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 ***A***llogeneic Mesenchymal Human Stem ***C***ell infusion therapy for ***E***ndothelial Dy***S***functi***O***n in diabetic subjects with Symptomatic ***I***schemic ***H***eart ***D***isease. (ACESO-IHD Study)

Study Product: Allogeneic Human Mesenchymal Stem Cells (MSCs)

Indication: Endothelial Dysfunction in Diabetes and Symptomatic Ischemic Heart Disease

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Protocol Agreement Signature:

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List of Abbreviations

ADA	American Diabetes Association
ACE	Angiotensin converting enzyme
AE	Adverse event
CFR	Coronary Flow Reserve
CHF	Chronic heart failure
CRF	Case report forms
CPL	Cell Processing Laboratory
CTCAE	Common Terminology Criteria for Adverse Events
CVD	Cardiovascular disease
DM	Diabetes Mellitus
EPC	Endothelial progenitor cells
EQ-5D	EuroQol - 5 dimension questionnaire
FDA	Food and Drug Administration
FFR	Fractional flow reserve
FMD%	Flow Mediated Diameter percent
GM-CSF	Granulocyte Macrophage Colony Stimulating Factor
BMMNCs	Bone marrow mononuclear cells
HEENT	Head, Eyes, Ears, Nose, & Throat
HF	Heart failure
HIPAA	Health Insurance Portability and Accountability Act of 1996
HLA	Human Leukocyte Antigen
hMSCs	Human mesenchymal stem cells
HSA	Human Serum Albumin
IFR	Instantaneous Wave-Free Ratio
IIEF	International Index of Erectile Function
IL	Interleukin
IRB	Institutional review board
IV	Intravenous Infusion
IVUS	Intravascular Ultrasound
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
QOL	Quality of Life
ROS	Reactive oxygen species
SAE	Serious Adverse Event
SAQ	Seattle Angina Questionnaire
SCF	Stem cell factor
SDF-1	Stromal derived factor 1
SF-36	Short Form 36
SLE	Systemic Lupus Erythematosus
SQOL-F	Sexual Quality of Life - Female
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
PRODUCT	Bone marrow-derived allogeneic human MSCs (MSC) versus placebo consisting of Cell-free PlasmaLyte-A medium.
PHASE OF DEVELOPMENT	I/II
MAIN CRITERIA FOR INCLUSION	Diabetic subjects with Symptomatic Ischemic Heart Disease (DM-IHD) and an indication for standard-of-care coronary angiography
STUDY OBJECTIVES	Test the hypothesis that intravenous (IV) delivery of MSCs improves coronary artery endothelial function (assessed by fractional flow reserve (FFR) and coronary flow reserve (CFR) at 6 months), systemic endothelial function (assessed by FMD and EPC-colony assay), and angina symptoms (assessed by Seattle Angina Questionnaire) in type 2 diabetic patients with symptomatic IHD compared to placebo. Test the hypothesis that allogeneic MSCs promote systemic and coronary endothelial repair through rescue of bone marrow progenitors in type 2 diabetic patients with symptomatic IHD compared to placebo.
STUDY DESIGN	Randomized, Double Blind, Placebo controlled, Pilot Trial
INVESTIGATIONAL PLAN	30 type 2 diabetic patients with symptomatic IHD that undergo standard-of-care coronary angiography with or without Percutaneous Coronary Intervention (PCI) and meet all the inclusion and none of the exclusion criteria will be randomized to receive, within 2 weeks post- standard-of-care coronary angiography , either allogeneic MSCs or placebo in a 1:1 blinded fashion: Group A (15 subjects): Allogeneic MSCs Fifteen (15) subjects will be treated with a single administration of intravenous allogeneic hMSCs (100 million). Group B (15 subjects): Placebo (Cell-free PlasmaLyte-A medium supplemented with 1% HSA) Fifteen (15) subjects will be treated with intravenous placebo infusion. - Patients will be followed for scheduled outcome and safety assessments at 1, 3 and/or 6 months and 12 months post-infusion. - A bone marrow biopsy will be obtained from participants in a sub-study at the 3-month follow up for cellular and molecular studies (separate informed consent document).
ROUTE OF ADMINISTRATION	Peripheral Intravenous Infusion
DURATION OF STUDY PARTICIPATION	12 months
SUBJECT POPULATION	30 subjects age >18 with type 2 diabetes and symptomatic IHD with an indication for standard-of-care coronary angiography with or without PCI

DEFINITION OF ENDPOINTS	<u>Primary Endpoint (Efficacy)</u> - Change in Brachial artery FMD% assessed at 2-weeks 1-, 3-, and 6-months post-infusion compared to baseline. - Change in EPC-CFUs at 2-weeks, 1-, 3-, and 6-months post-infusion compared to baseline. <u>Secondary Endpoints (Efficacy)</u> - Cardiac catheterization angiography IFR, CFR and FFR measurements to assess coronary artery endothelial function at 6 months post-infusion - Target lesion lumen loss by QCA at 6 months post-infusion. - Endothelialization and stent site vessel healing parameters by OCT or IVUS. - Circulating angiogenic and inflammatory markers at 1-, 3-, and 6-months post-infusion. - Seattle Angina Questionnaire (SAQ) Angina Frequency and Quality of Life scales at 1-, 3-, and 6-months post-infusion <u>Secondary Endpoints (Safety)</u> - Incidence (at one (1) month post-infusion) of any treatment-emergent serious adverse events (TE-SAEs), defined as the composite of: death, non-fatal MI, stroke, hospitalization for heart failure, sustained ventricular arrhythmias (characterized by ventricular arrhythmias lasting longer than 30 sec or with hemodynamic compromise) or atrial fibrillation. - Incidence of Major Adverse Cardiac Events (MACE), defined as the composite incidence of (1) death, (2) hospitalization for cardiovascular events, or (3) non-fatal MI at 12 months. - Treatment emergent adverse event (AE) and serious adverse event (SAE) rates through the duration of the study. - Target Vessel Failure (revascularization, death, or MI attributed to the target vessel) post-PCI (only applicable if participant undergoes PCI). - Abnormal hematology and clinical chemistry value results.
Inclusion Criteria	1. Be ≥ 18 years of age (males and females). 2. Provide written informed consent. 3. Have a diagnosis of symptomatic IHD and an indication for standard-of-care coronary angiography. 4. Have Type 2 diabetes mellitus documented by hemoglobin A1C > 7%, or on medical therapy for Type 2 diabetes mellitus.
Exclusion Criteria	1. Be younger than 18 years of age. 2. Have a baseline glomerular filtration rate (GFR) <30 ml/min 1.73m^2 estimated using the MDRD formula. 3. Indication for surgical revascularization or valve therapy. 4. Have known hypersensitivity or contraindication to aspirin; both heparin and bivalirudin; all available P2Y12 inhibitors (clopidogrel, prasugrel, and ticagrelor); or any zotarolimus, cobalt, chromium, nickel, tungsten, acrylic, or fluoro polymers; or hypersensitivity to contrast media that cannot be adequately premedicated. 5. Have a hematologic abnormality as evidenced by hematocrit < 25%, white blood cell < 2,500/uL or platelet values < 100,000/uL without another explanation.

