female gender
- No history of malignancy
- No active coagulopathy and/or hypocoagulable state
- No history of cardio/pulmonary conditions
- Negative tests for Hepatitis B Surface Antigen, Hepatitis C antibody, RPR or VDRL, Chagas, HIV 1 and 2 antibody screen, HTLV I and II antibody screen, Zika Virus, and West Nile Virus.
- Hemoglobin $\geq$ 13.0 g/dL if male; and if female donor hemoglobin $\geq$ 11.0 g/dL
- Platelet count 140,000 to 440,000/ul
- WBC 3.0 to 11.0 K/ul
- No anomalies on the CBC and differential suggestive of a hematopoietic disorder
- Creatinine $\leq$1.5 mg/dL
- ALT$\leq$ 112 IU/L
- Bilirubin < 1.5 mg/dL
- No diabetes
- Systolic blood pressure $\leq$ 170
- Diastolic blood pressure $\leq$ 90
- No history of autoimmune disorders
- Negative serum pregnancy test for female donors
- Body Mass Index (BMI) $\leq$ 30

Female donors would need to be screened for pregnancy as the procedure may be an added risk to a fetus.

5.2 Donor Consent

Informed consent will be obtained from all potential donors. The procedure will be explained in terms the donor can understand, and will include information about the significant risks of the procedure. Potential donors will have an opportunity to ask questions, the right to refuse or withdraw consent, and access to the results of all tests.

Donors will need to have virology's redrawn if BMA procedure not completed 7 days from the initial virology results.

5.3 Follow-up Schedule for Donors

After discharge from the hospital, the bone marrow donor will be contacted by the study team with a follow-up telephone call. This call will take place approximately 24 hours after leaving the hospital to determine the well-being and health status of the donor. The donor will be provided with contact telephone numbers in the consent form for any questions or comments.

5.4 Biomarker Assessment for Donors

A separate blood sample of about 10mL for gene expression profiling will be obtained at the donation visit. All samples will be identified so that they can be linked to individual subjects. These samples may be stored indefinitely. Individual results will not be returned to the subject or the study physician. The samples will be linked to subjects, but there will be no recontact. A separate genetic consent form will need to be completed for the donors' participation.

Data presented in publications will not contain individual subjects' clinical characteristics or outcomes; only aggregate data from the entire study will be disclosed.

6. TREATMENT OF SUBJECTS

6.1 Study Investigational Product

The investigational product (IP) consists of hMSCs obtained from a healthy donor of bone marrow or from a commercial clinical grade bone marrow source. Screening of allogeneic donors will follow standard transplant practices and all allogeneic donors will meet allogeneic donor eligibility criteria as outlined in 21 CFR Part 1271 and as specified in the POSEIDON-DCM trial (IND #: 14419; NCT01087996). BM will be obtained from normal volunteers and aspirated from the posterior iliac crest. The BM will be aspirated into heparinized syringes. The MNC fraction will be isolated using a density gradient with Lymphocyte Separation Media (specific gravity 1.077). The low-density cells will be collected and washed with Plasma-LyteA containing 1% HSA. The MNCs will be prepared with or without antibiotics. Subjects with a penicillin allergy will not receive antibiotic treated cells. The washed cells will be samples and viable cell numbers determined. The BM MNC will be seeded into 175 cm^2 tissue culture flasks in alpha MEM containing 20% FBS. After 14 days of culture, passage zero (P0) cells will be harvested by trypsin treatment and expanded into 60 flasks (P1 cells). After 7 to 10 days P1 cells are harvested by trypsin treatment (P1 cells). Cells from P1 will be cultured for 7 to 10 days and harvested by trypsin treatment and expanded into 180 flasks (P2 cells) with the option of

expanding them once again, to P3. After 7-10 days P3 cells would be harvested by trypsin treatment and cryopreserved.

Before dispensing the investigational product, Cell Therapy Lab staff will confirm the CMV status of eligible recipients. This information will be used to select the Allo-hMSC product. CMV status of the recipient and donor of the Allo-hMSC product will be matched. CMV positive or negative Allo-hMSC products may be infused to a CMV positive recipient. All CMV negative recipients will receive CMV negative Allo-hMSC product[91].

6.2 Dosing

The Allo-hMSCs are manufactured by the University of Miami Interdisciplinary Stem Cell Institute, as specified in the POSEIDON-DCM trial (IND #: 14419; NCT01087996).

6.3 Dosage Rationale

A safety profile for IV infusion of hMSCs was based on outcomes from previous completed toxicology results[8]. The results from these previous studies demonstrate that the product can be administered intravenously without toxic events at up to 65 x 10^6hMSC/kg dose delivered in one bolus infusion or at 100 x 10^6hMSC/kg cumulative dose delivered by 5 infusions (20 x 10^6hMSC/kg per infusion).

The evidence supports the conclusion that it is feasible to dose subjects in this study based on a standard dose of hMSCs rather than per kilogram of body weight. The total cell number corresponds to a range of 1.3 - 4.4 x 10^6 hMSCs per kg per infusion for subjects with 45kg to 150kg body weight.

In addition, the data from the administration of allogeneic MSCs in the DCM (IND #: 14419; NCT01087996) trial supports the clinical safety of the proposed MSC doses to be administered. Therefore, results from previous trials support the rationale on the safety and potential efficacy of the selected maximum dose of 100 x10^6 allo-hMSCs.

6.4 Administration Rate

Prior clinical trials have used rates up to 30×10^6 hMSC/min where no infusion related toxicity was observed.

In the proposed study, the cell dose to be delivered is 20 million (20 x 10^6) and 100 million (100×10^6) hMSC/infusion, reconstituted as 0.25 to 1.25 million hMSC/ml, in the following total volume:

80 ml for the 20 million dose (0.5 million hMSC/min)

80 ml for the 100 million dose (2.5 million hMSC/min)

Cells are placed in an 80ml bag and will be delivered at a rate of 2ml/min, and delivered at a maximum rate of 2.5×10^6 hMSC/minute and will last approximately 40 minutes for both doses

The infusion bag will be flushed with an additional 25 ml of 0.9% normal saline at the completion of allo-hMSC infusion and delivered at a rate of 2ml/min.

6.4.1 Infusion Monitoring

Subjects will be monitored in the Clinical Translational Research Site (CTRS) at the University of Miami Hospital (UMH) for two hours prior to infusion to establish baseline vital signs (oxygen saturation, heart rate, blood pressure, and temperature) every 15 minutes. Monitoring will also continue throughout the infusion.

Once the infusion is begun, 2L/min oxygen via nasal cannula will be provided if the oxygen saturation drops below 90% on room air. The infusion will be stopped if the oxygen saturation does not return to >93% within 3 minutes of initiating supplemental oxygen or if the subject requires greater than 2L/min supplemental oxygen to achieve the required saturation of >93%. If a subject requires the addition of oxygen, it will be continued for 4 hours after the completion of the infusion. At that time, oxygen will be weaned off to maintain a saturation >93% on room air.

6.5 Concomitant Therapy

6.5.1 Permitted therapy

Concomitant medications will be recorded on the case report form (CRF), which includes all FDA-approved medications and therapies.

6.5.2 Excluded therapy

Medications and therapies not approved by the FDA are prohibited for the duration of this trial, including participation with any investigational drug or device.

6.5.3 Subject monitoring

All aspects of the study will be conducted in accordance with Good Clinical Practice (GCP) as described in the ICH Guideline (CFR ICH Selected Regulations and Guidance for Drug Studies, CFR Title 21 Food and Drugs) all applicable national and local regulations. Monitoring will be conducted by a qualified outside source at the study site.

Monitoring of key safety endpoints will be conducted. If rates significantly exceed the pre-set threshold, then the DSMB will be advised.

6.6 Blinding and Unblinding

To ensure the primary objective of safety with infusing Allogeneic MSCs in diabetic subjects with endothelial dysfunction, the pilot phase of this trial will not be blinded.

In the Randomized Phase, subjects will be randomized into active groups and study personnel will be blinded to treatment assignments. The designated cell-processing technicians will prepare the allogeneic hMSCs for infusion. The investigational agent infusions will be prepared in identical infusion bags and labeled with the identical investigational drug labels as to preserve the blind. The designated technicians in the ISCI Cell Processing Laboratory (CPL) or designee will be responsible for maintaining the investigational product records including randomized treatment assignments by subject identification. Although the subjects and investigator will remain blinded to treatment allocation throughout the study, the blinding is necessary only for the first 28 days of the study, as these timepoints are what are necessary for the short-term efficacy and safety outcomes of the study. The follow up time points at 3 months, 6 months, and 12 months, are for long-term safety and long-term efficacy but do not require blinding. The 28 day data will be used to determine the dose and time point at which MSCs exert the greatest effect on FMD and EPC-CFUs, as designed in the study, in order to design the next study protocol. The 28 day data will be analyzed by the UM Biostatistics group to determine which dose and at which time point within the 28 day follow up period shows the greatest efficacy. These results will be submitted to the NIH DSMB and the FDA.

If, for important medical reasons, unblinding is thought to be necessary, the Investigator may identify the treatment assignment by obtaining the randomization assignment by contacting the Director of Experimental and Clinical Cell Based Therapies at ISCI who is responsible for maintaining randomization records for all subjects.