	6. Have liver dysfunction, as evidenced by enzymes (AST and ALT) greater than three times the upper limit of normal. 7. Have a bleeding diathesis or coagulopathy (INR > 1.3), cannot be withdrawn from anticoagulation therapy, or will refuse blood transfusions. 8. Be an organ transplant recipient or have a history of organ or cell transplant rejection. 9. Have a clinical history of malignancy within the past 2 years (i.e., subjects with prior malignancy must be disease free for 2 years), except curatively treated basal cell or squamous cell carcinoma, or cervical carcinoma. 10. Have a condition that limits lifespan to < 1 year. 11. Have a history of drug or alcohol abuse within the past 24 months. 12. Be serum positive for HIV, hepatitis B sAg, or viremic hepatitis C. 13. Be currently participating (or participated within the previous 3 months) in an investigational therapeutic or device trial. 14. Be pregnant, nursing, or of childbearing potential and not on contraception. (May participate if on 2 forms of acceptable contraceptives). 15. 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), but their prognosis is also 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[2-6] The persistent hyperglycemia and other metabolic abnormalities directly affect the endothelium, contributing to the pathophysiology of disease[2, 7, 8]. Numerous cell-based therapy clinical trials in subjects with ischemic heart disease (IHD) illustrate that mesenchymal stem cell (MSC) administration improves cardiac structure, function and quality of life[9-12]. 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[13], 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[13]. 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[13-15] Moreover, comorbidities and aging are potential factors underlying the difference in efficacy between autologous (subject-derived) and allogeneic (healthy donor) MSCs[16-18].

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 CVD and manifests in subjects with cardiovascular risk factors such as atherosclerosis, hypertension, chronic kidney disease, metabolic syndrome, and DM[19]. 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[6, 20, 21]. 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[22]. 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[23]. Subjects with CVD have decreased circulating EPC levels and bioactivity[24]. Indeed, circulating EPC levels serve as a predictor of CV events[25-27]. Low numbers of EPC-colony forming units (EPC-CFUs) have been linked to high Framingham risk scores for adverse cardiovascular

health outcomes[26]. Similarly, brachial reactivity measurements (FMD%) predict long-term cardiovascular events[28, 29].

Mesenchymal Stem Cells Transplantation for Patients with CVD and Endothelial Dysfunction

The field of cell-based therapy for CVD, particularly ischemic heart disease (IHD), has had major advances in the past few years[2, 9-13]. 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[9-13, 30, 31]. The type 2 DM subject population is at an especially high risk for IHD 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[9, 10, 12, 13, 32], have significant potential for clinical benefit in CVD by virtue of their antifibrotic[33], anti-inflammatory, and pro-angiogenic properties[34, 35], as well as their ability to stimulate endogenous progenitor cells and capacity to differentiate into endothelial cells[13, 14, 36]. Moreover, there is evidence that NO deficient environments stimulate MSC involvement in angiogenesis[13, 37]. We demonstrated in porcine models of ischemic cardiomyopathy that MSCs alone or in combination with cardiac-derived progenitors reduce infarct size by 35% and significantly improve global and regional left ventricular function[38-41]. These effects are due to cell engraftment, differentiation into myocytes and blood vessels, and stimulation of endogenous cardiac progenitor cell proliferation and differentiation[14]. Given this capacity of MSCs and the role of impaired EPCs in CVD[42, 43], 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[6, 13, 15]. 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[44, 45].

Numerous cell-based therapy clinical trials in subjects with IHD illustrate that MSC administration improves cardiac structure and function and quality of life[9-12, 30, 31]. Our 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[13], 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[13]. 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[6, 13-15]. Moreover, comorbidities and aging are potential factors underlying the difference in efficacy between autologous (subject-derived) and allogeneic (healthy donor) MSCs[16-18].

Our central hypothesis is that MSCs restore endothelial function by promoting endothelial repair through the secretion of proangiogenic and anti-inflammatory factors in a donor-dependent manner that stimulate EPC release from the bone marrow and vascular engraftment. The long-term goal of this study is to elucidate the mechanisms underlying the beneficial endothelial effects of MSCs and whether targeting endothelial function via MSC therapy ameliorates CVD outcomes in DM patients. Based on our previous clinical studies in heart failure[9-13, 30-32], frailty[46, 47], patients (ACESO completed pilot, preliminary data shown below), we propose to test allogeneic MSCs administered intravenously (IV) in a double blind, placebo-controlled clinical study of DM patients with endothelial dysfunction and IHD, as a proof of concept clinical investigation.

The POSEIDON[9] phase I/II NIH-sponsored trial evaluated the safety and efficacy of autologous vs. allogeneic MSCs in patients with IHD. It established that allogeneic MSCs do not stimulate significant donor-specific alloimmune reactions and they are safe and effective in reducing infarct scar size and cardiac remodeling. Moreover, we demonstrated that allogeneic MSCs restored endothelial function in patients with HF[13], including DM and non-DM patients. Accordingly, this phase I/II study is timely and warranted and could have a major health impact by addressing an unmet need in a large population of patients at risk for progressive IHD, HF, sudden cardiac death, and recurrent hospitalizations. This study will break new ground in the CV cell-therapy field in several respects. It will be the first rigorously conducted clinical trial of allogeneic MSC cell-based therapy for DM patients with symptomatic IHD. It will establish the efficacy of allogeneic MSCs on systemic (EPC function and FMD%) endothelial function in DM patients as well as on coronary artery flow reserve and fractional flow reserve, endothelialization and vessel healing post-percutaneous coronary intervention (PCI), and on bone marrow MSC and EPC survival and function. Importantly, although safety and efficacy has already been demonstrated by multiple previous phase I/II trials in HF patients, additional efficacy data will be determined from this DM population with IHD but normal left ventricular function.

The current clinical management of IHD relies upon restoration of blood flow through surgical interventions, such as coronary artery bypass graft (CABG) and percutaneous coronary intervention (PCI), and on pharmacological therapeutics with limited effect on endothelial repair. These treatments have only a modest influence on IHD progression, particularly in DM patients. Hence, there is a need for therapeutic interventions that can accelerate the repair of dysfunctional endothelium in the ischemic myocardium, promote the formation of collateral circulation, and provide enough oxygen to the ischemic tissue, leading to improved heart function. A promising novel therapeutic option is the replacement of damaged endothelial cells. Endothelial dysfunction has been shown to be an independent predictor of adverse cardiovascular event outcomes, emphasizing the important physiological role of this single layer of cells[6]. Direct replacement of the

damaged endothelial cells by stem/progenitor cells could allow reendothelialization, as well as neovascularization of ischemic tissues.

Follow-up coronary angiography after PCI is commonly implemented to assess the efficacy and safety of new revascularization devices and novel therapies. However, in clinical practice, routine follow-up coronary angiography has not been considered indicated in stable or asymptomatic post-PCI patients because the direct clinical benefits have been poorly defined. To address this question, Misumida et. al.[48], conducted a systematic review and meta-analysis of randomized trials to assess the potential clinical benefits associated with routine follow-up angiography post-PCI. They compared clinical outcomes after PCI between patients who underwent routine follow-up coronary angiography and those who only had clinical follow-up. Five randomized trials, totaling 4,584 patients met the inclusion criteria, including studies that used sub-randomization and ones that assigned consecutive patients per study protocol. The results showed that routine follow-up coronary angiography was associated with a lower rate of myocardial infarction (odds ratio [OR] 0.65; 95% confidence interval [CI] 0.46–0.91; P=0.01) without reduction in all-cause mortality (OR 0.87; 95% CI 0.59–1.28; P=0.48), and a higher rate of target lesion revascularization (OR 1.73; 95% CI 1.42–2.11; P<0.001). The investigators concluded that further studies investigating the potential role of routine follow-up angiography may be warranted.

The ACESO-IHD clinical trial is highly significant as cellular therapy offers the potential to restore endothelial function, decrease vascular inflammation, improve post-PCI vessel healing, and thereby reduce ischemic events and mortality. MSCs have a major advantage in that they may be used “off the shelf” as an allogeneic graft without requiring immunosuppressive therapy. Accordingly, this protocol is a phase I/II, double-blind, placebo-controlled, randomized trial to test the efficacy of intravenous allogeneic MSCs to restore endothelial function, reduce systemic inflammation, and provide CV clinical outcome benefits in patients with diabetic, symptomatic IHD. This clinical study will advance this promising cellular-based therapy and address a major unmet need in patients with diabetic IHD, one of the most common causes of death and disability in the USA and worldwide. This trial will be conducted with the high of ethical standards required for this growing field and will be conducted at our center by a highly qualified group of investigators.

A Randomized, Double Blind, Clinical Trial to Evaluate Safety and Efficacy of Allogeneic Mesenchymal Human Stem Cells Infusion Therapy for Endothelial Dysfunction in Diabetic Subjects (ACESO)