6.7 Study Investigational Therapy Management

6.7.1 Investigational Product Labeling and Storage

The product label contains the elements required by the CFR and other national and local authorities for investigational products. ISCI CMP will directly store and deliver the designated cell processing technologist in the CPL, and will be kept cryopreserved in liquid nitrogen vapor phase until shortly before administration must be stored in a securely locked enclosure. Access is strictly limited to unblinded CPL personnel prior to preparation for infusion. After preparation for infusion, the Investigator and his or her designees are permitted to administer the Investigational Product only to subjects participating in this protocol.

6.7.2 Investigational Product Accountability Procedures

In accordance with all applicable regulatory requirements, the Cell Processing Laboratory will maintain a record of the investigational products hMSCs received, dispensed, administered, destroyed, or returned. The final disposition of all unused, empty, and partially used Cryocyte™ bags will be handled in accordance with the drug preparation manual.

7.1 STUDY PROCEDURES

Time and Events Schedule

The Time and Events Schedule for the conduct of this study is shown in Table 1:

Schedule of Assessments

Table 1: Time and Events Table

VISIT		Screening	Baseline	Day 0	Day 3 Post-Infusion	Day 7 Post-Infusion	Day 14 Post-Infusion	Day 28 Post-Infusion	Month 3 Post-Infusion	Month 6 Post-Infusion	Month 12 Post-Infusion
		1-6 weeks prior to Baseline	0-14 Days Prior to Day 0		+ 2 days	± 2 days	± 2 days	± 2 days	± 2 weeks	± 2 weeks	± 62 weeks
Informed Consent		X									
Full Medical History		X									
Physical Exam and Vital signs		X	X	X	X	X	X	X	X	X	X
12-lead (ECG)		X		X				X	X	X	X
Concomitant Medications		X	X	X	X	X	X	X	X	X	X
Randomization			X								
Infusion Treatment (IP)				X							
QOL Questionnaires (IIEF, SQOL-F, EQ-5D, SF-36)[1]			X					X	X	X	X
Review Adverse Events				X	X	X	X	X	X	X	X
Brachial Artery Ultrasound[2]		X	X		X	X	X	X	X	X	X
Laboratory Testing	Urinalysis	X						X	X	X	X
	Hematology & Chemistry [3]	X		X				X	X	X	X
	Coagulation [4]	X									
	HbA1c	X							X	X	X
	Viral Serology[5]	X									
	Serum Pregnancy Test[6]	X		X							
	MNCs8			X			X	X		X	X
	Serum[9]		X		X	X	X	X	X	X	X
	Endothelial Progenitor Cells10		X		X	X	X	X	X	X	X
	Genetic RNA		X						X		
	Genetic DNA		X								

Time and Events Table Key:

1 – Questionnaires: IIEF will only be given to male subjects, and SQOL-F will only be given to female subjects.

2 – Brachial Artery Ultrasound to assess endothelial function should be performed in the morning, prior to 12 p.m.

3 – The minimal laboratory requirements for hematology and chemistry include:

Hematology Tests: Complete Blood Count with Differential (white blood cell count, platelet count, hemoglobin, hematocrit), and Glycated Hemoglobin (HbA1c)*.

HbA1C will not be collected for Day 0 and Day 28

Liver Function Tests: Albumin, alkaline phosphatase, alanine transaminase (ALT), asparate aminotransferase (AST), and bilirubin (fractionate if total >1.5 times normal).

Renal Function Tests: creatinine, blood urea nitrogen (BUN), glomerular filtration rate, sodium, potassium, chloride, calcium, total protein, carbon dioxide and glucose.

4 - **Coagulation Tests**: prothrombin time (PT/INR), activated partial thromboplastin time (aPTT) at screening.

5 – Viral Serology testing at screening will include the following: HIV 1 and HIV 2 antibody screen, Hepatitis B (Hep B surface antigen, surface antibody, and core antibody), Hepatitis C antibody, Syphilis (VDRL or RPR, with reflex FTA-antibody if positive), and CMV antibody.

6 – For females of childbearing potential: serum pregnancy test (βHCG) will be completed at screening and within 36 hours before infusion.

7 – During immune monitoring for graft rejection, **Panel reactive antibodies (PRA**) will be performed from the serum of the subjects to check for donor specific antibodies ***at Baseline and Month 3 only***.

8--IThe following markers may be used for analysis to assess for activated T-cells based upon a $CD3^+CD25^+$ (late/chronic T cell activation) or $CD3^+CD69^+$ phenotype (early T cell activation).

9 – The following biomarkers may be analyzed at any or all of the time points in Table 1:

Circulating pro-inflammatory markers: serum high sensitivity C-reactive protein (hs-CRP), IL-6, IL-1, and TNFα

Circulating angiogenic factors known to mobilize and recruit EPCs: VEGF, SDF-1α, and SCF

Transcriptomic/Proteome: DNA (at baseline only), RNA *(at Baseline and Month 3 only)*

10 – Endothelial Progenitor Cell-Colony Forming Units (EPC-CFUs): Blood will be collected in Lavender top tubes (EDTA) for EPC-CFU analysis.

11 - Postdoctoral (experimental laboratory) studies:
The separate blood samples for the immune markers, biomarkers, and EPC-CFUs described above in 7-9 will be obtained 1) to provide storage of critical biomaterials derived from subjects enrolled in ACESO 2) to provide long-term integrity of these biospecimens and samples, and 3) to provide management of samples for postdoctoral studies of immunologic, immunohistochemical, cellular, and molecular analyses of collected samples; including cell-surface markers (CXCR4, C-Kit, & Connexin 43), transcriptomic/proteonomic (DNA, RNA, miRNA, protein samples, and telomerase), growth factors (noted in 8, above), functional Assays (cell growth rate, CFU assay noted in 9 above), CD3, CD25, CD69 (noted in 7 above), and inflammatory (noted in 8 above), but not limited to these. These biospecimens will be used for research purposes only, will be stored without personal identifying information, and will be shared with approved researchers who will conduct studies to improve the understanding of the effects of cell therapies and/or of diabetes disease on cardiovascular function and outcomes.

12- Since trial participants may not be able to come to the investigational site for protocol specified visits during the “Coronavirus Disease 2019” (COVID-19) pandemic, alternative methods for assessments (e.g., phone contact, virtual visit, alternative location for assessment, including local labs or imaging centers) could be implemented when necessary and feasible, and would be sufficient to assure the safety of trial participants. Assessments could be completed as follows:

- Physical Examination could be done via telemedicine using a virtual platform.

- Vital Signs and 12-Lead EKG: If subject has completed an EKG or vital signs within the window specified per protocol with their PCP or any othe treating physciain a copy could be requested. Alternatively, subjects may record and report their own vital signs or EKG using home testing equipment (such as thermometer, automated blood pressure monitor, pulse oximeter, wearable health devices, or other method for recording vital signs.

- Questionnaires, concomitant medications, and review of adverse events may be completed by study team via email, phone contact, virtual visit or alternative methods.

- Laboratory assessments: A copy of SOC labs (urinalysis, hematology, chemistry, and HbA1c) may be obtained from PCP or treating physician if done within the protocol specified time window. If participant has not completed any laboratory assessments, they may complete this in any local laboratory facility.

- Brachial Artery Ultrasound, Immune Monitoring, EPC-CFUs and Biomarkers Assessment: If participant is willing to come to site to complete this assessment all necessary safety precautions must be taken. Study team will provide the subject with a mask and gloves upon arrival to the site. If subject prefers to complete this assessment at another time, the study team must document that the assessment will be completed out of the protocol specified window and report to the regulatory authorities accordingly.

8 Study Phases and Visits

8.1 Screening Visit

See Table 1 for the procedures and assessment to be performed during the screening visit of the study, which can take place over several days. All screening visit test and procedures will occur upon signing the informed consent form (ICF). No screening exams will take place until the subject is fully informed of the research and signs the consent form.

All subjects will be enrolled at the University of Miami (UM) hospital and clinics. At the time of enrollment, we will review the medical records to ensure that the ADA guidelines for diabetic care are being followed. We will inform the subjects' primary care physician that the subject is enrolled in our study and must continue to follow the ADA guidelines throughout the trial. All the investigators follow these guidelines, and monitoring of the subjects' care by the clinical investigator or principal investigator will be ongoing through the trial at each follow visit.

Physical exam

A complete physical examination will include general appearance, skin, head and neck, Lymph nodes, musculoskeletal/extremities, cardiovascular, chest/lungs, abdomen, and neurological assessment. At screening, information about the physical examination and any significant findings must be recorded in the source documentation at the study site.

Vital signs

Vital sign measurements will be performed at least once on each study visit up to time of discharge. These measurements will consist of height (screening only), weight, respiratory rate, heart rate, blood pressure, oxygen saturation, and temperature. Respiratory rate, heart rate, and blood pressure should be measured in a seated position.

Endothelial function

- Brachial ultrasound testing and blood collection will be performed to assess endothelial function in the diabetic population at screening, baseline and 3 days, 7, 14, 28 days, 3 months, 6 months, and 12 months post stem cell infusion. This will help provide cumulative data in assessing whether or not stem cell infusion improves endothelial function. This test will be done in the morning prior to 12p.m.

- Flow Mediated Diameter percent change (FMD%): All measurements of the brachial artery diameter and FMD will be performed in the morning, in a quiet and dark room and at controlled ambient temperatures between 20°C and 26°C. Studies will be conducted after an overnight fast of at least 10 hours (water is permitted), with the subject's supine and after 10 minutes of rest. The subject's right arm will be comfortably immobilized in an extending position, allowing for ultrasound scanning of the brachial artery 5–10 cm above the

antecubital fossa. In each examination, recording of vessel images will be followed by inflation of a cuff to supra-systolic pressure (40 to 50 mmHg above systolic pressure) for 5 minutes. Then the cuff will be deflated and the brachial artery diameter will be imaged and recorded for 3 minutes. FMD% more than 10% is considered a normal response. Lower than 7% FMD% reflects endothelial dysfunction, which means a high likelihood to develop cardiovascular event in the future. Subjects with negative FMD% results (the artery is constricted after stress and not dilated as was expected) have the worst prognosis.

8.2 Baseline Visit

See Table 1 for the procedures and assessment to be performed during the baseline visit of the study. This visit will occur after all screening tests are completed and it has been determined that the subject meets eligibility criteria. This visit should occur within 2 weeks prior to Day 0 (the infusion visit).

The following endothelial function tests will be performed:

- Brachial ultrasound testing and blood collection will be performed to assess endothelial function in the diabetic population at screening, baseline and 3 days, 7, 14, 28 days, 3 months, 6 months, and 12 months post stem cell infusion. This will help provide cumulative data in assessing whether or not stem cell infusion improves endothelial function.

- Flow Mediated Diameter percent change (FMD%): All measurements of the brachial artery diameter and FMD will be performed in the morning, in a quiet and dark room and at controlled ambient temperatures between 20°C and 26°C. Studies will be conducted after an overnight fast of at least 10 hours (water is permitted), with the subject's supine and after 10 minutes of rest. The subject's right arm will be comfortably immobilized in an extending position, allowing for ultrasound scanning of the brachial artery 5–10 cm above the antecubital fossa. In each examination, recording of vessel images will be followed by inflation of a cuff to supra-systolic pressure (40 to 50 mmHg above systolic pressure) for 5 minutes. Then the cuff will be deflated and the brachial artery diameter will be imaged and recorded for 3 minutes. FMD% more than 10% is considered a normal response. Lower than 7% FMD% reflects endothelial dysfunction, which means a high likelihood to develop cardiovascular event in the future. Subjects with negative FMD% results (the artery is constricted after stress and not dilated as was expected) have the worst prognosis.

- Blood drawn from fasting subjects for biomarker and PRA analysis will be separated and the serum will be frozen until processed as one batch towards the end of the study.

- Biomarker analysis: Soluble pro inflammatory cytokines (interleukin 1, interleukin-6, tumor necrosis factor alpha, high-sensitivity C Reactive Protein, and circulating angiogenic factors) (VEGF, SDF-1α, and SCF) using immunological and ELISA methods.

- Assay of colony forming units: Fresh blood will be processed for cell culture assays for endothelial progenitor stem cells colonies counting (a 5 days' protocol). Fifty milliliter of blood will be processed; peripheral-blood mononuclear cells will be isolated by Ficoll density-gradient centrifugation, will be washed twice in phosphate buffered saline with 5% fetal bovine serum and re-suspended in media (EndoCult basal media with supplements; StemCell Technologies, Vancouver, British Columbia, Canada) for EPC colony-forming assay. Cells will be planted on human fibronectin-coated plates (BIOCOAT; Becton Dickenson Labware, Bedford, Massachusetts) at a density of $5X10^6$ cells/well and incubated at 37^0C in humidified 5% CO_2. After 48 hours, the non-adherent cells will be re-plated onto fibronectin-coated 24 well plates at a density of $1X10^6$ cells/well. After 5 days, colony forming units (defined as a central core of rounded cells surrounded by elongated and spindle-shaped cells) will be counted manually in 8 wells out of a 24-well plate.

8.3 Day 0 Visit

See Table 1 for the procedures and assessment to be performed during the Day 0 visit of the study. The Day 0 visit will occur after all baseline tests are completed and it has been determined that the subject remains eligible. Once the subject is deemed eligible to continue in the study, the subject will be administered the investigational product.

8.4 Day 3, Day 7, Day 14, and Day 28 (Post-Intervention Visit)

See Table 1 for the procedures and assessment to be performed for Day 3 through Day 28 visit. Outpatient visits should be completed as close to the scheduled visit dates as possible. There will be a window of + 2 days for Day 3 visit, and ± 2 days for Day 7, Day 14, and Day 28 study visits.

8.5 Month 3, Month 6, and Month 12 (Post-Intervention Visit)

See Table 1 for the procedures and assessment to be performed for the Month 3, Month 6, and Month 12 study visits. Outpatient visits should be completed as close to the scheduled visit dates as possible. There will be a window of ± 2 weeks for the Month 3 and Manth 6 visits. Month 12 will have a window of ± 6 weeks.

8.6 Genetic Testing

A separate blood sample of about 10mL for gene expression (DNA) profiling of WBC (at baseline visit) and for RNA expression analysis (at baseline and month 3 visits) will be obtained from the study participants, as detailed on Table 1. A separate genetic consent form is completed by the study participants.
All samples will be identified so that they can be linked to individual subjects. These samples may be stored indefinitely. Individual results will not be returned to the subject

or the study physician. The samples will be linked to subjects, but there will be no recontact.
Data presented in publications will not contain individual subjects' clinical characteristics or outcomes; only aggregate data from the entire study will be disclosed.

8.7 Immune Monitoring for Graft Rejection

The studies planned in this protocol will utilize allogeneic mesenchymal stem cells (MSC) in subjects with endothelial dysfunction. The use of an allogeneic graft raises the potential of graft rejection through immune cells resulting in failure of the therapy. MSCs are ideal candidates for allogeneic transplantation because they show minimal MHC class II and ICAM expression and lack B-7 co-stimulatory molecules necessary for T-cell mediated immune responses[57, 58]. Indeed MSCs do not stimulate a proliferative response from alloreactive T-cells even when the MSCs have differentiated into other lineages or are exposed to proinflammatory cytokines. Previous studies have demonstrated that MSCs have significant immunomodulatory effects, inhibiting T-cell proliferation and prolonging skin allograft survival. Recently human MSCs were shown to alter the cytokine secretion profile of dendritic cells, T cells, and natural killer cells in vitro, inhibiting secretion of proinflammatory cytokines (e.g. TNF-a, IFN-γ) and increasing expression of suppressive cytokines (e.g.IL-10), possibly via a prostaglandin E2 mediated pathway.

In vivo studies of the fate of MSCs have shown that, when transplanted into fetal sheep, human MSCs engraft, undergo site-specific differentiation into various cell types, including myocytes and cardiomyocytes and persist in multiple tissues for as long as 13 months after transplantation in non-immunosuppressed immunocompetent hosts. Further, in vivo studies using rodents, dogs, goats, and baboons demonstrate that allogeneic MSCs can be engrafted into these species without stimulating systemic alloantibody production or eliciting a proliferative response from recipient lymphocytes. These findings, coupled with our demonstration of efficacy of these cells for cardiac repair, solidify the notion of using MSCs as an allograft for successful tissue regeneration.

As part of this protocol we will obtain peripheral blood samples from all subjects to evaluate the presence and/or development of Panel Reactive Antibodies (PRA) and the presence of activated T cells. Heparinized (green top) and Serum Separator Tube (SST) (which are either gold top or red tiger vacutainer tubes), approx. 15 cc total blood, will be collected at different time points during the study (please reference Table 1). Peripheral blood mononuclear cells (PBMC) will be isolated from heparinized blood by Eicoll sedimentation and will be viably cryopreserved for planned assessments of T cell activation.

Two of the best-accepted markers of T cell activation are CD69 and CD25 (IL-2 receptor α). We will monitor the activation of T cells by flow cytometric analysis of CD3+CD25+CD69+ cells in thawed PBMC. CD69 is an immediate/early marker of CD3+ T cell activation while CD25 expression increases within 1-2 days of activation and remains sustained over the intermediate-long term during chronic immune activation. Given the differences in the kinetics of CD69 and CD25 up regulation, assessment of

both activation phenotypes (CD3+CD69+ and CD3+CD25+) will maximize the sensitivity of detection of T cell activation following allogeneic hMSC infusion.

Additionally, in female subjects who receive allogeneic hMSCs, the stored baseline serum will be analyzed to evaluate the antibody responses to HLA and H-Y antigens.

9. SAFETY

9.1 Safety Variables

- Vital signs
- Physical examination
- Clinical laboratory tests
- Urinalysis
- Adverse events

9.2 Laboratory Evaluations

At screening, the HIV-1 and HIV-2 tests, Syphilis – VDRL (Confirmation with FTA-ABS if needed (Syphilis)), CMV, hepatitis screen, and β-HCG serum pregnancy tests (only for women of child-bearing potential) will be performed locally at the study site. Laboratory safety tests will consist of the following:

Serum chemistry: sodium, potassium, chloride, bicarbonate, BUN, creatinine, glucose, calcium, AST/SGOT, ALT/SGPT, total bilirubin (fractionate if total >1.5 times normal), alkaline phosphatase, albumin, Coagulation studies

Hematology (CBC): hemoglobin, hematocrit, platelets, WBC, WBC differential

The Investigator will review all clinically relevant laboratory results requested in the protocol. The diagnosis associated with any clinically significant laboratory deviations should be recorded as an AE and should indicate the underlying abnormality or diagnosis (such as renal insufficiency) as opposed to the observed deviation in laboratory results (such as elevated creatinine). If there is no underlying abnormality linked to a clinically significant abnormal laboratory value, the observed deviation should be reported as the AE.