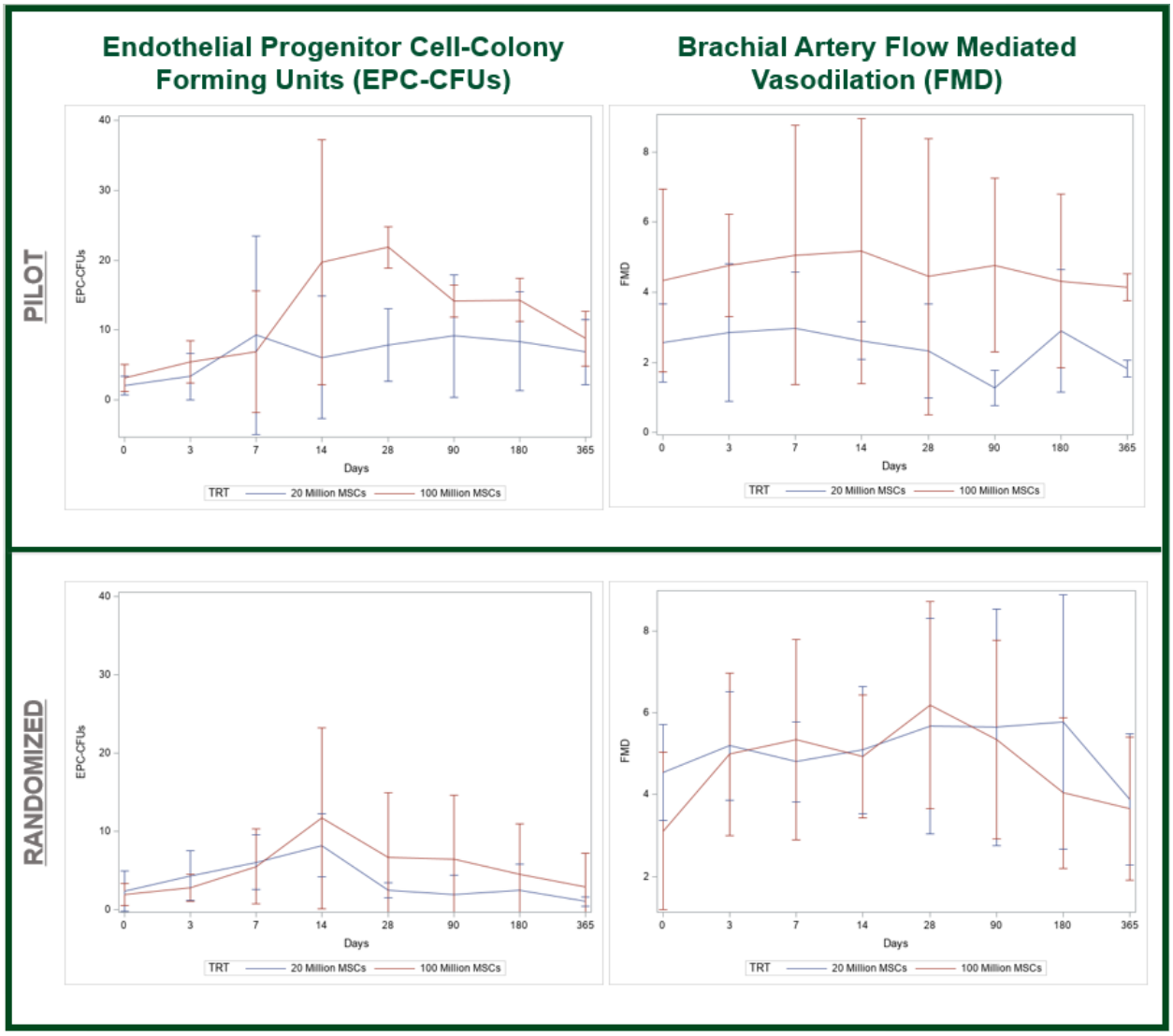
Introduction: Endothelial dysfunction underlies the pathophysiology of cardiovascular disease (CVD) in patients with diabetes mellitus (DM), but no known effective therapies exist. The randomized, double-blind ACESO clinical trial evaluated the safety and efficacy of allogeneic human mesenchymal stem cells (allo-MSCs) infusion to target endothelial dysfunction in Type 2 DM subjects.

Methods: An open label pilot phase (n=6) was followed by a blinded, randomized phase (n=10) study. DM subjects with endothelial dysfunction received a single peripheral intravenous infusion of either 20 or 100 million allo-MSCs (obtained from 3 healthy bone marrow donors) and followed for 1 yr. The primary endpoint was incidence of treatment-emergent serious adverse events (TE-SAE) within 1-month post-infusion. Secondary endpoints assessed endothelial function via endothelial progenitor cell-colony forming units (EPC-CFUs) and flow-mediated dilation (FMD%).

Results: Participants had well-controlled DM, blood pressure, and kidney function (GFR ≥50 ml/min), and most were taking statins. The 6 pilot-phase participants were men (1 Asian, 5 non-Hispanic white) with mean (±SD) age of 69.3 (9.6) years. The 10 randomized participants were 5 men and 5 women (5 Hispanic, 3 non-Hispanic Black), with mean age of 60.9 (10.6) years. No TE-SAEs were reported. None of the patients in the pilot phase developed Panel Reactive Antibodies (PRAs). One randomized subject had a moderate increase in PRAs after infusion. Both treatment groups (20 and 100M allo-MSCs) in the pilot and randomized phases showed increased EPC-CFUs over time, particularly at 14 and 28 days post-infusion, that was sustained at 6-12 months. FMD data followed a trend rising above baseline and peaking at days 7 to 14, and then subsided to pre-infusion levels by 1 yr.

Conclusion: Peripheral infusion of allo-MSCs for patients with DM and endothelial dysfunction is safe. The observed improvements in EPCs and FMD did not reach statistical significance in this pilot study. Larger clinical trials are warranted to assess the efficacy of allo-MSC therapy for cardiovascular outcomes in diabetic patients.

Figure 1



A 6 patient open-label pilot study was followed by a 10 patient, double-blinded, randomized trial to assess the safety and efficacy of 20 million vs. 100 million MSCs on endothelial dysfunction in DM patients. Results of these studies supports the safety and potential efficacy of peripheral infusion of allogeneic MSCs for DM patients with endothelial dysfunction.

2. STUDY OBJECTIVES AND ENDPOINTS

2.1 Study Objectives

2.1.1 Primary Objective

Test the hypothesis that intravenous (IV) delivery of MSCs improves coronary artery endothelial function (assessed by fractional flow reserve (FFR) and coronary flow reserve (CFR)
at 6 months), systemic endothelial function (assessed by FMD and EPC-colony assay), and angina symptoms (assessed by Seattle Angina Questionnaire) in type 2 DM patients with symptomatic IHD compared to placebo.

2.1.2 Secondary Objectives

Test the hypothesis that allogeneic MSCs promote endothelial repair through rescue of bone marrow progenitors in type 2 DM patients with symptomatic IHD compared to placebo.

2.2 Study Endpoints

2.2.1 Primary Endpoints (Efficacy)

- Change in Brachial artery FMD% assessed at 2 weeks,1-, 3-, and 6-months post-infusion compared to baseline.
- Change in EPC-CFUs at 2 weeks, 1-, 3-, and 6-months post-infusion compared to baseline.

2.2.2. Secondary Endpoints

Efficacy:

- Cardiac catheterization angiography, CFR and FFR measurement to assess post-coronary angiography coronary artery endothelial function at 6 months post-infusion
- Target lesion lumen loss by QCA at 6 months post-infusion
- Endothelialization and stent site vessel healing parameters by OCT (only applicable if participant undergoes PCI).
- Circulating angiogenic and inflammatory markers at 1-, 3-, and 6-months post-infusion.
- Seattle Angina Questionnaire (SAQ) Angina Frequency and Quality of Life scales at 1-, 3-, and 6-months post-infusion.