9.2.1 Pulse Oximetry

Pulse oximetry will be used to observe oxygen saturation when measuring vital signs at the infusion visit (Reference Appendix 1). Pulse oximetry will also be used throughout infusions and 2 hours following infusions. Subjects should have a resting oxygen saturation of ≥ 93% prior to infusion. Subjects requiring oxygen, need the peripheral artery oxygen saturation (SaO_2) to be ≥ 93% when given a maximum of 2L/minute supplemental

O_2 via nasal cannula. Infusion toxicity will be assessed based on decreases in oxygen saturation during infusion. The infusion will be stopped if the oxygen saturation does not return to >93% within 3 minutes of initiating supplemental oxygen or if the subject requires greater than 2L/min supplemental oxygen to achieve the required saturation of >93%. If this occurs, then subjects will be admitted to the hospital for observation.

9.2.2 Pregnancy

There is no information regarding allogeneic hMSCs and its effects or potential risks to a fetus or unborn child. The Principal Investigator and DSMB must be notified within twenty-four hours of investigator's awareness of the pregnancy via facsimile if a study subject becomes pregnant during the study. Any two of the enumerated contraceptive items will be acceptable for meeting the studies contraceptive requirements as listed in paragraph 2 of section 8.2.2. Females will be defined as non-childbearing potential if surgically sterilized (i.e. bilateral tubal litigation, bilateral oophorectomy, or complete hysterectomy) or post-menopausal (defined as 12 months no menses with an alternative medical cause and with a follicle stimulating hormone FSH $\geq$ 25.8 IU/L). Non-sterilized males who are sexually active with a female partner of childbearing potential must use any two of the acceptable forms of contraceptive items as listed below:

Acceptable forms of contraception include: 1) abstinence, 2) condoms (male or female) with a spermicidal agent, 3) diaphragm or cervical cap with spermicidal agent, 4) intrauterine device (IUD), 5) oral contraceptive, 6) injectable or transdermal hormonal contraceptive, 7) successful vasectomy with resulting azoospermia or azoospermia for any other reason, and 8) hysterectomy, bilateral oophorectomy, or tubal ligation.

Prior to study enrollment, women of childbearing potential must be advised of the importance of avoiding pregnancy during trial participation and the potential risk factors for a pregnancy. The subject must sign an informed consent and written authorization for use and disclosure of PHI document stating that the above-mentioned risk factors and the consequences were discussed with her.

9.2.3 Determination of Infusional Toxicity

Infusional toxicity will be evaluated by continuously monitoring the subject's vital signs and O_2 saturation by pulse oximetry from the time of allogeneic hMSCs administration until two hours after infusion is complete. Since there is no specific or antidotal therapy for AEs arising from allogeneic hMSCs, any toxicity that may arise during a subject's participation in this study should be managed with supportive measures at the discretion of the treating physician.

9.2.4 Subject Stopping Guidelines

This guideline is to be used to indicate boundaries requiring discussion by the Data and Safety Monitoring Board (DSMB) and is designed to assist the independent DSMB in

overseeing the study. The DSMB may also request additional interim analyses and develop other criteria including provision for monitoring of potential late effects to determine when to intervene in the enrollment or treatment of subjects in the study.

The first more conservative stopping guideline is to monitor subjects for unexpected SAEs where there is a reasonable possibility that the study product or administration procedure caused the event within 30 days of administration including cardiorespiratory distress during infusion, subject death, myocardial infarction, hemodynamically unstable ventricular tachycardia, stroke, or more than two related, ≥ Grade 3 AEs (based on the NIH CTCAEv4). Study accrual and further treatment of subjects will be put on hold (temporary suspension of study drug administration, pending a safety investigation) if any subjects experience one of these events. The DSMB will be notified within 24 hours of the occurrence of these events and will be convened within 3 business days to review the event and study.

The following are subject stopping guidelines:

- Any subject who develops persistent (that is, still existing more than 3 hours after the end of IP infusion) cardiorespiratory signs or symptoms (for example, shortness of breath, tachypnea, tachycardia, hypotension, or palpitations) will continue with all scheduled follow-up if such follow-up is considered safe in the opinion of the Investigator.

- Any subject whose infusion is stopped due to cardiorespiratory distress will receive no further IP infusions but will continue with all scheduled follow-up if such follow-up is considered safe in the opinion of the Investigator.

- Any subject who develops any sign or symptom that, at the discretion of the Investigator, warrants the discontinuation of infusion will receive no further IP infusions but will continue with all scheduled follow-up if such follow-up is considered safe in the opinion of the Investigator.

- Infusion of the IP may be stopped if there is an adverse event that the Investigator believes is related to the IP or if there is an issue with the IP infusion.

- The proportion of subjects experiencing TE-SAE as defined in Section 2.2.1 will be monitored within 30 days of injection. This guideline is designed to assist the independent DSMB in overseeing the study and indicate boundaries needing discussion by the DSMB. The DSMB may also request additional interim analyses and develop other criteria including provision for monitoring of potential late effects to determine when to intervene in the enrollment or treatment of subjects in the study.

- The stopping guidelines serve as a mechanism for consultation with the DSMB for additional review, and are not formal “stopping rules” that would mandate automatic closure of study enrollment. It is designed to assist the independent

DSMB in overseeing the study. The DSMB may also request additional interim analyses and develop other criteria including provision for monitoring of potential late effects to determine when to intervene in the enrollment or treatment of subjects in the study.

9.2.5 Subject observation and discontinuation after IP administration

The IP administration guidelines in **Appendix 1** list the study requirements for subject observation and discharge after IP administration.

9.3 Definition of an Adverse Event

An Adverse Event (AE) is any untoward medical occurrence in a subject or clinical investigation subject temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product. The occurrence does not necessarily have to have a causal relationship to the treatment received in the study. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease (new or exacerbated) temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

Examples of an AE include:

- Exacerbation of a chronic or intermittent pre-existing condition including either an increase in frequency or intensity of the condition.

- Significant or unexpected worsening or exacerbation of the condition/indication under study.

- A new condition detected or diagnosed after study therapy administration even though it may have been present prior to the start of the study.

- Pre- or post-treatment events that occur as a result of protocol-mandated procedures (e.g., invasive protocol-defined procedures, modification of a subject's previous treatment regimen).

An AE does **not** include:

- Medical or surgical procedures (e.g., colonoscopy, biopsy). The medical condition that leads to the procedure is an AE.

- Social or convenience hospital admissions where an untoward medical occurrence did not occur.

- Day to day fluctuations of pre-existing disease or conditions present or detected at the start of the study that do not worsen.

- The disease/disorder being studied or expected progression, signs, or symptoms of the disease/disorder being studied unless more severe than expected for the subject's condition.

9.4 Definition of Adverse Reaction

An adverse reaction is any adverse event caused by a drug. Adverse reactions are a subset of all suspected adverse reactions, for which there is reason to conclude that the drug caused the event.

9.5 Definition of Suspected Adverse Reaction

Suspected adverse reaction means any adverse event for which there is a reasonable possibility that the drug caused the adverse event. For the purposes of IND safety reporting, 'reasonable possibility' means there is evidence to suggest a causal relationship between the drug and the adverse event. A suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug.

9.6 Definition of Serious

An adverse event (AE) or suspected adverse reaction is considered "serious" if it:

1. results in death
2. is life-threatening (at risk of death at the time of the event)
3. requires in inpatient hospitalization or prolongation of existing hospitalization

   NOTE: Complications that occur during hospitalization are AEs. If a complication prolongs hospitalization or fulfills any other serious criteria, the event is serious. Hospitalization for elective treatment of a pre-existing condition that did not worsen from baseline is not considered to be an AE.
4. results in disability/incapacity

   NOTE: The term disability means a substantial disruption of a person's ability to conduct normal life functions. This definition is not intended to include experiences of relatively minor medical significance such as uncomplicated headache, nausea, vomiting, diarrhea, influenza, accidental trauma (i.e., sprained ankle) that may interfere or prevent everyday life functions but do not constitute a substantial disruption.
5. Is a congenital anomaly/birth defect.

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

9.7 Definition of Unexpected

An adverse event or suspected adverse reaction is considered “unexpected” if it is not listed in the investigator brochure or is not listed at the specificity or severity that has been observed; or, if an investigator brochure is not required or available, is not consistent with the risk information described in the general investigational plan or elsewhere in the current application.

9.8 Clinical Laboratory Assessments and Other Abnormal Assessments as Adverse Events and Serious Adverse Events

Abnormal laboratory findings (e.g. clinical chemistry, hematology) or other abnormal assessments (e.g., vital signs) that are judged by the Investigator as clinically significant will be recorded as AEs or SAEs if they meet the definition of an AE as defined in Section 8.3 (“Definition of an Adverse Event”) or SAE, as defined in Section 8.6 (“Definition of a Serious Adverse Event”) and will assess intensity based on the criteria defined in Section 8.10. Clinically significant abnormal laboratory findings or other abnormal assessments that are detected during the study or are present at screening and significantly worsen following the start of the study will be reported as AEs or SAEs. However, clinically significant abnormal laboratory findings or other abnormal assessments that are associated with the disease being studied, unless judged by the Investigator as more severe than expected for the subject’s condition, or that are present or detected at the start of the study but do not worsen, will not be reported as AEs or SAEs.

The Investigator will use the normal laboratory ranges (or a change in baseline in subject’s whose laboratory tests were outside of the normal range when enrolled), as well as the NIH CTCAEv4 when exercising medical judgment in assessing whether abnormal laboratory values are clinically significant.

9.9 Recording of Adverse Events and Serious Adverse Events

The Investigator should review all documentation (e.g., hospital progress notes, laboratory, or diagnostic reports) relative to the event being reported. The Investigator will then record all relevant information regarding an AE/SAE into the electronic data system. It is not acceptable for the Investigator to send photocopies of the subjects’ medical records in lieu of completion of the appropriate AE/SAE pages.

The Investigator will attempt to establish a diagnosis of the event based on signs, symptoms, and/or other clinical information. In such cases, the diagnosis should be documented as the AE/SAE and not the individual signs and symptoms.

SAEs will be reported to the IRB within 10 working days or within 24 hours if the event is life-threatening or results in death.

Pregnancies

Subject pregnancy must be reported to the Principal Investigator within 1 working day of knowledge of the event. Any subject that becomes pregnant during the study must be promptly withdrawn from the study. Follow-up information regarding the outcome of the pregnancy and any postnatal sequelae in the infant will be required.

9.10 Intensity of Adverse Events and Serious Adverse Events

The Investigator will make an assessment of intensity for each AE and SAE reported during the study. The assessment will be based on the Investigator's clinical judgment. The intensity of each AE and SAE should be assigned to one of the following categories:

Mild:	An event that is easily tolerated by the subject, causing minimal discomfort and not interfering with everyday activities.
Moderate:	An event that is sufficiently discomforting to interfere with normal everyday activities.
Severe:	An event that prevents normal everyday activities.
Life-threatening:	Immediate risk of death.

An AE that is assessed as severe should not be confused with an SAE. Severity is a category utilized for rating the intensity of an event; and both AEs and SAEs can be assessed as severe. An event is described as 'serious' when it meets one of the pre-defined outcomes as described in Section 8.6, "Definition of Serious."

9.11 Causality of Adverse Events and Serious Adverse Events

The Investigator is obligated to assess the causality between study therapy and the occurrence of each AE/SAE. The Investigator will use clinical judgment to determine if there is a reasonable possibility that the biological action of the study therapy was responsible for AE/SAE being reported. Alternative causes such as natural history of the underlying diseases, concomitant therapy, other risk factors, and the temporal relationship of the event to the study therapy will be considered and investigated. The Investigator will also consult the Clinical Investigator's Brochure and/or Product Information, for marketed products, in the determination of his/her assessment.

The Investigator will use the following questions when assessing causality of an adverse event to study therapy.

Is there a reasonable possibility that the study therapy caused the event? Reasonable possibility implies that there is evidence that the vent was caused by the study product. An affirmative answer designates the event as a suspected adverse reaction.

There may be situations when an SAE has occurred and the Investigator has minimal information to include in the initial report. However, it is very important that the Investigator always makes an assessment of causality.

The relationship between AEs and the study exposure will be classified by the investigator as:

- None: No relationship. Related to other known etiologies, conditions, or exposures.
- Unlikely: Current knowledge suggests that a relationship is unlikely.
- Possible: A plausible temporal sequence or response pattern exists but the AE may be related to other known etiologies, conditions, or exposures.
- Probable: A plausible temporal sequence or response pattern exists and the AE cannot be related other known etiologies, conditions, or exposures.
- Definite: A plausible temporal sequence or response pattern exists and the AE can be confirmed by re-challenge or with other supporting data.

9.12 Follow-Up of Adverse Events and Serious Adverse Events

After the initial AE/SAE report, the Investigator is required to proactively follow each subject and provide further information on the subject's condition. All AEs and SAEs documented at a previous visit/contact that are designated as ongoing will be reviewed at subsequent visits/contacts.

Adverse events and SAEs will be followed until resolution, until no further changes in the event are expected (i.e. the point at which a subject experiencing a critical adverse event is treated successfully and stabilized even though they may continue to experience lingering sequelae that may never resolve), until the subject is lost to follow-up, or until it is agreed that further follow-up of the event is not warranted (e.g. non-serious, study therapy unrelated, mild or moderate adverse events ongoing at a subject's final study visit). If a subject dies during participation in the study or during a recognized follow-up period, the Investigator will provide a copy of any post-mortem findings, including histopathology.

New or updated information will be recorded by modifying the AE forms in the electronic data system

9.13 Timeframes for Submitting SAE Reports

Once an Investigator becomes aware that an SAE has occurred in a study subject, he/she will record the information in the electronic data record within 48 hours. Any fatal or life-threatening event must be reported within 24 hours. If the Investigator does not have all information regarding an SAE, he/she will not wait to receive additional information before

recording the event in the data system and completing as much information known at the time of the submission. The reporting timeframes for any SAE occurring during the study are summarized in Table 2.

TABLE 2
Serious Adverse Event Reporting Requirements

	Initial Reports		Follow-Up Reports
Type of SAE	Fatal or Life-Threatening	Other SAEs	Any SAE
Reporting Timeframes	24 hours	48 hours	48 hours
Documents Required	24 hours: Complete as much information in the electronic data system that is known. 48 hours: Fully complete all AE forms	Fully completed AE forms	Updated AE Forms

9.14 Post-Study Adverse Events and Serious Adverse Events

The Investigator should report any death or SAE occurring at any time after a subject has completed or terminated a clinical trial, when such death or SAE may reasonably be related to the study therapy used in an investigational trial. Investigators are not obligated to actively seek AEs from former study participants.

9.15 Regulatory Aspects of Adverse Event Reporting

The Investigator will promptly report all SAEs within the timeframes specified in Section 8.13. Prompt notification of SAEs by the Investigator is essential so that UMMSM can meet legal obligations and fulfill ethical responsibilities towards the safety of all subjects participating in UMMSM-sponsored investigational trials.

The Investigator will comply with the applicable local regulatory requirements related to reporting of SAEs to his or her Institutional Review Board (IRB) or Independent Ethics Committee (IEC).

This protocol has been filed under an Investigational New Drug (IND) application with the FDA. A given SAE may qualify as an Expedited Safety Report (ESR) if the SAE is both at least possibly attributable to study therapy and unexpected. In this case, all Investigators participating in an IND study will receive an ESR.

The ESRs are prepared according to UMMSM policy and are forwarded to the Investigator as necessary. The purpose of the ESR is to fulfill specific regulatory and Good Clinical Practice (GCP) requirements regarding the product under investigation. Based on previous trials involving intravenous infusion of allogeneic human MSCs, no AEs have been attributed to treatment administration; therefore all AEs will be considered and documented as unexpected AEs.

All AEs occurring at any time during the trial will be collected, documented, and reported by the investigator. For each AE, the investigator will provide the date of onset and resolution, intensity, treatment required, outcome, seriousness, and potential causality with regards to the study exposure.

10. STATISTICAL ANALYSIS

10.1 Determination of Sample Size and Analysis Population

No formal statistical justification was performed to determine sample size in this Phase I/II study. The allocation ratio will be 1:1. All randomized subjects will be included in summaries of baseline characteristics, safety, and efficacy. Reasons for study discontinuation will be tabulated.

10.2 General Statistical Methods

All statistical tests will be performed at an $\propto$=0.05 level of significance, using two-sided tests. Because this is a Phase I/II study with only exploratory efficacy outcomes, no adjustments will be made for multiple analyses. Continuous variables will be presented by descriptive statistics. Categorical variables will be presented by counts. Two sided 95% confidence intervals will be calculated and presented where appropriate.

Analysis of AEs will include tabulation by frequency, severity, organ system affected, and relationship to study exposure. Lung function data will be summarized descriptively. Subject reported outcome data will be summarized according to the guidelines of each questionnaire.

10.3 Interim Analyses

Interim analyses will be conducted at times coincident with regularly scheduled meetings of the Data and Safety Monitoring Board (DSMB) at approximately six-month intervals. The DSMB Chair will be notified each time an SAE occurs. After all subjects in the low dose have been followed for 30 days, at that time an independent DSMB will review all available data to make an independent recommendation to either keep the specified randomized dose 1:1 or to recommend a dose modification.

Policies of the DSMB will be described in the DSMB Charter, which will be prepared by the DSMB prior to study initiation. The stopping guidelines serve as a trigger for consultation with the DSMB for additional review, and are not formal “stopping rules” that would mandate automatic closure of study enrollment.”

11 Data Safety and Monitoring Board (DSMB)

NHLBI Gene and Cell Therapy Data and Safety Monitoring Board

11.1 INTRODUCTION

This Charter describes the roles and responsibilities for the NHLBI Gene and Cell Therapy Data and Safety Monitoring Board (DSMB).
The DSMB may wish to review this Charter at regular intervals to determine whether any changes are needed.

11.2 ROLE OF THE DSMB

This study is designed to test the safety of hMSCs in diabetic subjects with endothelial dysfunction.

The purpose of the data safety monitoring board (DSMB), which is the NHLBI Gene and Cell Therapy DSMB, is to advise the investigators regarding the continuing safety of study subjects and those yet to be recruited to the study, as well as the continuing validity and scientific merit of the study.

The University of Miami Clinical Research Operations and Regulatory Support (CRORS) will be monitoring this trial. The Biostatistics Collaboration and Consulting Core will be the DCC for this trial.

This section describes the roles, responsibilities and operating procedures of the DSMB, and includes guidelines for communications and interactions between the DSMB and the investigators to schedule and format for meetings; format for presentation of data; specification of who will have access to interim data and who may attend all or part of DSMB meetings; procedures for assessing conflict of interest of potential DSMB members; and the method and timing of providing interim reports to the DSMB.