Safety:

- Incidence (at one (1) month post-infusion) of any treatment-emergent serious adverse events (TE-SAEs), defined as the composite of: death, non-fatal MI, stroke, hospitalization for heart failure, sustained ventricular arrhythmias (characterized by ventricular arrhythmias lasting longer than 30 sec or with hemodynamic compromise) or atrial fibrillation.

- Incidence of Major Adverse Cardiac Events (MACE), defined as the composite incidence of (1) death, (2) hospitalization for cardiovascular events, or (3) non-fatal MI at 12 months.
- Treatment emergent adverse event (AE) and serious adverse event (SAE) rates through the duration of the study.
- Target Vessel Failure (revascularization, death, or MI attributed to the target vessel) post-PCI (only applicable if participant undergoes PCI).
- Abnormal hematology and clinical chemistry value results.

Exploratory:

- Detailed molecular and functional assessment (genomic, proteomic, proliferation, senescence and differentiation, and angiogenic assays) of bone marrow aspirate at 3 months post-infusion.

3. STUDY DESIGN

3.1 Description of the Study

Thirty (30) patients meeting the inclusion/exclusion criteria will be randomized to receive either allogeneic MSCs or placebo in a 1:1 blinded fashion:

- Group A (15 patients) – Allogeneic MSCs: 100 million cells delivered intravenously.
- Group B (15 patients) – Placebo: Cell-free PlasmaLyte-A medium supplemented with 1% HSA

A separate sub-study will be conducted in which a bone marrow biopsy will be obtained at 3-month time point from patients participating in each group.

4. SUBJECT SELECTION

4.1. Inclusion Criteria

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

1. Be ≥ 18 years of age (males and females).
2. Provide written informed consent.
3. Have a diagnosis of symptomatic ischemic heart disease (IHD) and an indication for standard-of-care coronary angiography.
4. Have type 2 diabetes mellitus documented by hemoglobin A1C > 7%, or on medical therapy for type 2 diabetes mellitus.

4.2. Exclusion Criteria

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

1. Be younger than 18 years of age.
2. Have a baseline glomerular filtration rate (GFR) <30 ml/min 1.73m2 estimated using the MDRD formula.
3. Indication for surgical revascularization or valve therapy.
4. Have known hypersensitivity or contraindication to aspirin; both heparin and bivalirudin; all available P2Y12 inhibitors (clopidogrel, prasugrel, and ticagrelor); or

any zotarolimus, cobalt, chromium, nickel, tungsten, acrylic, or fluoropolymers; or hypersensitivity to contrast media that cannot be adequately premedicated.

5. Have a hematologic abnormality as evidenced by hematocrit < 25%, white blood cell < 2,500/uL or platelet values < 100,000/uL without another explanation (per investigator discretion).
6. Have liver dysfunction, as evidenced by enzymes (AST and ALT) greater than three times the upper limit of normal.
7. Have a bleeding diathesis or coagulopathy (INR > 1.3), cannot be withdrawn from anticoagulation therapy, or will refuse blood transfusions.
8. Be an organ transplant recipient or have a history of organ or cell transplant rejection.
9. Have a clinical history of malignancy within the past 2 years (i.e., subjects with prior malignancy must be disease free for 2 years), except curatively treated basal cell or squamous cell carcinoma, or cervical carcinoma.
10. Have a condition that limits lifespan to < 1 year.
11. Have a history of drug or alcohol abuse within the past 24 months.
12. Be serum positive for HIV, hepatitis B sAg, or viremic hepatitis C.
13. Be currently participating (or participated within the previous 30 days) in an investigational therapeutic or device trial.
14. Be pregnant, nursing, or of childbearing potential and not on contraceptive medications. (May participate if on 2 forms of contraceptives).
15. 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, cardiac 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 and Screening

Healthy volunteers (male or female) between the ages of ≥ 18 to ≤ 45 (inclusive) will be screened as potential bone marrow donors. 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), ACESO trial (IND#: 17054; NCT02886884), and FDA Drug Master File (DMF)# 17882. Donors will be consented and evaluated as per the current IRB approved DONOR protocol (eProst#: 20171033). All screening tests will be performed in accordance with the DONOR protocol (eProst#: 20171033).

6. TREATMENT OF SUBJECTS

6.1 Study Investigational Product

The investigational product (IP) consists of MSCs 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), ACESO trial (IND#: 17054; NCT02886884), and FDA Drug Master File (DMF)#: 17882. 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 Allo-hMSC product will only be infused to a CMV positive recipient. All CMV negative recipients will receive CMV negative Allo-hMSC product[91].

6.2 Dosing

The allogeneic MSCs are manufactured by the University of Miami Interdisciplinary Stem Cell Institute, as specified in the DONOR protocol (eProst#: 20171033) and FDA DMF#: 17882. The 100 million dose of MSCs has been validated in multiple published clinical trials by our group[9-13, 31, 32, 46, 47] and other groups as well as in the ongoing ACESO pilot study (IND#: 17054; NCT02886884; data shown above).

6.3 Dosage Rationale

A safety profile for IV infusion of MSCs was based on outcomes from previous completed toxicology results[10, 49]. The results from these previous studies demonstrate that the product can be administered intravenously without toxic events at up to 65 x 10^6 MSC/kg dose delivered in one bolus infusion or at 100 x 10^6 MSC/kg cumulative dose delivered by 5 infusions (20 x 10^6 MSC/kg per infusion).

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

A previous pilot study was conducted to compare the effects of two different doses of MSC given via IV infusion in type 2 diabetic subjects (ACESO). The results of this study showed that both the 20 and 100 million cell doses are safe but the 100 million dose was the optimal dose and was selected to be used for this study (results showed above).