The UM Biostatistics Collaboration and Consulting Core will prepare semi-annual summary reports of all AEs/SAEs for the NHLBI Project Officer and DSMB Chairman. Semi-annual reports will be made available as part of the semi-annual DSMB meeting materials.

11.3 Purpose of the DSMB

The primary function of the NHLBI Gene and Cell Therapy DSMB is to review the accumulating unblinded safety data from each study group and using the data as the basis for recommendations concerning the continuation and/or modification of the study. This will be accomplished through regularly scheduled formal meetings and/or additional meetings to review interim summaries of safety and efficacy data. The DSMB will make recommendations regarding modification or termination of the study in the event of significant study conduct issues or safety concerns. The DSMB will not stop the study

based on efficacy results favorable to hMSCs, other than for all-cause mortality as outlined below. The selected primary and secondary endpoints were chosen to measure major morbidity in subjects with diabetic subjects with endothelial dysfunction. Given the importance of mortality, a stopping boundary based on the all-cause mortality rate will be implemented to guide the DSMB. This stopping boundary will not be applied until after all subjects have enrolled in the study.

11.4 DSMB MEMBERSHIP

In the NHLBI Gene and Cell Therapy DSMB, rather than corresponding directly with the DSMB chair, AE reports will be sent to Dr. Denis Buxton as the NHLBI project officer with Charlene Schramm copied as the Executive Secretary of the DSMB. The NHLBI will then forward the reports to the DSMB. Charlene Schramm will be responsible for the minutes for DSMB meetings.

The DSMB will meet until the study's database has been locked and a final data review has been completed. If a member withdraws from the DSMB, the DSMB chairperson will be responsible for selecting an appropriate replacement.

11.4.1 Roles of NHLBI Staff in DSMB meetings

- NHLBI program staff members involved in the day-to-day conduct of the study may attend the open sessions of DSMB meetings. These Program staff may attend portions of a closed session as needed, but not when post-randomization outcome data by treatment group will be discussed.

- NHLBI's Office of Biostatistics Research has assigned one or more statisticians to this DSMB. The statistician(s) will not be involved in the day-to-day operations of the study, but will be involved in statistical aspects of protocol development, monitoring safety and efficacy data in an unmasked fashion, as well as working with the DCC on analytic plans and publications. The NHLBI study statistician(s) will also serve as a resource to the DSMB as needed.

- The NHLBI ES will be a federal employee or contractor with appropriate expertise and training who has no other involvement in the conduct of the trial and does not report directly to the lead program official.

- The ES is the only NHLBI staff member who can routinely be in the executive session. The DSMB can opt to have an executive session without the ES, but then will be responsible for minutes for that portion of the meeting. The DSMB can request to have other staff members attend the executive session to provide additional information as needed.

- The NHLBI ES and statistician(s) are expected to report issues of substantive concern to the NHLBI Division Director responsible for the trial. The NHLBI Division Director will communicate with the Office of the Director, NHLBI. Under special circumstances, and with the concurrence of the DSMB Chair, the Division

leadership and Director and Deputy Director of the NHLBI may see unmasked data presented at DSMB meetings.

- There may be occasions when it is appropriate for new staff not involved in the study to attend a DSMB meeting as a training opportunity. This will be discussed with the DSMB Chair before the meeting; the new staff member(s) would attend only the portions of the meeting outlined in the first bullet above.

11.4.2 DSMB Responsibilities

The DSMB is responsible for safeguarding the interests of study participants, assessing the safety and efficacy of study procedures, ensuring data quality, and for monitoring the overall conduct of the studies. For each of the clinical trials in its portfolio the DSMB is expected to evaluate candidate protocols for safety and equipoise, and monitor ongoing studies to ensure compliance with protocols, accurate and timely reporting of adverse events, and acceptable subject recruitment rates. In performance of these duties the DSMB may elect to review unblinded study data and/or request detailed information from the study Sponsor or Principal Investigator(s).

The DSMB is an independent body that provides recommendations to the Office of the Director, NHLBI, and is required to provide recommendations about starting, continuing, and stopping the studies.

In addition, the DSMB is asked to make recommendations or provide comments to the NHLBI regarding:

- Equipoise of the study
- Benefit/risk ratio of procedures and participant burden
- Potential issues related to selection, recruitment, and retention of participants
- Compliance with the protocol and informed consent procedures
- Completeness, quality, and feasibility of study endpoints
- Adequacy of the data and statistical analysis plan
- Amendments to the study protocol and consent forms, including whether any modifications may impact the equipoise of the study
- Performance of regional clinical centers or core labs
- Participant safety, including review of consent form
- Notification of and referral for unanticipated incidents or findings
- Participant safety and parent study burden of proposed ancillary studies, including whether the total burden of ancillary studies might compromise the parent study

11.4.3 DSMB Chair(s) Responsibilities

The Chair(s) is/are responsible for the conduct of meetings and ensuring that planned business, according to the agenda, is addressed. They should ensure that the atmosphere of board discussions fosters an open exchange of views and opinions, is

focused on salient issues, and is directed toward the formulation of recommendations. They should solicit the views of the member(s) with expertise appropriate for the issue at hand, and may request additional expertise or consultants if needed.

Members are expected to review reports of AE/SAE/Ups, new protocols, and/or protocol amendments that arise between meetings at the request of the NHLBI program staff and DSMB Chair.

11.4.4 DSMB Conflict of Interest

Members must submit an annual conflict of interest assessment, and promptly disclose any new conflicts of interest that may arise during their tenure. This DSMB will follow NIH and NHLBI conflict of interest rules. At the beginning of each meeting, all DSMB members will be asked to state whether they have developed any new conflicts of interest since the last formal annual report to NHLBI. If a new conflict is reported, the Chair and NHLBI program staff will determine if the conflict limits the ability of the DSMB member to participate in the discussion.

11.4.5 DSMB Confidentiality

Members are expected to maintain the security and confidentiality of study data and DSMB discussions.

Members are expected to review all materials prior to meetings and calls, and participate in discussions at all meetings and calls.

11.5 Investigator Responsibilities

The investigator has the responsibility to:

1. Make decisions based on DSMB recommendations in a timely fashion.
2. Notify study centers of the outcome of the DSMB meetings, and any DSMB recommendations addressing actions to be taken to ensure the integrity of the study.
3. Notify regulatory agencies of DSMB recommendations addressing any emerging safety concern not recognized at the start of the study.
4. Ensure that the unblinded DSMB support team is provided with the data necessary for the chosen analyses and reports.
5. Provide DSMB members with the current protocols and Investigator's Brochure.
6. Provide DSMB members with PSURs as published
7. Attend the open session of each DSMB data review meeting.

11.6 DSMB MEETINGS

DSMB meetings are usually held twice a year in the Washington, DC area. In addition to regular meetings, it may be necessary to convene the DSMB on an ad hoc basis to discuss new studies or new information related to monitored studies that raises questions

about equipoise, safety, or anything else in the trial. Conference calls are appropriate for conducting meetings, if the agenda permits.

The agenda for DSMB meetings and calls will be drafted by the ES in consultation with NHLBI staff. The ES will finalize the agenda after consultation with the DSMB Chair. The agenda and meeting materials should be distributed by the ES at least two weeks before each meeting or call. Documents will be distributed via a SharePoint site where all standing DSMB members will have reading and downloading privileges. Ad hoc (ex officio) members will receive from the ES only those documents relevant to the study(ies) monitored by each ad hoc member.

At the time that the agenda is sent out, and again at the beginning of the meeting, the ES will ask all DSMB members to state whether they have developed any new conflicts of interest since the last formal annual report to NHLBI. If a new conflict is reported, the Chair and staff will determine if the conflict limits the ability of the DSMB member to participate in the discussion.

During the meetings, and depending on the phase of each study, the DSMB will review adverse event data, other safety data, quality and completeness of study data, and enrollment data at each meeting to ensure proper trial conduct. Study personnel should provide any new literature particularly pertinent to the trial, along with their recommendation as to whether it affects the trial conduct or design. The DSMB will review the informed consent form when it reviews the protocol. The DSMB will review the consent periodically and/or as needed and consider whether the consent form requires revision in light of any new findings or amendments. At intervals, as noted above, the DSMB will also review formal interim analyses of the primary end point.

Each meeting culminates in a vote regarding a recommendation to the NHLBI whether the clinical studies should proceed as planned or with modifications, or be terminated according to the review and stopping guidelines outlined in this document.

It is expected that all DSMB members will attend every meeting and conference call. However, it is recognized that this may not always be possible. A quorum for voting is half of the standing members plus one. The Board may wish to decide if particular expertise is needed within the quorum for a particular meeting. All standing Monitoring Board members are voting members.

To ensure adequate expertise, the Board may elect to appoint ad hoc members for studies in its portfolio, and to confer voting rights for studies within their purview.

Outside entities may request inclusion of additional independent experts during discussions of their studies. The Board may elect to appoint such members in an ex officio capacity. Ex officio members may only be present during open sessions, and are not entitled to vote.

11.6.1 Discussion of Confidential Material

DSMB meetings and calls will be organized into open, closed, and executive sessions.

- During the open sessions, information will be presented to the DSMB by the DCC, study investigators, and NHLBI staff as appropriate, with time for discussion.

- During the closed sessions, the DSMB, DCC unmasked statistician, and unmasked NHLBI statistician(s) may discuss confidential data from the studies, including information on efficacy and safety by treatment arm. The NHLBI's expectation is that the DSMB will review unmasked data, but the Board has the discretion to remain blinded to study information. If the closed session occurs on a conference call, steps will be taken to ensure that only the appropriate participants are on the call, and to invite others to re-join the call only at the conclusion of the closed session.