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 100 million (100×10^6) MSC/infusion, reconstituted as 2 million MSC/mL, in the following total volume:

100 million dose (2.5 million MSC/min) and Placebo will be prepared in an 80ml bag

Investigational product is 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 MSC/minute, which will last approximately 40 minutes to complete.

The infusion bag will be flushed with an additional 25 ml of 0.9% normal saline at the completion of allo-MSC 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, respiration rate, and temperature) every 15 minutes. Vital signs will be recorded at the start and at 15-minute intervals 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

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

6.6 Blinding and Unblinding

Subjects will be randomized into active treatment groups and study personnel will be blinded to treatment assignments. The designated cell-processing technicians will prepare the investigational product 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.

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 the Cell Manufacturing Program 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 GMP Cell Processing Facility 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. Investigational product 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 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 Infusion	Week 2 Post-Infusion	Month 1 Post-Infusion	Month 3 Post-Infusion	Month 6 Post-Infusion	Month 12 Post-Infusion
		Within 7 days Pre or Post-Cath	0-30 days post-Cath	0-30 days post-Cath	± 5 days	± 2 weeks	± 2 weeks	± 2 weeks	± 2 weeks
Informed Consent[1]		X							
Medical History		X							
Physical Exam & Vital Signs[2]		X		X	X	X	X	X	X
12-lead Electrocardiogram (ECG)		X		X		X	X	X	X
Concomitant Medications[3]		X	X	X	X	X	X	X	X
Randomization[4]			X						
IV Infusion Treatment[5]				X					
Coronary Angiogram[6]		X						X	
FFR, CFR, IFR, IVUS, and/or OCT *(optional)*[7]		X						X	
Brachial Artery Ultrasound[8]			X		X	X	X	X	
Bone Marrow Biopsy[9]							X		
Questionnaires[10]			X		X	X	X	X	
Review Adverse Events			X	X	X	X	X	X	X
Laboratory Testing[11]	Hematology & Chemistry	X		X		X	X	X	
	Coagulation	X					X	X	
	Urinalysis	X					X	X	
	HbA1c	X					X	X	
	Biomarkers (SST) (includes PRA)		X		X	X	X	X	
	Immune Monitoring (MNC)		X		X	X	X	X	
	Genetic Testing (DNA and RNA)			X				X	
	EPC-CFU assay		X		X	X	X	X	
	Viral Serology	X							
	Serum Pregnancy Test	X		X					

Time and Events Table Key:

1. Informed consent can be obtained Pre- or Post- cardiac catheterization. The first angiogram is clinically indicated.

2. A physical examination will be performed and may include the following examinations: general appearance; skin and nails; limbs; head, eyes, ears, nose, throat (HEENT) and neck; respiratory; cardiovascular; and abdominal. Vital Signs measurements will consist of: height, weight, heart rate, blood pressure, respiratory rate, oxygen saturation, and temperature. Height is only required at screening. If physical exam and vitals were performed as "Standard of Care" within 7 days of signing ICF, those results may be extracted from patient medical records and used for the screening visit in this study. The screening visit physical exam can be completed by the participants' treating physician.

3. Concomitant medications will be recorded at screening visit and updated at each visit.

4. Randomization can be performed within 7 days prior to IP infusion.

5. Refer to Appendix 1 for Infusion guidelines.

6. Refer to hospital protocols for coronary angiogram guidelines. Results from the screening cardiac catheterization may be extracted from patient medical records and used for the purposes of this study. Subjects who did not have a stent placed at screening will not undergo the coronary angiogram (and associated measurements) at Month 6, but should complete the other assessment listed on Time and Events Table, per investigator's judgement.

7. Subjects who did not have a stent placed at screening may still undergo the coronary angiogram (and associated measurements) at Month 6, but should complete the other assessment listed on Time and Events Table. All procedures (CFR, FFR, IFR, IVUS, and/or OCT) will be performed in accordance with the established hospital protocols which are done as "Standard of Care". Subjects who undergo PCI at screening should have CFR, FFR, IFR, IVUS, and/or OCT measurements if available, as per investigator's judgement.

8. Refer to Appendix 2 for Brachial Ultrasound (Brachial Artery Ultrasound to assess endothelial function must be performed prior to 12pm).

9. Bone Marrow Biopsy will be performed at month 3 visit. Separate ICF will be used for this optional sub-cohort. The bone marrow biopsy can be performed in the Catheterization Laboratory or in Interventional Radiology department, either with or without imaging guidance (X-Ray or Ultrasound).

10. All subjects will be asked to complete the Seattle Angina Questionnaire (SAQ), EQ-5D, and SF-36 quality of life questionnaires. Only male subjects will be asked to

complete the IIEF questionnaire, and only female subjects will be asked to complete the SQOL-F questionnaire.

11. Subjects must be fasting for at least 8 hours prior to blood collection as required by standard of care. If labs were performed as "Standard of Care" within 7 days of signing ICF, those results may be extracted from patient medical records and used for the screening visit in this study. The minimal laboratory requirements include:

    Hematology Tests:
    Complete Blood Count with Differential (WBC, RBC, hemoglobin, hematocrit, MCV, and platelets; Diff: neutrophils, lymphocytes, monocytes, eosinophils, and basophils).
    Chemistry Tests: (fasting required)
    Comprehensive Metabolic Panel (glucose, sodium, potassium, chloride, bicarbonate, blood urea nitrogen, creatinine, total protein, albumin, alkaline phosphatase, total bilirubin [fractionate if total >1.5 times normal], alanine transaminase (ALT), aspartate transaminase (AST), and GFR).

    Coagulation Tests: Prothrombin time (PT/INR), activated partial thromboplastin time (aPTT).

    Urinalysis: Routine Urinalysis with reflex to culture.

    Glycosylated Hemoglobin: HbA1c

    Biomarkers: SST: (2 Gold or Tiger top serum separator tubes) Transcriptomic/proteome (RNA, mRNA, protein samples, and telomerase, akt); growth factors (sdf-1, notch); functional assays (cell-growth rate, VEGF, and CFU assays), PRA, and more.