- The DSMB may hold an executive session in which only the DSMB members are present. The NHLBI ES may attend the executive session at the invitation of the DSMB Chair. If the ES does not attend the executive session, the DSMB Chair will be responsible for summarizing the DSMB's discussion and recommendations to the ES. Based on an overall assessment of risk, the monitoring board will make one of three general recommendations: o Continue the study as planned, without modification;

    - Continue the study with recommendations – specifying the additions or modifications;
    - Stop enrollment in the study, either as a whole or for a particular arm.

Voting on recommendations will follow Robert's Rules of Order.

At the conclusion of the closed and executive sessions, the DSMB chair may provide a summary of the preliminary recommendations to the lead investigators and masked NHLBI staff to provide an opportunity for study investigators, the DCC, and NHLBI to ask questions to clarify the recommendations. Recommendations that would unmask results, such as a recommendation to close a study prematurely, should not be disclosed until approved by NHLBI leadership.

11.6.2 Reports of DSMB Deliberations

- Formal minutes: The NHLBI ES is responsible for preparation and transmission of the formal DSMB minutes to the Director of the applicable Division within 14 calendar days of each meeting or call. Minutes will document whether there is conflict of interest on the part of Board members and will summarize the key points of the discussion and debate, requests for additional information, response of the investigators to previous recommendations, and the recommendations from the current meeting.

- Following division and, when applicable, DCC review, the minutes are sent to:

    - DSMB Chair, who approves them on behalf of the DSMB.
    - Division Director, Division of Cardiovascular Sciences, NHLBI, for final Institute Approval.

- Once the Division Director, NHLBI has approved the minutes, they are considered final.

- The NHLBI Program Office will prepare a Summary Report of Board Recommendations and submit it to primary study investigators(s) or DCC within 30 calendar days of each meeting. Primary study investigators(s) or DCC will forward the Summary Report to each participating research site and are responsible for forwarding the DSMB reports to their respective IRBs.

- If the DSMB does not identify any safety or other protocol-related concerns, the Summary Report will state that:
    - A review of outcome data, adverse events, and information relating to study performance (e.g., data timeliness, completeness, and quality) across all centers took place on a given date
    - The observed frequency of adverse events did not exceed what was expected and indicated in the informed consent;
    - A review of recent literature relevant to the research took place, and;
    - The DSMB recommended that the study continue without modification of the protocol or informed consent

- If the DSMB does identify concerns, the NHLBI staff will distribute, as soon as feasible, preferably within 7 calendar days of the DSMB meeting, the Summary Report as outlined above, outlining the concerns and the basis for any recommendations that the DSMB has made in response to the concerns. Adverse event reporting will be consistent with NHLBI policy.

- The NHLBI Program Office will distribute the Summary Report to study investigators, or when applicable, the DCC. It is the responsibility of each clinical center to forward this information to the local IRB.

11.7 COMMUNICATION

The following description illustrates the relationship between the DSMB, NHLBI and other entities in the studies.

Communication of study investigators with DSMB members will be primarily through the NHLBI Program Office and, if applicable, the Data Coordinating Center (DCC). It is expected that study investigators will not communicate with DSMB members about the

study directly, except when making presentations or responding to questions at DSMB meetings or during conference calls.

If requested, this charter and accompanying list of Board members may be sent to an IRB. In the case, this charter will be marked as “not for dissemination” and be sent with a cover letter from the Principal Investigator (PI) to the IRB Chair.

Consistent with NHLBI policy, each DSMB is assigned an Executive Secretary (ES) to provide an unbiased staff interface between the DSMB members and other meeting participants, especially during closed and executive sessions. The ES is responsible for assuring the accuracy of the final recommendations and DSMB minutes and timely transmission to the NHLBI.

The NHLBI does not release Board members’ names in response to media inquiries until after publication of the main results of the study.

11.8 Reports to the DSMB

For each meeting, the study investigators, or when applicable, the DCC, with input from NHLBI staff, will prepare summary reports and tables to facilitate the oversight role of the DSMB.

The DSMB should discuss at the first or subsequent meetings what data they wish to review and how the data should be presented.

Reports will include at minimum:

1. Current protocol version
2. Name(s) of Principal investigator(s)
3. Organization/institution
4. Date of submission
5. Study title(s)
6. Brief description of study(s)
7. Recruitment status
8. Interim or final results (if available)
9. Description of next steps or plans
10. Summary of protocol amendments during the reporting period
11. Summary of serious adverse events
12. Summary of adverse events
13. List of relevant investigator publications during the reporting period

Adverse events will be presented in table format whenever possible and include all adverse events over the entire period of the study. For each event, the report will specify, at minimum:

1. Subject ID
2. Site
3. Date of Treatment
4. Date of AE
5. Description

6. Expectedness (Expected/Unexpected)
7. Severity or Grade (Mild/Moderate/Severe/Life-Threatening/Death; or Grade 1-5)
8. Relationship to Procedure (Unrelated/Unlikely/Possibly/Probably/Definitely)
9. Relationship to Study Product (Unrelated/Unlikely/Possibly/Probably/Definitely)
10. Current Status or Resolution (Ongoing/Resolved/Progressed/Unknown)

11.9 Expedited Adverse Event Review and DSMB Notification Process

The role of the DSMB is to is to provide an independent assessment of the severity of the event experienced by the individual subject and the significance of the event to the trial as a whole, that is, to weigh the potential risk of a similar event to other enrolled or future subjects, and to make recommendations regarding protocol and study operations to ensure subject safety, in an urgent manner when warranted.

The notification process is as follows:

- The PI, sponsor, or DCC reviews the occurrence in accordance with the DSM plan and federal regulatory requirements. The DCC medical monitor, PI, or designee, prepares a study AE report form, and sends the form to the NHLBI Program Officer in the timeframe established by NHLBI policy and FDA reporting requirements, according to whether the event requires expedited reporting. The AE report is often accompanied by a summary memorandum for the DSMB, and supporting clinical narratives, lab or exam reports.

- The NHLBI Program Officer notifies the ES to forward the report and any other documents to the appropriate members of the DSMB.

- The ES forwards the notification and documents to the board Chair and appropriate members of the DSMB.
- Each reviewer is requested to review and provide a statement of her/his assessment, in particular whether the event is expected or related, and if her/his assessment is in agreement, or in conflict, with that of the study medical monitor in any important respect.

- The Chair also determines whether the event is sufficiently serious as to require notification of the full board, and if the event requires a call for discussion. If so, the ES will notify the board with the information by e-mail, and handle arrangements for a conference call, as warranted.

- If the Chair and/or board require additional information on the event, the ES will contact the NHLBI Program Officer, who will communicate the request to the study personnel or DCC.

11.10 Clinical Holds

If at any time a study is placed on Clinical Hold by the FDA, IRB, or study investigators, the DSMB will be notified immediately, as follows:

- The PI, sponsor, or DCC notifies the NHLBI Program Officer that the study has been placed on Clinical Hold. For external Holds, correspondence from the FDA or IRB containing the justification for the Hold will be provided at the time of notification. Notification of internal holds will include a detailed justification provided by study investigators, sponsor, or DCC.

- The NHLBI Program Officer notifies the ES to forward the notification, justification(s), and any other documents to the appropriate members of the DSMB.

- The ES forwards the notification and documents to the board Chair and appropriate members of the DSMB.

If/when a Hold is lifted, the PI, sponsor or DCC will notify the NHLBI Program Officer and provide, as appropriate, documentation of FDA and IRB approvals, all data or other information provided in support of lifting the Hold, and revised protocol. The Program Officer will provide the materials to the ES to forward to the board Chair and appropriate members of the DSMB.

The study investigators will not resume accrual unless/until the DSMB reviews the materials and recommends to the NHLBI to allow the study to proceed. DSMB recommendations will be submitted to the Division and Institute by the ES and a summary returned to the study investigators as described above in Section 9: Reports of DSMB Deliberations.

11.11 Review of New Protocols

The DSMB will conduct a review of new protocols according to the following process:

- The NHLBI Program Officer notifies the ES when an NHLBI-supported study falls under the NHLBI policy for DSMB monitoring of high-risk gene and cell therapy clinical trials.

- The ES arranges a conference call to take place within approximately four weeks and sends the protocol, consent, and other documents to the board members. The members will be given at least two weeks for their review, if possible. At the discretion of the Chair, review and voting may be conducted by correspondence.

- While reviewing the documents, the board members will record their comments and questions and forward them to the ES.

- The ES will collate the comments and questions into one document. The ES sends the document to the NHLBI Program Officer.

- The Program Officer forwards the comments and questions to the study investigators and/or DCC for their response, which is then sent to the ES to share with the DSMB prior to the meeting.

If notification is received within four weeks of a regular meeting of the DSMB, the review of the new protocol may occur as part of the regular meeting.

If a study team wishes to provide an informational introduction to an anticipated protocol, a presentation may be included in the agenda of a regular meeting. However, the DSMB will not make recommendations to the NHLBI until provided with the FDA- and IRB-approved protocol and informed consent forms; investigator brochure, if applicable; documentation of FDA review of the protocol; and for gene therapy studies, documentation of RAC review.