    Immune monitoring: MNCs: (2 Green top sodium heparin tubes) CD3*CD25* or CD3*CD69* and more.

    Gene Expression: peripheral blood will be collected prior to infusion on Day 0, and at the 6 month follow up visit to analyze DNA & RNA (DNA to be collected at one time point only).

    EPC-CFU Assay: Blood samples (5 Lavender top tubes) to measure Endothelial Progenitor Cell-Colony Forming Units (EPC-CFU).

    Viral Serology: Infectious disease tests – HIV, HIV 2, Hep B (HBsAg), Hep C (Anti-HCV), and CMV.

    Serum Pregnancy Test: Serum βHCG test will be completed at screening and within 36 hours prior to infusion in women of childbearing potential only.

If subjects are unable to come to the site for specified visits alternative methods for assessments (e.g., phone contact, virtual visit, alternative location for assessments, including local labs or imaging centers) could be implemented when necessary.

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. Screening visit can take place within 7 days before or after coronary angiogram. 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.

A subject may be rescreened based on the Investigators judgement and will at a minimum need to reconsent and complete screening laboratory tests. The subject will remain with the same subject number throughout this process.

If any of the screening assessments (coronary angiogram, vasoreactivity testing, OCT, physical exam, labs, EKG, etc.) were performed as “Standard of Care” within 7 days of signing ICF, those results may be extracted from patient medical records and used for the screening visit in this study.

8.2 Baseline and Day 0 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 0 - 30 days post-coronary angiogram.

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 within 0 - 30 days post-coronary angiogram 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. Refer to Appendix 1 for Infusion Guidelines.

8.3 Week 2, Month 1, Month 3, Month 6, and Month 12 (Post-Infusion Visit)

See Table 1 for the procedures and assessment to be performed for the Week 2, Month 1, Month 3, Month 6, and Month 12 study visits. Subjects who had a stent placed at screening will have a follow up coronary angiography at 6 months post-investigational product infusion to evaluate the treated lesion lumen loss with quantitative coronary angiography (QCA), physiology with fractional flow reserve (FFR) and endothelial function with coronary flow reserve (CFR). Outpatient visits should be completed as close to the scheduled visit dates as possible. There will be a window of ± 5 days for week 2 and ±2 weeks for the Month 1, 3, 6, and 12 study visits.

An optional sub-study will be conducted on at least 5 participants from each treatment group in which they will undergo a bone marrow biopsy at Month 3 for a detailed molecular (genomic, proteomic, proliferation, senescence and differentiation assays) and functional assessment (angiogenic assays) of the effect of allogeneic MSCs on bone marrow derived autologous MSC and EPCs. All subjects will be asked to sign a separate ICF before participating in this optional sub-study. Due to the blinded nature of the study, more than 10 subjects will be enrolled in this sub-study and Cell Manufacturing lab will inform the clinical team once 5 subjects from each treatment complete the bone marrow biopsy. In the interest of participant safety, the bone marrow biopsy may be performed under ultrasound guidance (X-Ray or Ultrasound) by interventional radiology department, if indicated by investigator's clinical judgement.

8.4 Assessment Details

8.4.1 Medical History and Physical Exam

A complete medical history will be conducted during screening testing including: vital signs, height and weight; medical, surgical, and smoking history; and review of current use of prescription and OTC medications. Physical exam performed by the treating physician can be used as part of the screening assessments. Similar physical exams will be conducted at each additional clinic visit during the study including vital signs, weight, review of AEs, and concomitant medications

8.4.2 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 oxygen saturation, weight, respiratory rate, heart rate, blood pressure, and temperature. Height will only be collected at the screening visit.

8.4.3 Flow Mediated Diameter percent change (FMD%)

A Flow Mediated Diameter percent change will be performed four times during the study: at baseline, and at the Week 2, Month 1, 3, and 6 follow-up visits. This exam will assess systemic endothelial function. All measurements of the brachial artery diameter and FMD will be performed in the morning, in a quiet and dark room. (refer to appendix 3)

8.4.4 Coronary Angiography

Coronary angiography will be done by the interventional cardiologist 6 months after infusion of the investigational product to assess post-PCI coronary artery endothelial function, assessed by IFR, CFR, and/or FFR, as available. Participants will also have evaluation of target lesion lumen loss by IVUS or OCT, as available.

8.4.5 Laboratory Testing

Gene Expression

A separate blood sample of approximately 17mL for gene expression (DNA) profiling of WBC (at Day 0 OR Month 6 visit) and a separate blood sample of approximately 5.0mL for RNA expression analysis (at Day 0 AND month 6 visits) will be obtained from the study participants, as detailed on Table 1. 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. Refer to SOP ISCI QAL-006 (Collection and Transport of Blood Specimens for Clinical Research).

Biomarkers/Immune Monitoring for Graft Rejection

The studies planned in this protocol will utilize allogeneic human bone marrow derived MSCs in subjects with endothelial dysfunction and ischemic heart disease due to the Type 2 DM. 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 responses57, 58. Indeed both types of cells do not stimulate a proliferative response from alloreactive T-cells even when the cells 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, prolonging skin allograft survival, and decreasing graft-versus-host disease (GVHD). 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. TNFα, 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 the ACESO IHD protocol we will obtain peripheral blood samples from all subjects to evaluate the presence of activated T cells. Two heparinized (green top) vacutainer tubes (approx. 15 cc total blood) will be collected at different time points during the study (Reference Table 1 for schedule). Peripheral blood mononuclear cells (PBMC) will be isolated from heparinized blood by Ficoll sedimentation and will be viably cryopreserved for planned assessments of T cell activation. Refer to SOP ISCI QAL-006 (Collection and Transport of Blood Specimens for Clinical Research).

Two of the best-accepted markers of T cell activation are CD69 and CD25 (IL-2 receptor). We could 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 MSC infusion.