11.12 Statistical Monitoring Guidelines

Review of new protocols will include review of the adequacy of the statistical monitoring plan. The final plan, whether part of a research protocol or separate document, will be maintained as an appendix to the DSMB Charter. The DSMB should discuss the statistical monitoring procedures that will be followed to guide recommendations about termination or continuation of the trial. These procedures could include guidelines for early termination for benefit, termination for futility, and termination for safety reasons.

12. STUDY ADMINISTRATION

12.1 Regulatory Authority Approval

This study will be conducted in accordance with Good Clinical Practice (GCP) requirements described in the current revision of International Conference on Harmonization of Technical Requirements of Pharmaceuticals for Human Use (ICH) Guidelines and all applicable regulations, including current United States Code of Federal Regulations (CFR), Title 21, Parts 11, 50, 54, 56, and 312 and Title 45, Part 164. Compliance with these regulations and guidelines also constitutes compliance with the ethical principles described in the current revision of the Declaration of Helsinki. This study will also be carried out in accordance with local legal requirements.

12.2 Ethics Approval

It is the Investigator’s responsibility to ensure that prior to initiating this study; this protocol is reviewed and approved by the appropriate local IRB. The composition and conduct of this committee must conform to the United States CFR.

The IRB/IEC must also review and approve the site’s informed consent form (ICF), other written information provided to the subject and all advertisements that may be used for subject recruitment.

If it is necessary to amend the protocol or the ICF during the study, the Investigator will be responsible for ensuring that the IRB/IEC reviews and approves these amended documents. An IRB/IEC approval of the amended protocol and/or ICF must be obtained in writing before implementation of the amended procedures and before new subjects are consented to participate in the study using the amended version of the ICF.

12.3 Subject Informed Consent

Before being admitted to the clinical study, all subjects must consent in writing to participate. An ICF will be given to each subject, which will contain all United States federally required elements, all ICH-required elements, and Health Insurance Portability and Accountability Act Authorization (HIPAA) information in language that is understandable to the subject.

The process of obtaining the informed consent will be in compliance with all federal regulations, ICH requirements, and local laws.

The investigator or designee will review the study with each subject. The review will include the nature, scope, procedures, and possible consequences of the subject's participation in the study. The ICF and review must be in a form understandable to the subject. The Investigator or designee and the subject must both sign and date the ICF after review and before the subject can participate in the study. The subject will receive a copy of the signed and dated form, and the original will be retained in the site study files. The Investigator or his/her designee must emphasize to the subject that study participation is entirely voluntary and that consent regarding study participation may be withdrawn at any time without penalty or loss of benefits to which the subject is otherwise entitled.

If the ICF is amended during the study, the Investigator must follow all applicable regulatory requirements pertaining to approval of the amended ICF by the IRB/IEC. The site must use the amended consent form for all new subjects and repeat the consent process with the amended ICF for any ongoing subjects.

If a subject is unable to come to site due to COVID-19, the following methods will be used to obtain informed consent that meets the requirements of local regulations, ICH guidelines, and the IRB/EC or study center, where applicable:

- Obtain the informed consent electronically; OR
- Obtain the informed consent by teleconference/video conference in alignment with local regulatory guidance.

How the consent was obtained and reason why it was obtained using that particular method should be documented in the eCRF. The signature may be obtained via a secure email or digital signature. If it is not possible to obtain a digital image of the signed page, a statement of consent can be obtained via cellphone text message or via secure email. This does not preclude a site from obtaining consent via paper, if such arrangements can be made, e.g. fax, mail. During the remote informed consent process the subject, a witness, and someone from the study team will be present.

12.4 Confidentiality of Information

Subjects' names will remain confidential and will not be included in the database. Only subject number, subject initials, and birth date will be recorded in the data system. If the

subject name appears on any other document collected (e.g., hospital discharge summary), the name must be deleted before the document is transmitted. All study findings will be stored in electronic databases. The subjects will give explicit permission for representatives of regulatory authorities and the IRB/IEC to inspect their medical records to verify the information collected.

Subjects will be informed that all personal information made available for inspection will be handled in the strictest confidence and in accordance with all state, local, and federal data protection/privacy laws, including, without limitation, the HIPAA.

Participants will be asked to voluntarily provide written authorization prior to requesting or disclosing private health information either as part of the written ICF or as a separate authorization form. The authorization will contain all required elements specified by 45 CFR 164, and will allow the site to access study-related private health information until the conclusion of the clinical study. The authorization will remain valid and in full force and effect until the first to occur of (1) the expiration of two years after the study therapy is approved for the indication being studied, or (2) the expiration of two years after the research program is discontinued. Individual subject medical information obtained during this study is confidential and its disclosure to third parties (other than those mentioned in this Section) is strictly prohibited. In addition, medical information obtained during this study may be provided to the subject's personal physician or to other appropriate medical personnel when required in connection with the subject's continued health and welfare.

The investigator will maintain a personal subject identification list (subject and treatment numbers with the corresponding subject names) to enable records to be identified.

12.5 Payments to Subjects

Subjects will be reimbursed $25 at the end of each follow-up visit (Day 3 – Month 12) for a total remuneration of $175. These disbursements are meant to cover the time required to complete these study visits and all necessary travel and parking expenses.

APPENDIX 1: Infusion Guidelines

Prior to the start of the infusion the following procedures and assessments will be conducted on the study subject:

1. Vital Signs: Blood pressure, heart rate, respiratory rate, and temperature, will be measured within 15 minutes prior to the initiation of the infusion.
2. Oxygen saturation will be continuously monitored by pulse oximetry for at least 30 minutes prior to initiation of IP infusion.
3. Confirm that IV access is established and that the IV catheter is no smaller than 20 gauge
4. Study personnel needs to verify that the following pre-medications have been administered 30 minutes to an hour prior to infusion, unless otherwise determined by the physician:

    - Hydrocortisone 25 – 50 mg IV
    - Diphenhydramine (Benadryl) 25 – 50 mg IV

    Note: No other medications should be given during the infusion unless determined medically necessary by the Investigator.

5. Document pre-medications given prior to infusion on the source documents
6. Required IV Infusion materials as follows:
    - 0.9 % normal saline IV infusion bag
    - IV Pump tubing
    - IV extension tubing (unless using a central line)
    - Volumetric infusion pump
    - Gloves
7. Remove 0.9% normal saline infusion bag and connect IV tubing to the volumetric infusion pump
8. Cover the IV tubing with the blinding material provided with the infusion bag by the drug preparation technician.

During the IP infusion the following procedures and assessments will be conducted on the study subject:

1. Monitor the subject continuously with pulse oximetry
2. Hang the blinded infusion bag. Investigational product (IP) should not be “piggybacked” through another line
3. Intravenously administer the IP at a rate of 2ml/min.
   Note: Study personnel administering the IP must be present throughout the infusion process. The Investigator must be available at the site during the infusion process in case an emergency should arise.
4. Record the start time of the infusion bag
5. Gently squeeze the infusion bag several times every 15 minutes to assure uniform dispersion of contents

6. Vital signs and O_2 saturation will be measured every 15 minutes until the end of IP infusion
7. Record the total volume infused from the IP bag
8. At the end of the IP infusion, close the line and flush 25ml of 0.9% normal saline into the luer lock connector on the bottom of the IP bag, reopen line and allow to infuse at a rate of 2mL/min until completion.
9. Discard IV tubing according to established guidelines
10. Return the blinded IP infusion bag to the cell-processing technician.

Procedures post-infusion:

1. Vital signs will be monitored at 15 minutes, 30 minutes, 1 hour, and 2 hours post IP infusion
2. The subject will be monitored for a minimum of 2 hours post IP infusion with continuous pulse oximetry
3. If the O_2 saturation decreases to < 93% over a continual period of 3 – 5 minutes then supplemental oxygen may be added or increased during the two hours post-infusion observation period.
4. If at the end of the 2 hour observation period, if a subject's O_2 saturation stays below 93% then the subject will be provided additional oxygen to maintain a saturation of >93% at room air up to 4 hours post infusion.
5. After the minimum two-hour observation period, the subject will be discharged, if no complaints are experienced, such as shortness of breath or other objective signs of cardiorespiratory compromise.
6. Subjects not meeting criteria for discharge will be assessed by the Investigator during the observation period to further determine hospitalization otherwise not specified in the protocol.

<u>Subject Stopping Guidelines:</u>

- Any subject who develops persistent (that is, still existing more than 3 hours after the end of IP infusion) cardiorespiratory signs or symptoms including hypoxemia (defined per oxygenation criteria of 93% on room air at rest, or shortness of breath, tachypnea, tachycardia, hypotension, or palpitations) will continue with all scheduled follow-up if such follow-up is considered safe in the opinion of the Investigator. The infusion will be stopped if the oxygen saturation does not return to >93% within 3 minutes of initiating supplemental oxygen or if the subject requires greater than 2L/min supplemental oxygen to achieve the required saturation of >93%. If a subject requires the addition of oxygen, it will be continued for 4 hours after the completion of the infusion. At that time, oxygen will be weaned off to maintain a saturation >93% on room air.

- Any subject whose infusion is stopped due to cardiorespiratory distress will receive no further IP infusions but will continue with all scheduled follow-up if such follow-up is considered safe in the opinion of the Investigator.

- Any subject who develops any sign or symptom that, at the discretion of the Investigator, warrants the discontinuation of infusion will receive no further IP

infusions but will continue with all scheduled follow-up if such follow-up is considered safe in the opinion of the Investigator.

- Infusion of the IP may be stopped if there is an adverse event that the Investigator believes is related to the IP or if there is an issue with the IP infusion.

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