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

These samples will 1) provide storage of critical biomaterials derived from subjects enrolled in ACESO IHD 2) provide long-term integrity of these biospecimens and samples, and 3) provide management of samples for postdoctoral studies of immunologic, immunohistochemical, cellular, and molecular analyses of collected samples; as well as cell-surface markers (CXCR4, C-Kit, & Connexin 43), transcriptomic/Proteome (DNA, RNA, miRNA, protein samples, and telomerase, akt), growth factors (Sdf-1, notch,), functional Assays (cell growth rate, VEGF, and CFU assay), CD3, CD25, CD69, Inflammatory (IL-1, TGF-) , (but not limited to 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 Type 2 DM.

Endothelial Progenitor Cell-Colony Forming Units (EPC-CFUs) Assay

Blood collection for EPC-CFU assay will be performed to assess endothelial function at baseline, week 2 and 1-, 3-, and 6-months post study product infusion. This will help provide cumulative data in assessing whether stem cell infusion improves endothelial function. Blood collection for EPC-CFU assay will be performed in the morning prior to 12pm.

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. Refer to SOP ISCI QAL-006 (Collection and Transport of Blood Specimens for Clinical Research) and SOP ISCI QAL-007 (EPC Protocol).

9. SAFETY

9.1 Safety Variables

- Vital signs

- Physical examination
- Clinical laboratory tests
- Adverse events

9.2 Laboratory Evaluations

At screening, the HIV-1 and HIV-2 tests, CMV, hepatitis screen, and β-HCG serum pregnancy tests (only for women of child-bearing potential) will be performed. 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, GFR, total protein, HbA1C

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 be monitored throughout infusion and continuously for 2 hours post-infusion. 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 MSCs 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 ≥ 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 Infusion Toxicity

Infusion 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 CTCAEv5). 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 investigational product (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, protocol, ICF, 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 9.3 (“Definition of an Adverse Event”) or SAE, as defined in Section 9.6 (“Definition of a Serious Adverse Event”) and will assess intensity based on the criteria defined in Section

9.10. 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 CTCAEv5 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 24 hours of awareness 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 and CTCAEv5.

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 9.6, "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 event 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.

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 MSCs in type 2 diabetic subjects with ischemic heart disease.

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 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:

  i. Continue the study as planned, without modification
  ii. Continue the study with recommendations – specifying the additions or modifications
  iii. 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.

As an additional safeguard, an impartial third-party witness will be required to observe the informed consent process if the either: 1) Consent is obtained using a remote process that does not include a video conference call; 2) Participant is considered likely to be vulnerable to coercion or undue influence, such as children, prisoners, individuals with impaired decision-making capacity, or economically or educationally disadvantaged persons, as described in 45 CFR 46; 3) The subject, parent or LAR is unable to read or sign the consent document due to physical limitations; and/or 4) Consent is obtained using the short form process, and this consent document is the summary.

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

- Review of the informed consent process will be provided via telehealth, phone, and/or videoconference (zoom health) in alignment with local regulatory guidance.

During the remote informed consent process the participant, a witness, and someone from the study team will be present. Signatures of the informed consent can be obtained in any of the following ways:

- Electronically (Docusign or Adode Certificate); OR
- Secure email or picture of the signed informed consent.

This does not preclude a site from obtaining consent via paper, if such arrangements can be made (fax, mail, etc.). The completed document will be printed out and saved in the research binder. A copy of the signed informed consent will be sent to the subject via secure email, mail, or fax. How the consent was obtained and reason why it was obtained using that method should be documented in the eCRF.

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.

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 after completion of follow-up visits Month 1 – Month 12. Subjects who participate in the optional Bone Marrow Biopsy sub-group will be reimbursed an additional $100 after completion of bone marrow collection visit. Subjects who undergo cardiac catheterization at the month 6 visit will receive an additional $50. Maximum total remuneration amounts to $250.These disbursements can be given in the

form of gift card, check, or cash, and are meant to cover the time required to complete these study visits and parking expenses.

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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:
   a. Hydrocortisone 25 – 50 mg IV
   b. 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
   - Remove 0.9% normal saline infusion bag and connect IV tubing to the volumetric infusion pump
   - 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.

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

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.

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 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.

APPENDIX 2: Flow Mediated Diameter percent change (FMD%) Instructions

A Flow Mediated Diameter percent change (FMD%) will be performed four times during the study: at baseline, Week 2, Month 1, 3, and 6 follow-up visits. This exam will assess vascular function.

Participant should be instructed in pre-session requirements prior to the session. These requirements include:

a) no coffee, tea, soda or other caffeinated beverages in the 4 hr preceding session;
b) no smoking cigarettes or tobacco use in the 2 hr preceding session;
c) no alcohol since midnight;
d) no exercise in the 4 hr preceding session;
e) for men, no use of erectile function medicines in the 24 hr preceding the session;
f) wear a short sleeve shirt for the session.

Procedure Details:

- 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 temperature.

- Studies will be conducted after an overnight fast of at least 10 hours (water is permitted), with the subject 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.

- Blood flow is manipulated in the brachial artery by a pneumatic cuff placed around the forearm distal to the segment of artery being imaged inflated to a suprasystolic pressure (240 mm Hg) for 5 min and released, resulting in a brief episode of reactive hyperemia. Changes in brachial artery diameter in response to the increase in blood flow once the forearm cuff is deflated are recorded.

- Recovery of brachial artery diameter is assessed for an additional 5 min. Images are all digitized and stored on portable storage device following recording for later off-line scoring.

- Two repeated tests are performed. An inter-test rest period of 5 min is used and the second test is not conducted until mean arterial pressure is within 5 mmHg of the initial resting mean arterial pressure taken following the initial rest period.

- Recordings are digitized continuously for 5 min of baseline recording, 5 min of cuff inflation to supra-systolic pressure, and for 5 min after cuff deflation when brachial flow increases to accommodate the dilated resistance vessels.

- FMD% more than 7.1% is considered a normal response[60]. Lower than 7.1% 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.